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Laboratory Diagnosis of 37 Cases of *Bartonella* Endocarditis Based on Enzyme Immunoassay and Real-Time PCR

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ABSTRACT *Bartonella* spp., mostly *Bartonella quintana* and *B. henselae*, are a common cause of culture-negative endocarditis. Serology using immunofluorescence assay (IFA) and PCR performed on cardiac tissues are the mainstays of diagnosis. We developed an enzyme immunoassay (EIA) and a novel multiplex real-time PCR assay, utilizing *Bartonella* genus-specific, *B. henselae*-specific, and *B. quintana*-specific SimpleProbe probes, for diagnosis of *Bartonella* endocarditis. We aimed to evaluate the performance of these assays. Thirty-seven patients with definite endocarditis, 18 with *B. henselae*, 18 with *B. quintana*, and 1 with *B. koehlerae*, were studied. Diagnosis was confirmed by conventional PCR and DNA sequencing of surgical cardiac specimens. Similar to the case with IFA, anti-*Bartonella* IgG titers of ≥1:800 were found in 94% of patients by EIA; cross-reactivity between *B. henselae* and *B. quintana* precluded species-specific serodiagnosis, and frequent (41%) but low-titer cross-reactivity between *Coxiella burnetii* antibodies and *B. henselae* antigen was found in patients with Q fever endocarditis. Low-titer (1:100) cross-reactivity was uncommonly found also in patients with brucellosis and culture-positive endocarditis, particularly *Enterococcus faecalis* endocarditis. Real-time PCR performed on explanted heart valves/vegetations was in complete agreement with results of sequence-based diagnosis with characteristic melting curves. The genus-specific probe identified five additional endocarditis-associated *Bartonella* spp. at the genus level. In conclusion, EIA coupled with a novel real-time PCR assay can play an important role in *Bartonella* endocarditis diagnosis and expand the diagnostic arsenal at the disposal of the clinical microbiologist. Since serology remains a major diagnostic tool, recognizing its pitfalls is essential to avoid incorrect diagnosis.

KEYWORDS *Bartonella* sp., *Bartonella henselae*, *Bartonella quintana*, *B. koehlerae*, immunofluorescence assay, IFA, enzyme immunoassay, EIA, ELISA, real-time PCR, endocarditis, development, diagnosis, human, *Bartonella* spp., diagnostics

Following Q fever, *Bartonella* spp. are the second most common cause of culture-negative endocarditis, accounting for up to 28% of cases (1–3). Most reported cases have been attributed to *Bartonella quintana*, followed by *B. henselae*, while other *Bartonella* spp. have been implicated in single cases of endocarditis, including *B. elizabethae* (4), *Bartonella vinsonii* subsp. *berkhoffii* (1, 5, 6), *Bartonella vinsonii* subsp. *arupensis* (7), *B. koehlerae* (8), *B. alsatica* (1, 9, 10), and "*Candidatus* Bartonella mayotimonensis" (11). *Bartonella*

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spp. are fastidious bacteria with notoriously poor sensitivity of axenic cultures performed on clinical specimens of human blood or tissue. Laboratory diagnosis of *Bartonella* endocarditis is therefore based mainly on serological assays and PCR performed on infected tissues. Microscopic immunofluorescence assay (IFA) is the most commonly used serological method utilized for diagnosis of *Bartonella* infections, although other methods, such as enzyme immunoassay (EIA), are used as well (12–16). Compared with EIA, IFA is laborious and time-consuming, and interpretation is subjective. A limitation of all serological assays for the diagnosis of *Bartonella* infection is cross-reactivity between *B. henselae* and *B. quintana*. *Bartonella* IFA has also been reported to have cross-reactivity with *Coxiella burnetii* and *Chlamydia pneumoniae* (17–19). The Bernard Pridan Laboratory for Molecular Biology of Infectious Disease at Tel Aviv Sourasky Medical Center serves as the national reference laboratory for human *Bartonella* infections in Israel and has developed over the past 30 years serological and molecular tools for the diagnosis of *Bartonella* infection, including an EIA and various conventional and real-time PCR assays. Although we have occasionally used these assays for non-*henselae Bartonella*-associated infections, they have been developed and utilized primarily for the diagnosis of cat scratch disease, which is caused predominantly by *B. henselae* (12, 14, 15, 20–24). A systematic evaluation of the performance of our assays for the diagnosis of *Bartonella* endocarditis has not been conducted thus far. The aim of the current study was to evaluate an EIA and molecular assays, including a novel multiplex real-time PCR assay, for the diagnosis of *Bartonella* endocarditis.

