Bartonella Native Valve Endocarditis: The First Brazilian Case Alive and Well

Lamas C.[1,2,3], Favacho A.[2], Ramos R.G.[1], Santos M.S.[1], Ferravoli G.I.[1], Weksler C.[1], Rozental T.[2], Bóia M.N.[3] and Lemos E.R.S.[2]
[1]National Institute of Cardiology; [2]Laboratory of Hantaviruses and Rickettsiosis (FIOCRUZ); [3]Department of Tropical Medicine, FIOCRUZ; Rio de Janeiro, RJ, Brazil

***Bartonella* is an important cause of blood culture-negative endocarditis in recent studies. Seroprevalence studies in the States of Minas Gerais and Rio de Janeiro have shown Bartonella IgG positivity around 14% in healthy adults and 40% in HIV seropositive adults, respectively. A case report of a 46-year-old white male with moderate aortic regurgitation (AR) due to rheumatic heart disease (RHD), admitted due to worsening heart failure, is presented. Clinical features were apyrexia, anemia, polyclonal hypergammaglobulinemia, hematuria and splenomegaly. He was submitted to surgery due to worsening AR. Histopathology of the excised valve showed active bacterial endocarditis and underlying RHD. Routine blood cultures were negative. Indirect immunofluorescence (IFI) assays for *Coxiella burnetii* were non-reactive. *Bartonella henselae* IgG titer was 1:4096 prior to antibiotics and 1:512 14 months after treatment. History of close contact with a young cat during the months preceding his admission was elicited.**
Key-Words: *Bartonella* spp., infective endocarditis, blood culture-negative infective endocarditis, rheumatic heart disease, serology, indirect immunofluorescence.

Bartonella endocarditis has been recognized since the first reports from the 1990's [1-8] and its importance in blood culture-negative infective endocarditis (IE) has been reinforced in recent studies [9-14]. Although there are three national rickettsial diseases reference laboratories in Brazil (FIOCRUZ, Rio de Janeiro, Adolpho Lutz, in São Paulo, and FUNEDE, in Minas Gerais), only two probable fatal cases of *Bartonella* endocarditis have been reported so far in our country [14]. There are also case reports of *Bartonella* disease such as bacillary angiomatosis in HIV-positive individuals, disseminated disease in children and cat-scratch disease in Brazil, and there are also groups who work experimentally with *Bartonella* [15-21]. Two seroprevalence studies, one in the State of Minas Gerais [22], and the other in the city of Rio de Janeiro [23], show that the Brazilian population is significantly exposed to *Bartonella.* We present the first case of *Bartonella* endocarditis from Brazil, whose diagnosis was done ante-mortem and who had an extremely favourable outcome.

Case Report

A 46-year-old white male patient was admitted to Instituto Nacional de Cardiologia (INC), Rio de Janeiro, due to worsening heart failure in October 2005. He was born and resided in São Gonçalo, a town situated one hour from Rio de Janeiro. He became ill in 1994, during his job as a security officer. Essential hypertension was diagnosed then, as well as valvular disease. He was followed up in INC outpatient clinic. His first transthoracic echocardiography (TTE) in 1995 showed moderate aortic regurgitation and a calcified aortic valve. He retired a few years later due to heart failure. He was a poor historian, but his present admission was due to progression of fatigue and dyspnea on minimal exertion over the past four months. He denied fever. He was on use of captopril and frusemide. On examination, he was pale, height was 1.82 m, weight 76 Kg; carotid pulses were slow with a rapid descent, heart rate was 88 bpm, bp = 120x70 mmHg, cardiac rhythm was regular, right ventricular ictus was palpable as well as left ventricular ictus, which was located in the anterior axillary line, measuring three digital pulps and was propulsive. Heart sounds were soft, and a left ventricular third sound was heard. A systolic fremitus was palpable in the aortic area. Lungs were clear and abdomen was unremarkable. His lower limbs showed edema and were hyperchromic due to chronic venous insufficiency. First impression was of worsening cardiac function due to progression of his valvulopathy. TTE showed moderate aortic regurgitation, with an average aortic valve gradient of 47 mmHg. Electrocardiogram showed 1st degree atrioventricular (AV) block, 3rd degree right bundle branch block, left ventricular hypertrophy, an enlarged left atrium, ventricular and supraventricular ectopic beats. Hemoglobin was 11.5 g/dL, hematocrit was 25%, and mean corpuscular volume was 74. White cell count and platelets were normal. Creatinine was 2.2 mg/dL, urea was 102 mg/dL, potassium was 5.6 mEq/L and sodium was 134. Albumin was 2.7 g/dL, globulins were elevated at 5.4 (protein electrophoresis performed later showed polyclonal hyperglobulinemia; urinary Bence-Jones protein was absent). Thyroid function tests and B12 vitamin were requested because of a "neuropsychiatric condition", and were normal.

