Article DOI: http://dx.doi.org/10.3201/eid2108140343

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

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. (*glt*A). 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* (IS*1111*). 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
Bartonella spp. conventional PCR (ITS 1)	16S–23S rRNA gene ITS	(*2*)
Bartonella spp. conventional PCR (ITS 2)	16S–23S rRNA gene ITS	(*3*)
Bartonella spp. conventional PCR (srA)	Transfer–mRNA (ssrA)	(*4*)
Bartonella henselae nested PCR (FtsZ)	Cell division protein *FtsZ*	(*5*)
Bartonella spp. real-time PCR (gltA)	Citrate synthase gene	(*6*)
C. burnetii 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**

	Serology (IFA) IgG			PCR (tissue or serum)					
				Bartonella spp.					*C. burnetii*
Bartonella Cases	*B. henselae*	*B. quintana*	Antiphase I *C. burnetii*	ITS 1	ITS 2	ssra	FtsZ	gltA	IS*1111*
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.

Publisher: CDC; Journal: Emerging Infectious Diseases
Article Type: Dispatch; Volume: 21; Issue: 8; Year: 2015; Article ID: 14-0343
DOI: 10.3201/eid2108.140343; TOC Head: Dispatch

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

Patients by infection type	Age, y/sex	Epidemiology	Valve/position	Antimicrobial drug treatment (d) †	Endocarditis-related complications	Surgical treatment	Cause of death	*C. burnetii* serology	
								At diagnosis	At end of treatment
Bartonella 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	Oxacilin + 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	
C. burnetii									
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 *C. burnetii* 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. burnettii* endocarditis (ciprofloxacin and doxycycline for 72 mos.) was used.

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Article Type: Dispatch; Volume: 21; Issue: 8; Year: 2015; Article ID: 14-0343
DOI: 10.3201/eid2108.140343; TOC Head: Dispatch

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	*Bartonella* spp. endocarditis no. (%)	Non–*Bartonella* spp. endocarditis no. (%)	*p* 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
≥1affected 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.