

***Bartonella* spp. and *Coxiella burnetii* Associated with Community-Acquired, Culture-Negative Endocarditis, Brazil**

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We evaluated culture-negative, community-acquired endocarditis by using indirect immunofluorescent assays and molecular analyses for *Bartonella* spp. and *Coxiella burnetii* and found a prevalence of 19.6% and 7.8%, respectively. Our findings reinforce the need to study these organisms in patients with culture-negative, community-acquired endocarditis, especially *B. henselae* in cat owners.

Worldwide, *Bartonella* spp. and *Coxiella burnetii* endocarditis have varied prevalences and clinical effects (1,2). Detection is difficult in routine blood cultures, so different diagnosis methods are needed. Our study investigated the frequency of and the risk factors for *Bartonella* spp. and *C. burnetii* infection in cases of culture-negative, community-acquired endocarditis.

The Study

During January 2004–January 2009, the Infection Control Team from the university hospital at São Paulo, Brazil (Instituto do Coração–Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo) used active surveillance to identify 369 patients with endocarditis. The study focused on community-acquired endocarditis caused by fastidious bacteria. Patients >18 years of age with confirmed endocarditis were included as a prospective inception cohort of patients (3). Excluded were

patients with health care–associated endocarditis (i.e., patients with prosthetic valve endocarditis in the first postoperative year, hemodialysis patients, and nosocomial endocarditis patients) (4).

Indirect immunofluorescence assays (IFAs) were performed for all patients with negative blood cultures ≤ 7 days after admission at a referral center for rickettsial infections (Adolfo Lutz Institute, São Paulo). The same observer analyzed all assays; IgG titers $\geq 1:800$ for *B. henselae* and *B. quintana* (5) and anti-phase I IgG titers $\geq 1:800$ for *C. burnetii* (6) were considered positive (online Technical Appendix, <http://wwwnc.cdc.gov/EID/article/21/8/14-0343-Techapp1.pdf>). New diagnostic criteria for Q fever endocarditis were used (6).

Immunohistochemical and molecular methods were applied to valve tissue specimens and serum samples of patients whose serum samples were positive for *Bartonella* spp. or *C. burnetii*. DNA from paraffin-embedded valve tissue specimens and serum samples were extracted. Samples positive for *Bartonella* by IFA were analyzed by using 5 different PCRs to 4 distinct regions. Tissue and serum DNA from patients positive for *C. burnetii* by IFA were tested by quantitative PCR (online Technical Appendix Table 1) (7).

Of the 369 identified endocarditis patients, 221 (59.9%) were included in the study; median age of included patients was 53 years. Of included patients, 144 (65.2%) were male; 107 (48.4%) had prosthetic valves; 209 (94.6%) had left-sided endocarditis; and 152 (68.8%) had concurrent conditions. Of patients with concurrent conditions, 62 (40.8%) had hypertension, 17 (11.2%) had diabetes, 53 (34.9%) had heart failure, and 36 (23.7%) had other conditions. Of the 221 patients included in the study, microorganisms were identified in 170 (76.9%); specimens from 51 (23.1%) patients were culture negative.

A standardized questionnaire regarding exposure to cats, ectoparasites, or farm animals was administered to patients with culture-negative endocarditis. For the 170 samples in which microorganisms were found, the most commonly identified bacteria were *viridans*-type *Streptococci* (81 [47.6%]), *Streptococcus bovis* (17 [10.0%]), *S. pneumoniae* (6 [3.5%]), *S. agalactiae* (2 [1.2%]), *S. pyogenes* (2 [1.2%]), *Enterococcus fecalis* (13 [7.6%]), *E. faecium* (3 [1.8%]), other enterococci (4 [2.4%]) and *Staphylococcus aureus* (14 [8.2%]).