Urinalysis showed hematuria and presence of hemoglobin. HIV and hepatitis B and C serological assays were non-reactive. He was immune to hepatitis A. Ultrasound scan of the kidneys showed preserved cortex and medulla, and kidney length was 10 cm. Despite conventional treatment for heart failure, three weeks after admission he was in New York Heart Association (NYHA) functional class III. Cardiac catheterization done five weeks after admission showed normal

Received on 10 May 2007; revised 16 October 2007.
Address for correspondence: Dr. Cristiane Lamas. Rua Presidente Carlos de Campos 81/101, Laranjeiras 22231-080. Rio de Janeiro-RJ/ Brazil. Phone number: 5521-2237-7955. Fax number: 5521- 2556-8455. Email: cristianelamas@gmail.com. Financial support: CAPES, Brazil.

coronaries. A repeated TTE showed worsening of the aortic gradient (68 mmHg) and more severe aortic regurgitation. He had surgery six weeks after admission. Surgical notes described heavily calcified aortic cusps; he needed temporary pacing due to complete heart block. A metallic St Jude 23 valve was placed in the aortic position. Extracorporeal circulation time was 150 min, clamping time was 120 minutes. He presented mild hypertension post-operatively, but he was discharged from intensive care two days later. Five days after surgery he presented atrial flutter which was reversed with 100 J shock and amiodarone. Moderate left ventricular dysfunction and a small pericardial effusion were seen on a TTE performed two days later. His cardiac function deteriorated and one week later transesophagic echocardiography (TEE) showed severe aortic regurgitation. Histopathology of the excised native aortic valve showed vegetations and was suggestive of active bacterial endocarditis. Aqueous penicillin and gentamicin were started the following day, after collection of three blood culture sets. Insertion of a peripherally inserted central catheter (PICC) was requested. Chest and abdominal tomography scans showed a small pericardial effusion and homogeneous splenomegaly. Blood cultures were negative and were discarded on the 7th day of incubation. Blood was sent off to the Rickettsial Reference Laboratory in FIOCRUZ for *Coxiella* and *Bartonella* serological assays. His antibiotics were changed to ceftriaxone, gentamicin and doxycycline two days after being started. On the 8th day of antibiotics, his hematocrit fell, and hemolysis was considered. Due to severe paraprosthetic leak, he has done another surgery one month after first valve replacement. Surgical notes confirmed paravalvular leak in the coronary and non-coronary cusps, and severe calcification of all valve ring, with injury to the anterior mitral valve leaflet. No signs of endocarditis were seen. Surgical time was 155 minutes, clamping time was 120 minutes. He presented complete AV block and was briefly asystolic in the immediate post-operative intensive care admission, but was well enough to be discharged three days later. He was treated with six weeks of intravenous ceftriaxone, two weeks of gentamicin and six weeks of oral doxycycline. He was discharged home two and a half months after admission, clinically well. He has kept appointments in the outpatient clinic, the last one being on February 2007. When re-called for repeating serology on April 2007, he reported having had close contact with a young cat, which was his wife's, during the year of 2005. He denied being scratched, licked or bitten by the cat, or being bitten by cat fleas. He divorced his wife a few months ago, and found out that the cat had died, for reasons unknown.

Materials and Methods

Routine blood cultures were performed, as well as culture of the excised native aortic valve on chocolate blood agar plates at 37ºC with 5% CO_2 in a humid incubator, and the cultures were checked regularly for bacterial growth.

Indirect immunofluorescence assays for *Coxiella burnetii* (Panbio®) and *Bartonella henselae* (Bion®)-specific immunoglobulin G (IgG) and IgM were done in three different serum samples, one dated from December 1st, 2005, the other from December 26, 2005, and the last one from April 4th, 2007.

