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# Infectious endocarditis by *Bartonella* species. Report of two cases

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## ABSTRACT

*Infectious endocarditis (IE) by Bartonella species is an emerging problem worldwide. We report two cases of native valve Bartonella-associated IE events, both affecting adult male patients with a history of alcohol abuse and a low socioeconomic status. Admissions were due to pancytopenia and bleeding in one case and embolic stroke in the other. Blood cultures were negative and IgG indirect immunofluorescence assays (IFA) were positive for B. henselae/B. quintana in high titers (1/16,384-1/16,384, and 1/32,768 -1/16,384, respectively). Cases were classified as definitive IE events according to modified Duke criteria due to the presence of valve vegetations with at least three minor criteria. One patient required aortic mechanical valve replacement and survived, and the other died after a massive hemorrhagic transformation of his stroke. PCR amplification and sequencing of the 16S ribosomal bacterial DNA from a valve tissue sample obtained at surgery in the patient who survived, confirmed B. quintana as the etiological agent. Bartonella-associated IE is an emerging problem in Chile, present in disadvantaged populations. It should be suspected in patients with culture-negative IE. IFA does not discriminate between B. henselae and B. quintana infection, but high titers suggest IE. Complementary PCR techniques may help to elucidate the final causative agent.*
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*Key words: Bartonella; Endocarditis, Bacterial; Fluorescent Antibody Technique, Indirect; Sequence Analysis, DNA; Stroke.*

## Endocarditis infecciosa por *Bartonella*. Descripción de dos casos en Chile

*La endocarditis infecciosa(EI) asociada a Bartonella es un problema emergente a nivel mundial. Publicamos los 2 primeros casos de EI en válvula nativa asociados a Bartonella en Chile, los que afectaron a pacientes masculinos con historia de consumo de alcohol y bajos ingresos. La hospitalización fue provocada por pancitopenia y hemorragias en un caso y por un evento cerebrovascular en el otro. Se solicitó serología para Bartonella por inmunofluorescencia indirecta (IFI) para ampliar el estudio ante hemocultivos negativos y en ambos casos se reportaron resultados intensamente positivos para B. henselae y B. quintana1/16.384-1/16.384 y 1/32.768 -1/16.384, respectivamente). Los casos se clasificaron como eventos definitivos de EI según los criterios modificados de Duke debido a la presencia de vegetaciones valvulares con al menos 3 criterios menores. Un paciente requirió reemplazo valvular aórtico y sobrevivió, y el*

*otro falleció tras una transformación hemorrágica masiva del infarto cerebral. La amplificación del ADN ribosomal 16S por RCP y posterior secuenciación de una muestra de tejido valvular confirmó la presencia de B. quintana. La EI por Bartonella sp. es un problema emergente en Chile, probablemente asociada a poblaciones desfavorecidas, la que debe ser sospechada en pacientes con cultivos negativos. La IFI no permite discriminar infecciones por B. henselae o B. quintana pero los títulos altos sugieren EI. Técnicas complementarias por RCP pueden ayudar a dilucidar el diagnóstico.*

**Palabras clave:** *Bartonella; Endocarditis Bacteriana; Accidente Cerebrovascular.*

Blood-culture-negative infective endocarditis (IE) can be explained by sterilized cultures in previously treated patients, fastidious or slow growing agents (HACEK group, deficient *Streptococci*, *Candida* sp.), or true blood culture-negative microorganisms[1,2]. Several agents like *Coxiella burnetti*, *Bartonella sp., Brucella sp. Tropheryma whipplei*, *Chlamydiophila*, and *Mycobacterium chimaera* belong to the latter group[2]. *Bartonella* species are considered emergent pathogens in culture-negative IE with increasing reports in recent years[3-10].

Bacteria of the genus *Bartonella* includes facultative Gram-negative intracellular pathogens, able to form biofilms, and its diagnosis has been made possible by serology or molecular methods such as detection of *Bartonella* 16S rRNA gene sequences or other PCR gene targets in tissue or blood samples[3,8].