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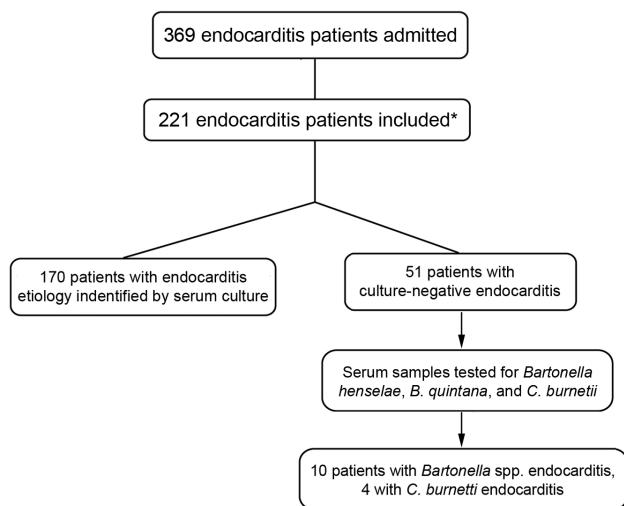


Figure. Distribution of patients etiologically diagnosed with endocarditis and admitted to the heart institute (Instituto do Coração) at the University of São Paulo Medical School, Sao Paulo, Brazil, January 2004–January 2009. *A modified Duke criteria (3) was used to determine inclusion of 221 patients. Excluded were 148 patients: 58 with unconfirmed endocarditis, 28 with endocarditis caused by cardiac implantable electronic devices, 47 with nosocomial endocarditis, and 15 hemodialysis patients.

For the 221 patients in the study, findings from 10 (4.5%; 95% CI 3.96%–5.09%) patients (Figure) showed *Bartonella* spp., and 4 (1.8%; 95% CI 1.58%–2.04%) showed *C. burnetii* endocarditis. For the 51 culture-negative endocarditis patients, *Bartonella* spp. was found in cultures from 10 (19.6%; 95% CI 9.8%–33.1%), and *C. burnetii* was found in 4 (7.8%; 95% CI 2.2%–18.9%). The Table shows the immunohistochemical and molecular biology analyses for patients with positive IFA results. *Bartonella* spp. DNA was detected with ≥ 1 PCR in all 6 patients whose paraffin-embedded valve tissue samples were found positive for *Bartonella* spp. For the other 4 patients with *Bartonella* spp., DNA was detected in 2 serum samples. Amplicons were sequenced, and their analyses showed that the cultures from 2 patients had 100% similarity with *B. quintana* (GenBank accession no. BX897700.1); cultures from 4 patients had 100% similarity with *B. henselae* infection (GenBank accession no. BX897699.1). Cultures from 2 patients were positive for *Bartonella* spp. by using IFA but negative by using PCR.

All patients used antimicrobial drugs for 7 days before sample collection. All endocarditis patients whose cultures were found to be positive for *C. burnetii* by using IFA were also positive by using quantitative PCR: 3 by serum samples and 2 by paraffin-embedded valve tissue specimens (online Technical Appendix Table 2).

Clinical and follow-up findings from *Bartonella* spp. and *C. burnetii* endocarditis patients are shown in online

Technical Appendix Table 3. *Bartonella* spp. infection was associated with low levels of C-reactive protein on admission and chronic symptoms related to endocarditis (online Technical Appendix Table 4). Three (75%) of 4 patients with *Bartonella henselae* endocarditis were associated with a cat living in the patient's home, compared with 6 (12.8%) of 47 patients with culture-negative *Bartonella henselae* negative endocarditis ($p = 0.015$ by Student *t*-test). Hydroxychloroquine was unavailable in our facility; therefore, we used a second-line therapy for *C. burnetii* endocarditis. Hydroxychloroquine was replaced with ciprofloxacin, and treatment was extended for 72 months (8). Subsequently, symptoms resolved, and antibody titers reduced substantially, considered a favorable response (9) (online Technical Appendix Table 3).

Conclusions

In this study, the systematic use of IFA detected a 4.5% (10/221) prevalence of community-acquired endocarditis due to *Bartonella* spp. and a 1.8% (4/221) prevalence due to *C. burnetii*. For the 51 culture-negative endocarditis patients, IFA enabled recognition of the endocarditis etiology in 14 (27.5%) patients (*Bartonella* spp. in 10 [19.6%] and *C. burnetii* in 4 [7.8%]). Some of these patients have been recognized as having the first cases of endocarditis caused by these microorganisms in Brazil (10,11).