MATERIALS AND METHODS

Patients and clinical samples. As part of a surveillance study of *Bartonella* infections ongoing in Israel since 1991, all clinical specimens from patients with suspected *Bartonella* endocarditis, including serum samples and tissue for PCR assays and cultures, are referred to a single laboratory at Tel Aviv Sourasky Medical Center, thus allowing identification of essentially all laboratory-confirmed cases of *Bartonella* endocarditis in Israel. The referring hospital-based physicians are requested to fill out a questionnaire providing data on patients' demographic and clinical features and are contacted to obtain additional information. For the purpose of the current study, we included patients with culture-negative endocarditis who met the following two criteria: (i) they have undergone valve surgery for endocarditis-associated valve dysfunction complicated by heart failure and/or the presence of intracardiac abscess, and (ii) *Bartonella* was identified in the valvular/paravalvular tissue or vegetation by PCR and DNA sequencing. The study included 37 patients with *Bartonella* endocarditis diagnosed in hospitals throughout Israel in 2001 to 2020: 18 patients with *B. henselae*, 18 with *B. quintana*, and 1 with *B. koehlerae*. All patients had definite endocarditis based on at least two major criteria of the Duke score: (i) echocardiogram positive for endocarditis as defined in the modified Duke criteria (25) and (ii) *Bartonella*-positive PCR from infected cardiac tissue corroborated by DNA sequencing, that is, if one accepts *Bartonella*-positive PCR as a finding with equal significance to a major criterion of the modified Duke criteria (25, 26). Patients with *Bartonella* endocarditis without PCR-based diagnosis for species identification were excluded from the study. The study was approved by the institutional review board (Helsinki Committee) of the Tel Aviv Sourasky Medical Center.

Bacterial strains and cultures. Bacterial strains used in this study are described in Table 1, including 7 *Bartonella* spp. that have been implicated in human endocarditis and non-*Bartonella* bacterial species that have been associated with culture-negative endocarditis (27). All bacterial strains were obtained from known reference collections (American Type Culture Collection [ATCC], USA, National Collection of Type Cultures [NCTC], United Kingdom, Collection Nationale de Cultures de Microorganismes [CNCM], Institute Pasteur, France, and Collection of the Institute Pasteur, France [CIP]). Exceptions included Nine Mile phase I *Coxiella burnetii* DNA (Vircell, Spain), *B. henselae* isolate BhTA-4, cultured in our laboratory from lymph node biopsy specimen of a patient with typical cat scratch disease, and *B. koehlerae* isolate C-508, cultured in our laboratory from a bacteremic cat and found to have *ribC* and *gltA* DNA sequences identical to those of *B. koehlerae* amplified from a valve of an endocarditis patient reported by our group (8). *Bartonella* spp. and cardiac tissue specimens, whenever sufficient material was available, were cultured on chocolate agar plates as previously described (12). *Bartonella* spp. were identified by PCR targeting various genes with amplicon sequencing (Table 2).

Serology. EIA for the detection of anti-*Bartonella* antibodies was performed essentially as reported previously, with some modifications (12, 14, 23). The EIA antigen was prepared as sarcosyl-insoluble, presumably outer membrane protein extracts of agar-derived *B. henselae* strain 87-66 (ATCC 49793), *B. quintana* Fuller strain (CIP 107027), or *B. koehlerae* strain C-508 (8), as needed. Sera were initially screened at a 1:100 dilution, and positive sera at this dilution, as determined previously (12), were tested at incremental dilutions to determine their endpoints. To assess assay specificity, serum specimens from the following control groups were subjected to *Bartonella* EIA. (i) The first group consisted of patients with blood culture-positive endocarditis ($n = 62$), including 31 cases of *Enterococcus faecalis* endocarditis and 31 cases with other pathogens, including *Streptococcus viridans*, *Streptococcus sanguinis*, *Streptococcus mutans*, *Streptococcus mitis*, *Streptococcus infantarius*, *Streptococcus gallolyticus*, *Streptococcus pneumoniae*, *Aggregatibacter aphrophilus*,

TABLE 1 Results of the real-time PCR assay performed on *Bartonella* spp. and other bacterial species associated with culture-negative endocarditis