Serology methods involve indirect fluorescent antibody assay (IFA) or ELISA tests. Both have problems of cross-reactivity between *B. quintana* and *B. henselae*[3]. Of note, IFA titers ≥ 1/3,200 have a high positive predictive value for IE by these agents (100%)[4]. Western blotting with cross-adsorption circumvents problems of cross-reactivity and increases both sensitivity and specificity[4]. In large clinical series dedicated to IE secondary to *Bartonella* sp, *B. quintana* lightly surpasses *B. henselae* in frequency[4].

*B. quintana*, the agent of trench fever, has a human reservoir with worldwide distribution and nowadays is mostly associated to alcoholic and homeless populations in urban settings[3]. Infection between humans is transmitted by body lice and possible by fleas[11,12]. *B. henselae* is endemic in Chile, and transmission to humans has been linked to cats[3,13]. Infections have mainly involved children with cat scratch disease and other *Bartonella* infections have been rarely described in our country[14-16].

In recent years, we have observed two patients with IE by *Bartonella* species, a condition not reported in Chile before. Patients gave their informed consent for this report.

## Case 1

A 57-year-old male patient, with a past medical history of chronic renal failure (CRF) stage 3, tobacco smoking, and alcohol and drug abuse (cannabis and cocaine pasta base),was initially hospitalized during April 2017 by weight loss, gastrointestinal bleeding, epistaxis, gross hematuria, pancytopenia (hemoglobin 7.8 g/dL, WBC count 3,980/μL; platelets count 14,000 μL), and hypoalbuminemia. A mild midsystolic murmur was registered. He received a short course of ceftriaxone and clindamycin, did not require transfusions and was discharged after being stabilized. No blood cultures were taken, and murmur was attributed to anemia. He was readmitted one month later with effort dyspnea, cough with hemoptoic sputum but without fever.

Exams revealed the persistence of macrocytic anemia, thrombocytopenia, and appearance of microscopic hematuria with dysmorphic red blood cells (100%). CT imaging detected bilateral pleural effusion and fluid analysis showed a transudate. Erythrocyte sedimentation rate (ESR) was 66 mm/h. Microbiological analysis from a bronchoalveolar lavage discarded tuberculosis and fungal infections. A bone marrow examination discarded a lymphoproliferative disorder. Not a definitive diagnosis was reached and was discharged with vitamin B12-folic acid therapy and follow-up controls. After three weeks, he was

readmitted, with constipation, lower limb edema, hemoptysis, abdominal pain due to hepatomegaly and Janeway lesions. Pancytopenia persisted, and a direct Coombs test was positive. Bilateral pleural effusion and cardiomegaly was detected by chest-x-ray. A transthoracic echocardiogram (TTE) indicated global contractile dysfunction (ejection fraction 35%) and a degenerative aortic valve disease with two vegetations.

After blood cultures, empirical treatment with ceftriaxone and ampicillin was initiated as part of the global management of his heart failure. He evolved with hypotension, fever, tachypnea, and persistent heart failure being transferred to the ICU where he received vasoactive drugs and non-invasive mechanical ventilation. As blood cultures were negative, serological studies for *Bartonella sp* were requested and resulted positive at