DNA extraction: total genomic DNA was extracted from serum and paraffin-embedded valvular tissue**.** QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) was used for DNA preparation, following the manufacturer's instructions. Briefly, two hundred microliter aliquots of serum were homogenized in AL lysis buffer and protease K and incubated overnight (ON) at 56ºC. The mixture was added, washed and purified on a spin Qiagen column for 1 min at 14,000 x g in a mini centrifuge (Eppendorf 5245). One hundred microliters of elution buffer was used to resuspend the purified DNA. To obtain DNA from paraffin-embedded valvular tissue, 20 mg of tissue was homogenized in xylene, mixed with a vortex machine and centrifuged. Overnight incubation at 56ºC in ATL lysis buffer and protease K was done. After incubation, AL buffer was added, homogenized and incubated for 10 min at 70ºC. Separation of DNA was obtained by centrifugation of samples in a Qiagen column for 1 min at 14,000 x g. Extracted DNA was eluted with 100 µL of the elution buffer (Qiagen). All genomic DNA preparations were stored at 4ºC until used as a template in PCR assay.

PCR Assays

PCR assay for the 60-kDa heat shock protein was performed as described previously [24,25]. DNAs, prepared from serum and valvular tissue, were used as template for the PCR assays. The extracted DNA was amplified with degenerate primer pair CAT-1 (GATTCAATTGGTTTGAAGGAGGCT) and CAT-2 (TCACATCACCAGGACGTATTC), for amplification of fragment of the 60-kDa heat shock protein (*htrA*), which defines a 414 bp fragment from both *B. henselae* and *B. quintana*. The PCR reaction mixture (total volume of 25 µL) contained 5 µL of the isolated DNA, 2.5 µL of 10-fold PCR buffer, 0.8 µM concentrations of each primer (IDT/ PRODIMOL), 200 µM concentrations of each nucleotide, and 0.65 U of *Taq* polymerase (Platinum *Taq*, INVITROGEN). The PCR was accomplished by predenaturing for 5 min at 95ºC followed by a total of 40 cycles of 94ºC for 1 min, primer annealed at 50ºC for 1 min, and extended at 72ºC for 1 min in an automated DNA 2,400 thermal cycler (Applied Biossystem). Amplification was completed by holding the reaction mixture at 72ºC for 7 min to allow complete extension of the PCR products. Each of the PCR experiments included DNA extracted from either *B. henselae* or *B. quintana* as positive control and water was used as negative control. Ten microliters from each PCR assay was electrophoresed through a 1% agarose gel, stained with ethidium bromide and documented with a gel documented system. The presence of a 414 bp band was considered positive.

Results

Routine blood cultures were negative, as was routine culture of the excised valve. Histopathology of the excised native aortic valve showed vegetations with neutrophilic exudates suggesting bacterial infective endocarditis; there were calcification and fibrosis suggesting underlying rheumatic disease (Figures 1 and 2). Warthin-Starry and PAS stains did not show any microorganism. PCR in serum and paraffin-embedded valvular tissue were negative. Serology IFA IgG and IgM were non-reactive for *C. burnetii*.

Serum titers for *Bartonella henselae* IgG were 1:4096 in the sample collected on December 1st, 2005, and 1:2048 on that of December 26, 2005. Serum collected on April 2007 (14 months after completion of antibiotics) showed a titer of 1:512.

Figure 1. Native aortic valve, hematoxylin-eosin 40x magnified. Vegetation with underlying tissue showing fibrosis and calcification (chronic rheumatic damage).

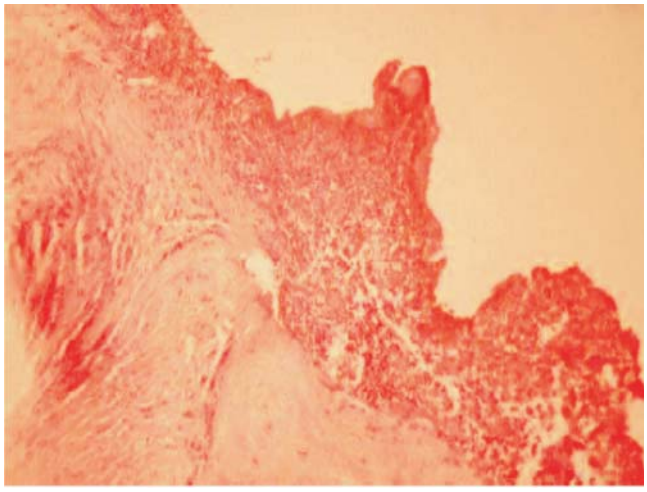
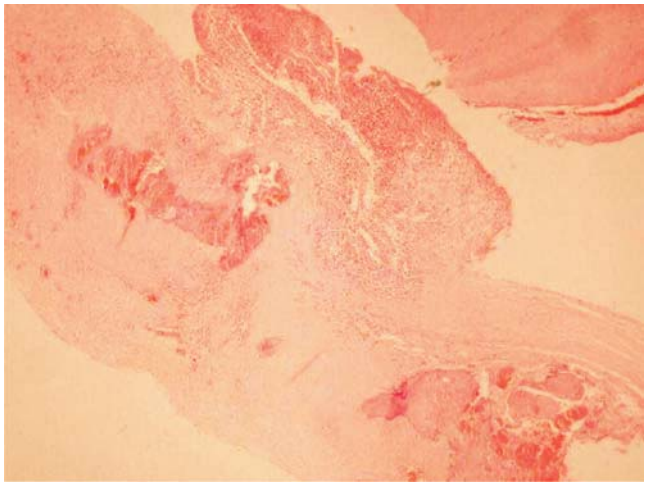