Bacterial species/subsp.	Strain designation(s)[b]	Probe T_m[a] (°C) or status		
		Bartonella genus	*Bartonella henselae*	*Bartonella quintana*
B. henselae	BhTA-4, ATCC 49793 (87-66)	58–61	47–51	Negative
B. quintana	CIP 103739 (Toulouse), CIP 107027 (Fuller)	57–61	Negative	68–71
B. koehlerae	ATCC 700693	57–59	Negative	Negative
B. vinsonii subsp. *berkhoffii*	ATCC 51672	57	Negative	Negative
B. vinsonii subsp. *arupensis*	ATCC BAA-2250	58	Negative	Negative
B. elizabethae	ATCC 49927	43	Negative	Negative
B. washoensis	ATCC BAA-2254	50	Negative	Negative
Coxiella burnetii	ATCC VR-615 (Nine Mile phase I)	Negative	Negative	Negative
Tropheryma whipplei	CNCM I-2202T (Twist-Marseille)	Negative	Negative	Negative
Legionella pneumophila subsp. *pneumophila*	ATCC 33152	Negative	Negative	Negative
Aggregatibacter aphrophilus	ATCC 33389	Negative	Negative	Negative
Aggregatibacter paraphrophilus	NCTC 10557	Negative	Negative	Negative
Aggregatibacter actinomycetemcomitans	ATCC 700685	Negative	Negative	Negative
Aggregatibacter segnis	NCTC 10977	Negative	Negative	Negative
Cardiobacterium hominis	NCTC 10426	Negative	Negative	Negative
Eikenella corrodens	ATCC 23834	Negative	Negative	Negative
Kingella kingae	NCTC 10529	Negative	Negative	Negative
Abiotrophia defectiva	ATCC 49176	Negative	Negative	Negative
Granulicatella adiacens	ATCC 49175	Negative	Negative	Negative
Granulicatella elegans	ATCC 700633	Negative	Negative	Negative
Gemella bergeriae	ATCC 700627	Negative	Negative	Negative
Gemella haemolysans	ATCC 10379	Negative	Negative	Negative
Gemella morbillorum	NCTC 11323	Negative	Negative	Negative
Gemella sanguinis	ATCC 700632	Negative	Negative	Negative

[a]T_m, melting temperature.
[b]ATCC, American Type Culture Collection, USA; CIP, Collection of the Institute Pasteur, France; CNCM, Collection Nationale de Cultures de Microorganismes, Institut Pasteur, France; NCTC, National Collection of Type Cultures, Central Public Health Laboratory, United Kingdom.

Aggregatibacter paraphrophilus, *Aggregatibacter actinomycetemcomitans*, *Abiotrophia defectiva*, *Gemella*, *Staphylococcus aureus*, coagulase-negative staphylococci, and *Aspergillus*. The reason for enriching this control group with *E. faecalis* is a recent publication suggesting a cross-seroreactivity, based on immunoblotting, between *E. faecalis* and *Bartonella* (28). (ii) The second group consisted of patients suspected to have chronic Q fever ($n = 34$), with anti-phase I IgG titers of 1:1,600 to 1:51,200, determined using IFA performed at the national reference laboratory for Q fever, the Israel Institute for Biological Research, Ness-Ziona, Israel. (iii) The third group consisted of patients seropositive for *Chlamydia pneumoniae* (either IgG $\geq$ 1:64, IgA $\geq$ 1:32, or both) ($n = 20$) and seronegative for *Chlamydia trachomatis* and *Chlamydia psittaci*, using IFA performed at the National *Chlamydia* and *Mycoplasma* Center, Rabin Medical Center, Petah Tikva, Israel. (iv) The last group consisted of patients with brucellosis and serum agglutination test value of $\geq$1:160 ($n = 10$).

DNA extraction and PCR. Genomic DNA was extracted from tissue specimens and bacteria using the QIAamp DNA minikit (Qiagen, Valencia, CA) or E.Z.N.A. bacterial DNA kit (Omega Bio-Tek) and plasmid DNA was extracted using the QIAprep spin miniprep kit (Qiagen), all according to the manufacturers' instructions, and kept at −20°C until further analysis. Conventional PCR was performed using primers targeting various genes (Tables 2 and 3), followed by DNA sequencing of 1 to 4 PCR products per specimen (DNA Sequencing Unit at the G. S. Wise Faculty of Life Sciences, Tel Aviv University, and Hy Laboratories Ltd., Rehovot, Israel) and analysis using Chromas (version 2.6; Technelysium, South Brisbane, Australia) and BLAST search in the GenBank database (https://www.ncbi.nlm.nih.gov/GenBank/).