**Table 1. Features observed in two cases of infectious endocarditis associated to *Bartonella* species**

| Parameter | Case 1 | Case 2 |
|---|---|---|
| Age in years, gender | 57, male | 67, male |
| Habits | Cannabis, cocaine pasta base and alcohol abuse | Alcohol abuse |
| Contact with cats | Positive | Positive |
| Socioeconomic status | Low, no income | Low income group, below percentile 25th |
| Symptoms initiation before admission | 4 months (weigth loss) | 3 months (effort dyspnea) |
| Main clinical picture | Pancytopenia with multiple site bleeding. Congestive heart failure thereafter | Cerebrovascular accident |
| Findings associated to infectious endocarditis during physical exam | Mild systolic murmur Janeway lesions | Holosystolic murmur of recent appearance. |
| ESR | 66 mm/h | 61 mm/h |
| Hematuria | Present, 100% dysmorfic cells | Present, 90% dysmorfic cells |
| C3-C4/ Rheumatoid factor (RF) | Not done | Hypocomplementemia and elevated RF |
| Blood cultures | Negative | Negative |
| Main echocardiogram findings | Aortic valve vegetations, global contractile dysfunction | Mitral valve tendineous cord rupture with vegetations |
| *Bartonella* serology by IFA | *B. henselae* IgG 1/16,384 *B. quintana* IgG 1/16,384 | *B. henselae* IgG 1/32,768 *B. quintana* IgG 1/16,384 |
| 16S Ribosomal DNA amplification and sequencing | Positive for *B. quintana* | Not done |
| Antibiotic treatment after Bartonella infection diagnosis | Ceftriaxone + doxycycline + azithromycin. Gentamycin was not used by CRF Stage 3 | Doxycycline + rifampin + gentamycin |
| Outcome | Aortic valve replacement. Discharged alive | Brain infarct with massive hemorrhagic transformation, Deceased |
| Case classification according to modified Duke criteria | Definitive case 1 major criterion (valve vegetations, abscess) + 3 minor criteria (vascular: Janeway lesions; immunological: glomerulonephritis; microbiological: Positive *B. quintana* detection in valve tissue) | Definitive case 1 major criterion (valve vegetations) + 3 minor criteria (vascular: embolic brain infarct; immunological: hycomplementemic glomerulonephritis; microbiological: serological evidence of *Bartonella sp*. infection) |

high titers for *B. henselae* and *B. quintana* (IFA IgG 1/16,384 - 1/16,384). History of contact with a domestic cat was obtained (Table 1). Doxycycline (100 mg oral route BID) was added to therapy. A follow-up echocardiography revealed a phlegmonous aortic valve with an abscess on the aortic valve, and azithromycin was added as salvage therapy. He was referred to another hospital where he underwent a mechanical aortic valve replacement. A tissue sample was obtained during surgery for PCR amplification of the 16s ribosomal DNA followed by sequencing. Results indicated the presence of *B. quintana* in the valve tissue sample. He evolved satisfactorily but remained with severe myocardial dysfunction (left ventricle ejection fraction 22%). This case was defined as a definitive endocarditis (Table 1). The patient had a low socioeconomic status according to health insurance information. HIV test was negative.

## Case 2

A 67-year-old male patient with heavy alcohol consumption was admitted on June 2019 by a cerebrovascular accident with left hemiparesis and effort dyspnea in the preceding three months. He was conscious, had fever (37.5°C) and no arrhythmia. There was not past history of smoking or obesity. Laboratory evaluation showed mild anemia (hemoglobin 13.1 g/dL) without leukocytosis and an elevated ESR (61 mm/h). Total cholesterol and triglycerides were normal. Brain tomography indicated a right frontoparietal hypodensity suggesting a right posterior frontal infarction. (Figure 1A). Due to a mild increase in hemiparesis, a new brain CT obtained at 72 h showed a hemorrhagic zone in the infarcted area (Figure 1B). A new holosystolic mitral murmur was detected, and IE was suspected.