Prevalences of *Bartonella* spp. endocarditis vary worldwide by region studied (1). In a broad series of 759 culture-negative endocarditis patients in France, serum samples showed high sensitivity for detection of *C. burnetii* and *Bartonella* spp. infections, compared with other diagnostic tools, such as PCR, cell culture, and immunohistochemical analysis (2). In Brazil, studies of *Bartonella* spp. infection among culture-negative endocarditis patients have shown varied results. A retrospective case series of 51 surgically treated, culture-negative endocarditis patients found 2 cases of *Bartonella* spp. and 1 case of *C. burnetii* by using PCR on valvular tissue (12). Another series of 46 culture-negative endocarditis patients from the city of São Paulo used PCR to investigate *Bartonella* spp. in blood and found 13 (28%) patients with positive results (13).

We found an association between *B. henselae* endocarditis and the presence of a cat living at a patient's home, a risk factor indicating that clinicians should consider this infection when assessing endocarditis patients. The relatively small sample of patients with endocarditis caused by *Bartonella* spp. and *C. burnetii* limited the statistical analyses of factors associated with these infections. Serologic investigations of infections by these agents were applied only to patients with negative cultures. Although rare (2,14), co-infection by these microorganisms in culture-positive endocarditis is possible, so frequency of *Bartonella* spp. and *C. burnetii* infections in

Table. Serologic, immunohistopathologic, and molecular test results for patients with infective endocarditis caused by *Bartonella* spp. or *Coxiella burnetii*, Brazil*

Patient no., by infection type	Serum IgG ≥800 by IFA†	Immunohistochemical analysis of cardiac valve vegetation		Microorganism by histologic analysis	PCR‡	Species of <i>Bartonella</i>
		<i>Bartonella</i> spp.	<i>C. burnetii</i>			
<i>Bartonella</i> spp.						
1	+	+	Neg	Gram-negative coccobacilli	+	<i>B. quintana</i>
2	+	+	Neg	Gram-negative coccobacilli	+	<i>B. henselae</i>
3	+	+	Neg	None	+	<i>B. henselae</i>
4	+	NA	NA	NA	Neg	NA
5	+	+	Neg	Gram-negative coccobacilli	+	NA
6	+	+	Neg	Gram-negative coccobacilli	+	<i>B. quintana</i>
7	+	NA	NA	NA	+	<i>B. henselae</i>
8	+	NA	NA	NA	+	NA
9	+	NA	NA	NA	Neg	NA
10	+	+	Neg	Gram-negative cocci	+	<i>B. henselae</i>
<i>C. burnetii</i>						
	Serum anti-phase I IgG ≥800 by IFA§	Immunohistochemical analysis of cardiac valve vegetation		Microorganism by histologic analysis	PCR‡	Q fever endocarditis (6)
		<i>Bartonella</i> spp.	<i>C. burnetii</i>			
11	+	Neg	+	Small gram-negative coccobacilli	+	Definite
12	+	Neg	+	None	+	2A criteria
13	+	Neg	+	None	+	Definite
14	+	NA	NA	NA	+	2A criteria
						Definite
						2B criteria¶

*IFA, immunofluorescence assay; +, positive; Neg, negative; NA, material not available for analysis.

¶Serologic result >6,400 and vegetation on ecocardiography.

†*B. henselae* or *B. quintana*.

‡Serum or tissue sample.

§*C. burnetii*.

these patients may be higher than shown. Our study indicates that systematic serologic research for *Bartonella* spp. and *C. burnetii* in community-acquired, culture-negative endocarditis may be clinically useful, particularly in screening for *B. henselae* in cat owners.

Dr. Siciliano is an infectious disease specialist working in infection control at the Heart Institute at the university hospital at São Paulo, Brazil, a tertiary care hospital dedicated to care of heart disease patients. His clinical research interest is endocarditis.

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Technical Appendix

Indirect Immunofluorescence Assays

As recommended (1), we used the following antigens: *Bartonella henselae* RA2552, Lot 08–0039; *Bartonella quintana*, RA2551, Lot 08–0038 obtained from the Centers for Disease Control and Prevention (Atlanta, GA, USA). We also used *C. burnetii* antiphase I (SCIMEDX Corporation, Denville, NJ, USA).

Immunohistochemical Analyses

The immunohistochemical analysis for *Bartonella henselae* was performed by Biocare Medical (Concord, CA, USA; clone H2A10). That for *Bartonella quintana* and *Coxiella burnetii* was performed by the Rickettsial Zoonoses Branch, Centers for Disease Control and Prevention.