Real-time PCR was developed in our research laboratory and has been used by the clinical microbiology laboratory since 2017. This assay was designed to specifically identify *B. henselae* and *B. quintana*, which account for >95% of all *Bartonella* endocarditis cases, and also identify other *Bartonella* spp. implicated in endocarditis at the genus level. *Bartonella* genus-specific degenerate primers (Sigma-Aldrich, Rehovot, Israel) were used to amplify a 185-bp fragment of the *ribC* gene. The resulting PCR product was detected postamplification using 3 SimpleProbe probes for the identification of *Bartonella* genus, *B. henselae*, and *B. quintana* DNA (TIB MOLBIOL, Berlin, Germany, and Dyn Diagnostics, Migdal HaEmeq, Israel) (Table 3). Once hybridized to its target sequence, the SimpleProbe probe emits more fluorescence than the free probe, which is self-quenched. Each probe has a distinctive melting temperature (T_m) at which the probe no longer binds the amplified target, resulting in an instantaneous drop in the fluorescent signal. As a result, changes in fluorescence are based solely on the hybridization status of the probe. Even minor changes in the target DNA sequence, such as single nucleotide polymorphisms, might result in a detectable shift of the melting curve, thus facilitating pathogen identification, without sequencing the amplicon (technical note no. LC 18/2004, Roche Applied Science). PCR conditions were

TABLE 2 Results of the real-time PCR assay (expressed as melting temperatures) and the enzyme immunoassay (expressed as the reciprocal titers) among 37 patients with *Bartonella* endocarditis

Patient no.	Age (yrs)/ sex[a]	Molecular diagnosis[b]	Conventional PCR target gene(s)	Real-time PCR T_m (°C)[c]			Maximal reciprocal titer (anti-*Bartonella* IgG antibodies)		
				T_m-1	T_m-2	T_m-3	*B. henselae*	*B. quintana*	*B. koehlerae*
1	60/M	*B. koehlerae*	*gltA*, *ribC*		59		400	800	1,600
2	63/M	*B. henselae*	*gltA*	49	59		1,600	400	800
3	71/M	*B. henselae*	*gltA*	49	59		3,200	1,600	3,200
4	37/M	*B. henselae*	*gltA*	49	59		3,200	1,600	1,600
5	58/M	*B. henselae*	*ribC*, *pap31*	NA[d]	NA	NA	12,800	6,400	ND[e]
6	63/M	*B. henselae*	*gltA*	49	60		400	NA	ND
7	22/F	*B. henselae*	*gltA*	49	59		3,200	1,600	ND
8	71/M	*B. henselae*	*pap31*	NA	NA	NA	3,200	3,200	ND
9	74/F	*B. henselae*	*ribC*	49	59		3,200	800	ND
10	75/F	*B. henselae*	*ribC*	49	59		12,800	12,800	ND
11	73/M	*B. henselae*	*ribC*	49	59		1,600	800	ND
12	43/M	*B. henselae*	*pap31*, 16S rRNA	51	61		NA	NA	ND
13	56/M	*B. henselae*	*gltA*	49	60		1,600	ND	ND
14	71/F	*B. henselae*	*gltA*, 16S rRNA	49	59		6,400	6,400	ND
15	20/M	*B. henselae*	*ribC*	51	60		6,400	3,200	ND
16	56/F	*B. henselae*	16S rRNA	49	59		12,800	12,800	ND
17	50/M	*B. henselae*	*gltA*	49	59		3,200	1,600	ND
18	42/F	*B. henselae*	16S rRNA	50	60		12,800	6,400	ND
19	56/M	*B. henselae*	*gltA*	49	59		1,600	800	ND
20	32/M	*B. quintana*	*gltA*, *groEL*, *rpoB*, *ftsZ*		59	70	400	1,600	400
21	20/M	*B. quintana*	*gltA*, *groEL*, *rpoB*, *ftsZ*		59	70	400	1,600	200
22	56/M	*B. quintana*	*gltA*, *groEL*, *rpoB*, *ftsZ*		59	69	1,600	6,400	800
23	43/M	*B. quintana*	16S rRNA, *gltA*, *pap31*	NA	NA	NA	800	1,600	ND
24	72/M	*B. quintana*	*gltA*	NA	NA	NA	3,200	3,200	1,600
25	56/M	*B. quintana*	*ribC*		59	70	200	1,600	ND
26	48/F	*B. quintana*	*gltA*, *groEL*, *rpoB*, *ftsZ*		59	70	1,600	6,400	ND
27	12/F	*B. quintana*	*gltA*, *groEL*, *rpoB*, *ftsZ*		58	69	100	1,600	ND
28	7/F	*B. quintana*	*gltA*, *groEL*, *rpoB*, *ftsZ*		61	71	1,600	NA	ND
29	28/M	*B. quintana*	*gltA*, *groEL*, *rpoB*, *ftsZ*		61	71	3,200	6,400	ND
30	52/M	*B. quintana*	16S rRNA		58	69	12,800	25,600	ND
31	16/F	*B. quintana*	*gltA*, *groEL*, *rpoB*, *ftsZ*		58	68	12,800	6,400	ND
32	9/F	*B. quintana*	*gltA*, *groEL*, *rpoB*, *ftsZ*		59	70	3,200	6,400	ND
33	75/M	*B. quintana*	*gltA*, *groEL*, *rpoB*, *ftsZ*		58	69	25,600	25,600	ND
34	65/M	*B. quintana*	*gltA*, *groEL*, *rpoB*, *ftsZ*		57	68	3,200	6,400	ND
35	20/F	*B. quintana*	*ribC*		59	70	200	200	ND
36	11/M	*B. quintana*	*gltA*, *groEL*, *rpoB*, *ftsZ*		59	70	12,800	25,600	ND
37	75/F	*B. quintana*	16S rRNA		58	69	3,200	6,400	ND