Figure 2. Detail of vegetation with neutrophilic infiltrate.



Discussion

Bartonella disease has a wide spectrum, ranging from asymptomatic to features of fever of unknown origin, uveitis, cat-scratch disease, bacteremia, bacillary angiomatosis and/or peliosis hepatis; the last two manifestations in immunocompromised hosts. Endocarditis has been well described, the first case being published 13 years ago; so far *B. quintana* has been the most frequently identified species, followed by *B. henselae* [26-30]; *B. vinsonii* subsp. *berkhoffi* [30] and *B. elizabethae* [2], which have been infrequently reported. There is a close relationship with acquiring *B. henselae* disease and cat exposure [9,10,26,31-33]. In Brazil, several clinical manifestations of *Bartonella* disease have been reported. Most importantly, seroprevalence studies have shown around 14% *B. quintana* and 13% *B. henselae* IgG antibodies in healthy adults [22] and 40% for *Bartonella sp.* in HIV-positive individuals [23]. In this last study, breeding cats was the only variable associated with *Bartonella* positive serology, despite high exposure to lice, fleas, ticks, dogs, rats and mice in the studied population. Despite the availability of serological studies by reference laboratories in Brazil, only two probable cases of *Bartonella* endocarditis have been reported so far; one involved a native aortic valve and the other a prosthetic aortic valve; both had domestic cats. Diagnosis was based on only one serological sample analysis, no details on associated conditions were mentioned and patients died rapidly (seven and ten days post-admission). This rapid evolution is not usual in *Bartonella* endocarditis [9,10,26,27]; there are several endemic conditions in our country such as Chagas disease and tuberculosis, among other infectious diseases, which may potentially present serological cross-reactions. The present case shows features of *Bartonella* endocarditis, which were similar to what was already published: predisposing valvular condition, absence of fever, exposure of the patient to a young cat at home in the months preceding his progressive heart failure, the need for valvular surgery and a good response to antibiotic regimen containing penicillin, gentamicin and doxycycline [9,10,26,27,32,33]. The diagnosis was made retrospectively by very high IgG titers (1:4096) to *B. henselae* in serum collected prior to antibiotic therapy, and lower levels (1:512) 14 months after completion of specific treatment. There are no antibody kinetic studies on *Bartonella* endocarditis to our knowledge, though a work has been published on cat-scratch disease and HIV-infected individuals, which showed titers declining rapidly over one year [34]. Cross-reactivity to *Coxiella burnetii* was ruled out by specific serology [35]. We can not rule out *B. quintana* cross-reactivity and we are aware of the significant predominance of this organism in countries such as Algeria and Tunisia [30,31]. Also the identification of *B. quintana* in cat fleas in France [36] and in cats [37] and the description of infective endocarditis caused by *B. quintana* in dogs [38] show that *B.quintana* is more widespread than previously thought. PCR was negative on the patient's serum as well as on paraffin-embedded valvular tissue; it has been reported that yield from such tissue is low [10]; besides, the process of decalcifying the valve might have further interfered with DNA extraction.

Bartonella sp. seems to be a prevalent microorganism in Brazil according to two recent seroprevalence studies; rheumatic heart disease is still frequently encountered, and rheumatic valvulopathy is the most important predisposing heart condition to endocarditis in our country. Therefore we assume more cases of *Bartonella* endocarditis are occurring but diagnosis is not being considered by clinicians, possibly because of lack of information regarding the ability of reference labs to perform serology and even molecular biology studies. We suggest investigation of all patients with blood culture-negative endocarditis for *Bartonella* in Brazil.

Acknowledgements

We would like to thank all the Rickettsial Laboratory Staff, especially lab technician Luzia Bezerra de Lima, for their dedication, and all the medical staff at Instituto Nacional de Cardiologia who helped look after the patient reported in this work.

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