Molecular Methods

Molecular tests were used to confirm the serologic results and identify *Bartonella* species. From 10 *Bartonella* spp. positive patients, the serum was subjected to DNA extraction by using QIAamp DNA Mini Kit (QIAGEN, Valencia, CA, USA). From 4 patients whose cultures were positive for *coxiella*, nucleic acid was extracted by serum samples by using PureLink Viral RNA/DNA Mini Kit-Life Technologies (Invitrogen, Grand Island, NY, USA). We also performed DNA extraction from formalin-fixed, paraffin-embedded valve tissue specimens from 6 patients with specimens positive for *Bartonella* spp. and 3 positive for *C. burnetii* by using PureLink Genomic DNA Mini Kit - Life Technologies (Invitrogen).

Screening of samples was performed by using 3 *Bartonella* genus-specific single tube PCR and 1 nested PCR specific for *B. henselae*; amplicons generated were sequenced. Formalin-fixed, paraffin-embedded valve tissue specimens were also submitted to real-time PCR to detect *Bartonella* spp. (*gltA*). Serum samples and formalin-fixed, paraffin-embedded valve tissue specimens of patients whose results were positive for *C. burnetii* by immunofluorescence assay

were submitted to real-time PCR to detect *C. burnetii* (IS1111). The molecular methods used follow the protocol described in the Technical Appendix Table 1.

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Technical Appendix Table 1. PCR types and references used in *Bartonella* spp. and *C. burnetii* analysis, Brazil*

Application	Target gene	Reference
<i>Bartonella</i> spp. conventional PCR (ITS 1)	16S–23S rRNA gene ITS	(2)
<i>Bartonella</i> spp. conventional PCR (ITS 2)	16S–23S rRNA gene ITS	(3)
<i>Bartonella</i> spp. conventional PCR (srA)	Transfer-mRNA (ssrA)	(4)
<i>Bartonella henselae</i> nested PCR (FtsZ)	Cell division protein <i>FtsZ</i>	(5)
<i>Bartonella</i> spp. real-time PCR (gltA)	Citrate synthase gene	(6)
<i>C. burnetii</i> real-time PCR (IS 1111)	Insertion element	(7)

*ITS, intergenic transcribed spacer.

Technical Appendix Table 2. Serologic titles and PCR results for patients with infective endocarditis caused by *Bartonella* spp. or *C. burnetii**

Bartonella Cases	Serology (IFA) IgG			PCR (tissue or serum)					
	<i>B.</i>		<i>C. burnetii</i>	<i>Bartonella</i> spp.				<i>C. burnetii</i>	
	<i>henselae</i>	<i>B. quintana</i>	Antiphase I <i>C. burnetii</i>	ITS 1	ITS 2	ssra	FtsZ	gltA	IS1111
1	≥1,600	≥1,600	<800	+	+	+	Neg	Neg	NP
2	≥1,600	≥1,600	<800	Neg	+	+	+	+	NP
3	≥1,600	≥1,600	<800	Neg	+	+	+	+	NP
4	≥1,600	≥1,600	<800	Neg	Neg	Neg	Neg	NP	NP
5	≥1,600	≥1,600	<800	Neg	+	Neg	Neg	+	NP
6	≥1,600	≥1,600	<800	Neg	+	+	Neg	Neg	NP
7	≥1,600	≥1,600	<800	Neg	+	Neg	+	NP	NP
8	800	<800	<800	Neg	+	Neg	Neg	NP	NP
9	≥1,600	≥1,600	<800	Neg	Neg	Neg	Neg	NP	NP
10	800	≥1,600	<800	Neg	+	Neg	Neg	Neg	NP
11	<800	<800	25,600	NP	NP	NP	NP	NP	+
12	<800	<800	6,400	NP	NP	NP	NP	NP	+
13	<800	<800	25,600	NP	NP	NP	NP	NP	+
14	<800	<800	51,200	NP	NP	NP	NP	NP	+

*IFA, immunofluorescence assay; ITS, intergenic transcribed spacer; +, positive; Neg, negative; NP, not performed.