[a]M, male; F, female.
[b]Molecular diagnosis was determined by sequencing of the conventional PCR products and by real-time PCR melting-curve analysis. All *B. henselae* strains were Huston-1 type as determined by DNA sequencing.
[c]T_m-1, melting temperature generated by the *B. henselae*-specific probe; T_m-2, melting temperature generated by the *Bartonella* genus probe; T_m-3, melting temperature generated by the *B. quintana*-specific probe.
[d]NA, not available. Specimen was not available for further testing.
[e]ND, not done.

TABLE 3 Sequences of oligonucleotides relevant to this study

Oligonucleotide use and name	Oligonucleotide sequence (5′→3′)	Target organism	Target gene	Product size (bp)	Reference
Conventional PCR					
BhCS.781p	GGGGACCAGCTCATGGTGG	*Bartonella* sp.	*gltA*	379	37
BhCS.1137	AATGCAAAAAGAACAGTAAACA	*Bartonella* sp.			
gltA-F	GCTATGTCTGCATTCTATCA	*Bartonella* sp.	*gltA*	790	38
gltA-R	GATCYTCAATCATTTCTTTCCA	*Bartonella* sp.			
Barton-1	TAACCGATATTGGTTGTGTTGAAG	*Bartonella* sp.	*ribC*	588	39
Barton-2	TAAAGCTAGAAAGTCTGGCAACATAACG	*Bartonella* sp.			
PAPn1	TTCTAGGAGTTGAAACCGAT	*B. henselae*	*pap31*	277	40
PAPn2	GAAACACCACCAGCAACATA	*B. henselae*			
ftsZ-F	ATTAATCTGCAYCGGCCAGA	*Bartonella* sp.	*ftsZ*	935	38
ftsZ-R	ACVGADACACGAATAACACC	*Bartonella* sp.			
groEL-F	GAACTNGAAGATAAGTTNGAA	*Bartonella* sp.	*groEL*	1,459	38
groEL-R	AATCCATTCCGCCCATTC	*Bartonella* sp.			
rpoB-F	CGCATTGGCTTACTTCGTATG	*Bartonella* sp.	*rpoB*	893	38
rpoB-R	GTAGACTGATTAGAACGCTG	*Bartonella* sp.			
16S-27f	AGAGTTTGATCMTGGCTCAG	Eubacteria	16S rRNA	862-3	41
16S-907r	CCGTCAATTCMTTTRAGTTT	Eubacteria			42
Real-time PCR					
ribC-bart-F1	GGTTTGGCTGAGATYATTGA	*Bartonella* sp.	*ribC*	185	Present study
ribC-bart-R1	TCAAGTGTATGRCGAATAAT	*Bartonella* sp.			Present study
Bartonella genus SimpleProbe	GAAGGTGAXITGCAGTTCGTT-Ph	*Bartonella* sp.	*ribC*		Present study
B. henselae SimpleProbe	TTTCCTXIACAAGTACCAAA-Ph	*B. henselae*	*ribC*		Present study
B. quintana SimpleProbe	TCCTGCAAGTXITCCAACAAAATTCACGCCCTT-Ph	*B. quintana*	*ribC*		Present study
β-globin-F	TACACATATTGACCAAATCAGGGTA	Human	*β-globin*	165	Present study
β-globin-R	TTTAGAATGGTGCAAAGAGGC	Human	*β-globin*		Present study
β-globin-probe	Cy5-TACTTTCCCTAATCTCTTTCTTTCAGGGCAAT-BBQ	Human	*β-globin*		Present study