No dental procedures, urinary, intestinal or skin disorders were detected. Blood cultures remained negative, and empirical treatment with cloxacillin, ampicillin and gentamicin was started. Additional exams showed microhematuria (5-10 per high power field) of dysmorphic predominance (90%), hypocomplementemia (C3 81.2 mg/dL [Normal Value 90-180 mg/dL]; C4 4.2 mg/dL [Normal value 10-40 mg/dL]), and an elevated rheumatoid factor (102 UI/mL; [Normal value 0-14 UI/mL]). After antibiotic treatment started, fever disappeared, ESR decreased to 38 mm/h, and the patient remained stable. A TTE indicated a mitral degenerative valve disease with no vegetations or abscesses, but a transesophageal echocardiogram (TEE) showed a mitral valve tendinous cord rupture with moderate insufficiency and vegetations. Serologic studies for *B. henselae* revealed a high positive IgG IFA positive result (1/32,768) that was cross-reactive with *B. quintana*
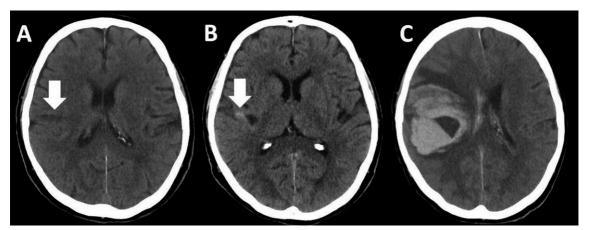


**Figure 1. A:** CT brain image without contrast media taken at admission. A front-parietal infarction is signaled by a white arrow. **B:** Image taken 3 days later showing a hyperdense zone inside the infarction zone, indicating a hemorrhagic transformation (white arrow). 1C. A late intracerebral hemorrhage occurring after near 1 month. Hemorrhage is associated with midline displacement.

at one lower dilution (1/16,384). Treatment was changed to doxycycline 100 mg BID, rifampin 300 mg BID both by the oral route, maintaining iv gentamicin. An attempt to amplify gene targets of *B. henselae* from a blood sample a week after gentamicin started was unsuccessful. The patient admitted contact with cats from his neighbor that he fed every night but without touching them. He remained in stable conditions, but unfortunately, he suddenly presented a massive intracerebral hemorrhage on the 29th day of admission, out of surgical scope (Figure 1C), and died. He was not receiving anticoagulants nor antiaggregant drugs. The event was classified as a definitive IE case (Table 1). HIV test was not studied, and serological tests for *C. burnetii* serology were negative. His monthly income was low (Table 1).

## Discussion

Cases presented here illustrate the emergence of IE by *Bartonella* species in Chile, enlarging the list of possible etiological agents involved in this condition in our country[17-19]. To the best of our knowledge, *Bartonella*-associated IE has not been described in Chile before. Patients shared similar features: both were males, had a history of alcohol consumption, and a low socioeconomic status, a profile that has been described in large series[3,4]. For *B. quintana*, a pathogen of human reservoir, these conditions may be a proxy of debilitated patients living in urban trench conditions[3]. IFA does not discriminate between *B. henselae* and *B. quintana* infection, but high titers, as in our patients, are only associated with IE, and rarely to disseminated disease[4,20].

Our small series also illustrates the relevance of including studies beyond classical blood cultures in pursuing the etiological agent involved in IE cases, especially when they are negative. Serological studies, together with PCR amplification and sequencing of different gene targets of blood or valve samples, may enhance the diagnostic yield[4,8,9]. This strategy may recognize not only *Bartonella* infections but also those provoked by *Brucella* and *Coxiella burnetii*[2]. All of these techniques are currently available in Chile. Specific living conditions, habits, and zoonotic exposures may improve the clinical suspicion.

Clinical manifestations and prognosis of IE by

*Bartonella* species are somewhat similar to other causes. Diagnostic limitations already commented, together with special therapeutic requirement, are the most relevant differences. Only observational studies are available that have compared different compounds and outcomes for *Bartonella* IE, so a definitive therapeutic regimen is not yet defined[21]. Gentamycin has been associated with higher recovery rates when compared to other antibiotics and less mortality if at least 14 days are completed[21]. Doxycycline has a lower performance if not accompanied by aminoglycosides. One of our patients died despite receiving this compound but after neurological complications.

In conclusion, *Bartonella* species provoke IE in Chile, and serological and PCR techniques are available for diagnosis. These agents must be searched when blood cultures remain negative or when specific behaviors or zoonotic exposures are detected. Prognosis is similar to other causes of IE, but therapy appears to rely on aminoglycosides compounds.

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