Technical Appendix Table 3. Clinical and evolving characteristics of 14 patients with endocarditis caused by *Bartonella* spp. and *C. burnetii*, Brazil*

Patients by infection type	Age, y/sex	Epidemiology	Valve/position	Antimicrobial drug treatment (d) †	Endocarditis-related complications	Surgical treatment	Cause of death	<i>C. burnetii</i> serology	
								At diagnosis	At end of treatment
<i>Bartonella</i> spp.									
1	35/M	Flea	Prosthesis/aortic	Oxacillin + ceftriaxone (30) Gentamicin (17)	–	Yes	NA		Neg
2	65/M	Domestic cat	Native/aortic	Oxacillin + ceftriaxone (19) Gentamicin (11)	Paravalvular abscess	Yes	Heart failure		Neg
3	52/M	Domestic cat; cat scratch	Prosthesis/aortic	Oxacillin + Penicillin (5)	–	No	Malignant tachyarrhythmia		Neg
4	31/F	Domestic cat; cat scratch	Prosthesis/mitral	Oxacillin + ceftriaxone (42)	–	No	NA		Neg
5	60/M	Domestic cat	Native/aortic	Ceftriaxone (45) Gentamicin (30)	Paravalvular abscess + fistula	Yes	NA		Neg
6	58/M	Homelessness	Native/aortic	Oxacillin + ceftriaxone (42) Gentamicin (30)	–	Yes	NA		Neg
7	70/M	Domestic cat	Native/aortic	Penicillin (24)+ Gentamicin (24)	CNS emboli + Paravalvular abscess	No	Heart failure and septic shock		Neg
8	41/M	Lice; scabies	Prosthesis/aortic	Oxacillin + penicillin (62) Gentamicin (28)	Paravalvular abscess	Yes	NA		Neg
9	21/M	Domestic cat	Native/mitral	Penicillin (28) Gentamicin (14)	–	No	NA		Neg
10	51/M	Homelessness	Native/aortic	Ceftriaxone (30) Gentamicin (3)	–	Yes	Heart failure		Neg
<i>C. burnetii</i>									
11	41/M	Rural residence; consumption of raw milk	Native/mitral	Ciprofloxacin + doxycycline*	Heart failure	Yes	Septic shock caused by nosocomial pneumonia (day 5 after cardiac surgery and day 26 after start of <i>C. burnetii</i> treatment)	25,600	NP
12	45/M	Rural residence; consumption of raw milk	Prosthesis/aortic	Ciprofloxacin + doxycycline*	Heart failure	Yes	NA	6,400	<800
13	34/F	Rural residence; consumption of raw milk	Prosthesis/mitral	Ciprofloxacin + doxycycline*	–	Yes	NA	25,600	3,200
14	64/M	Rural residence; consumption of raw milk	Prosthesis/aortic	Ciprofloxacin + doxycycline*	–	No	NA	51,200	12,800

*–, no identified complications; NA, not applicable (patient did not die); Neg, negative; NP, not performed (patient died before end of treatment).
 †Hydroxychloroquine was unavailable; second-line treatment for *C. burnetii* endocarditis (ciprofloxacin and doxycycline for 72 mos.) was used.

Technical Appendix Table 4. Distribution of clinical, laboratory, and echocardiographic features of 221 patients with community-acquired endocarditis, according to whether *Bartonella* spp. infection was involved*

Characteristic	<i>Bartonella</i> spp. endocarditis no. (%)	Non- <i>Bartonella</i> spp. endocarditis no. (%)	<i>p</i> value
Male sex	9 (10)	135 (64.0)	0.092
Age ≥60 y	3 (30)	87 (41.2)	0.480
Body mass index ≥25 kg/m ²	1 (10)	71 (37.2)	0.081
Concurrent conditions	8 (80)	144 (68.2)	0.433
Valvular heart disease	7 (70)	177 (83.9)	0.250
Previous endocarditis	1 (10)	25 (11.8)	0.859
Duration of symptoms ≥30 d	8 (80)	95 (45.2)	0.048
Fever	9 (90)	188 (89.5)	0.992
≥1 affected valve	0 (0)	17 (8.1)	NA
C-reactive protein ≥80 mg/L	2 (25)	90 (62.5)	0.032
Severe sepsis	4 (40)	76 (36.0)	0.798
Moderate or severe valvular regurgitation	8 (80)	153 (73.2)	0.634
Vegetation on echocardiography	9 (90)	162 (77.5)	0.351
Glomerulonephritis	1 (10)	43 (24.9)	0.285

**p* value determined by Pearson χ^2 or Fisher exact test; NA, not applicable.