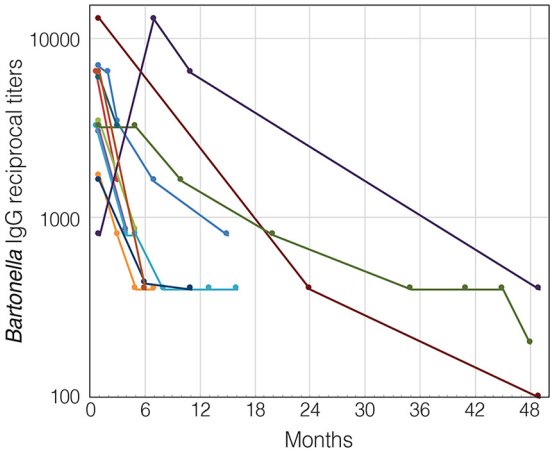

FIG 1 Kinetics of anti-*Bartonella* IgG antibodies expressed as reciprocal titers in a logarithmic scale in patients with *Bartonella* endocarditis ($n = 12$). Only results for patients with 2 or more serum specimens taken at least 1 month apart are presented. Each circle represents the mean enzyme immunoassay optical density of triplicate determinations for 1 patient. Each patient is marked with a different color.

as follows: 5 min at 95°C followed by 50 cycles of 10 s at 95°C, 20 s at 55°C, and 10 s at 72°C. The melting-curve analysis step was performed postamplification and consisted of 60 s at 95°C and 120 s at 37°C, with gradual increase to 80°C and cooling for 30 s. Real-time PCR and analysis were performed using the LightCycler 96 instrument and software version 1.1 (Roche Diagnostics, Basel, Switzerland). For the construction of positive-control plasmids, we cloned the *ribC* PCR amplicons of *B. henselae* and *B. quintana* into high-copy-number plasmids designated pMR-ribC185-Bh and pMR-ribC185-Bq, respectively (PCR cloning kit; Qiagen, Valencia, CA). Thirty, 300, and 3000 copies of both were used as positive controls along with the clinical specimen DNA in a multiplex real-time PCR assay. The analytical sensitivity was determined to be 3 to 30 plasmid copies per reaction (M. Giladi, unpublished data). To identify *Bartonella*, melting peaks of unknown specimens were compared to those of known standards (positive-control plasmids). Each run of clinical specimens also included a negative no-template control and an internal control gene (human *β-globin*) to verify template DNA integrity and absence of PCR inhibitors in the specimens. For the purpose of this study, all clinical specimens were subjected to both real-time PCR and conventional PCR except for four patients for whom tissue or DNA samples were not available for real-time PCR (Table 2). All PCR products of both real-time PCR and conventional PCR assays were sequenced to corroborate the identification of the *Bartonella* sp. To determine its specificity, the real-time PCR assay was performed on DNA extracted from non-*Bartonella* bacteria known to be associated with culture-negative endocarditis as presented in Table 1.

RESULTS

Serology. Of 17 patients with *B. henselae* endocarditis who were tested with EIA using *B. henselae*-derived antigen, 16 (94%) had anti-*B. henselae* IgG titers of ≥1:800 and 1 patient had an IgG titer of 1:400 (range, 1:400 to 1:12,800). Of 17 patients with *B. quintana* endocarditis who were tested using *B. quintana*-derived antigen, 16 (94%) had anti-*B. quintana* IgG titers of >1:800 and 1 patient had an IgG titer of 1:200 (range, 1:200 to 1:26,600). One patient with *B. koehlerae* endocarditis had an anti-*B. koehlerae* IgG titer of 1:1,600 when tested using the corresponding antigen. Significant cross-reactivity between *B. henselae*, *B. quintana*, and *B. koehlerae* was found in all sera tested (Table 2). Two or more serum specimens taken from the same patient at least 1 month apart were available for 12 patients, 10 with *B. henselae* and 2 with *B. quintana* endocarditis (Fig. 1). The median duration to achieve IgG titer decrease of two dilutions, observed in 11 patients, was 5 months (range, 2 to 41 months).

The anti-*Bartonella* IgG titer distribution among patients with *Bartonella* endocarditis and patients in the control groups tested to evaluate specificity is presented in Fig. 2. Of 62 patients with blood culture-positive endocarditis, 58 (94%) were considered seronegative, with an anti-*Bartonella* IgG titer of <1:100, while 4 patients (6%), 3 of them with *Enterococcus faecalis* endocarditis, had an IgG titer of 1:100. Of 34 patients with serological profile consistent with chronic Q fever, 20 (59%) were seronegative, while 5 patients were seropositive for *Bartonella henselae* and/or *B. quintana* with IgG titers of 1:100, 4 patients with 1:200, 4 patients with 1:400, and 1 patient with an anti-

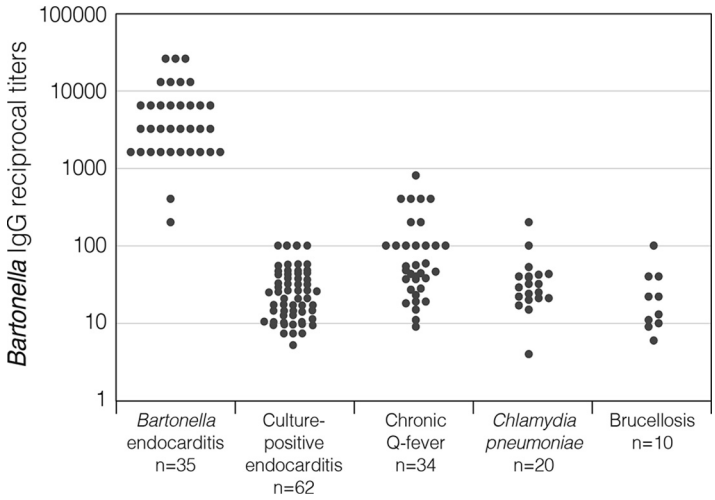

FIG 2 Distribution of anti-*Bartonella* IgG reciprocal titers, expressed in a logarithmic scale, in patients with *Bartonella* endocarditis and 4 control groups: patients with culture-positive endocarditis, patients with anti-*Coxiella burnetii* IgG phase I antibody titers of ≥1:1,600, *Chlamydia pneumoniae*-seropositive patients, and patients with brucellosis and serum agglutination test values of ≥1:160. Each circle represents the mean enzyme immunoassay optical density of triplicate determinations for 1 patient.

Bartonella IgG titer of 1:800. Of note, among the 14 patients with chronic Q fever and cross-reactivity with *Bartonella*, anti-*Bartonella* IgG titers were 3 to 7 dilutions lower than the matching anti-*Coxiella burnetii* phase I IgG titers. Of the patients with *Bartonella* endocarditis, only one patient (3%), with an anti-*Bartonella* IgG titer of 1:3,200, tested positive for Q fever, with a phase I IgG titer of 1:200. Of 20 patients with *Chlamydia pneumoniae* infection, 18 (90%) were seronegative for *Bartonella* (IgG < 1:100) and 2 were seropositive, with IgG titers of 1:100 and 1:200. Of 10 patients with brucellosis, 9 (90%) were seronegative for *Bartonella* and 1 had an IgG titer of 1:100.

Molecular diagnosis. We first evaluated the performance of the *Bartonella* genus-specific probe to identify *Bartonella* spp. known to cause endocarditis. All species tested were identified, each with a single fluorescent peak, some with a unique T_m (e.g., *B. elizabethae*, T_m, 43°C, and *B. washoensis*, T_m, 50°C) and others with overlapping T_ms which do not allow interspecies separation (*B. henselae*, *B. quintana*, *B. koehlerae*, and *B. vinsonii*, T_m, 57 to 58°C) (Fig. 3 and Table 1). DNA sequencing confirmed the species identity.

The real-time PCR assay performed on infected cardiac specimens was positive for all 33 tested specimens and was in complete agreement with results of the sequence-based diagnosis of the conventional PCR. The assay specifically identified all cases of *B. henselae* and *B. quintana* endocarditis based on the fluorescent pattern and T_m values. T_m-1 (range, 49°C to 51°C) and T_m-3 (68°C to 71°C) were generated by the specific *B. henselae* and *B. quintana* probes, respectively. T_m-2 (57°C to 61°C), representing the genus-specific probe, was common to *B. henselae*, *B. quintana*, and *B. koehlerae* (Table 2). Figures 4 and 5 present the identification of *B. quintana* DNA in infected native aortic valve and *B. henselae* DNA in infected native mitral valve, respectively, of two patients with culture-negative endocarditis. Specificity of the real-time PCR assay was established when the multiplex assay, including *Bartonella* genus, *B. henselae*, and *B. quintana* probes, was performed using DNA of non-*Bartonella* culture negative-associated bacteria and resulted in negative fluorescent signals (Table 1).

Cultures. Of the 28 clinical cardiac specimens available for culture, 1 (4%) was positive, growing *B. henselae*. Two blood cultures were positive, one growing *B. henselae* and the other *B. quintana*.

DISCUSSION

This report describes the modalities used in a national reference center for the diagnosis of *Bartonella* endocarditis. The fact that all *Bartonella* endocarditis cases in Israel are referred for diagnoses to a single laboratory permitted analysis of the performance

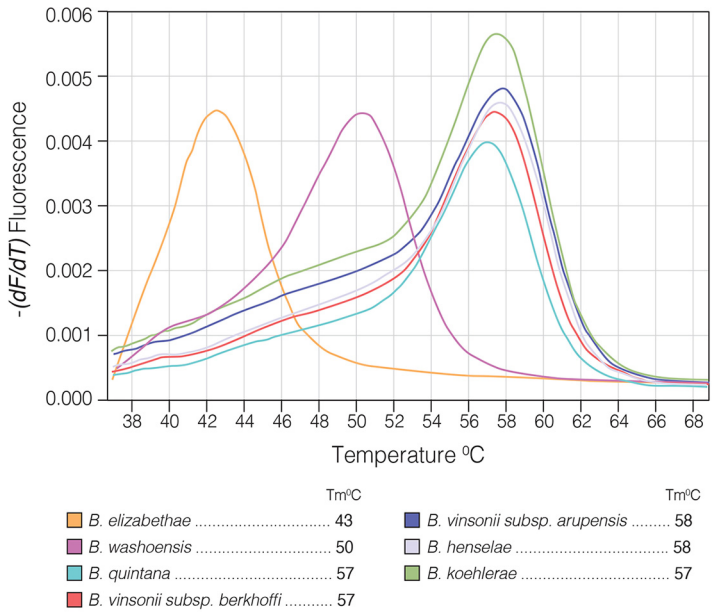

FIG 3 Multiplex real-time PCR performed on 7 *Bartonella* spp. reported to cause endocarditis in humans, utilizing a *Bartonella* genus-specific probe. The melting temperature (T_m) was calculated by plotting the negative derivative of fluorescence over temperature ($-dF/dT$). The T_m value was defined as the peak of the curve. Values were rounded to the nearest integer.

of these methods for 37 patients diagnosed with *Bartonella* endocarditis during a 20-year period. To date, the mainstay of the literature on *Bartonella* endocarditis, and in particular description of diagnostic methods, stems from series originating in France and one series from the United Kingdom, with limited data reported elsewhere (1, 18, 29–31). In fact, a recent review published in 2019 identified only 13 cases of *B. quintana* endocarditis reported from North America, although *Bartonella* spp. are believed to be the most common cause of culture-negative endocarditis in the United States (32, 33).

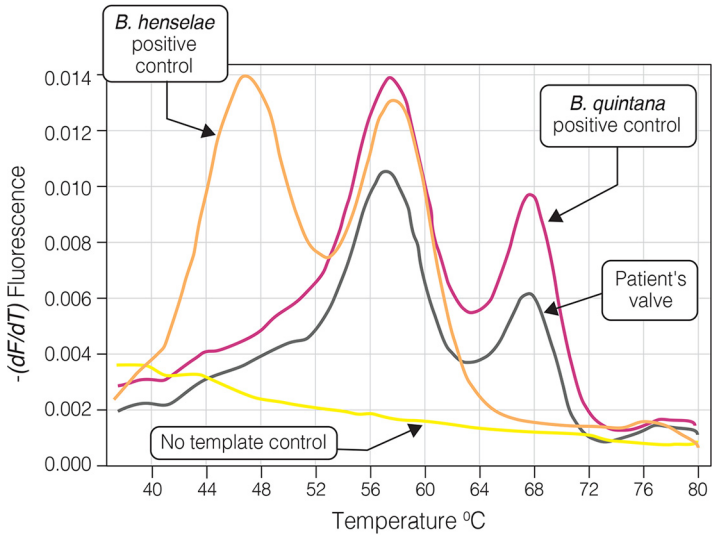

FIG 4 Multiplex real-time PCR using 3 SimpleProbe probes: *Bartonella* genus-specific, *B. henselae*-specific, and *B. quintana*-specific probes. PCR was performed on valvular tissue of a 65-year-old patient with native aortic valve *Bartonella quintana* endocarditis (patient number 34 [Table 2]). Two fluorescent peaks corresponding to melting temperatures (T_ms) of 57.2°C and 67.6°C are the result of detachment of the genus-specific and *B. quintana*-specific probes from the PCR product, respectively. Plasmids pMR-ribC185-Bq and pMR-ribC185-Bh were used as positive controls for *B. quintana* and *B. henselae*, respectively.

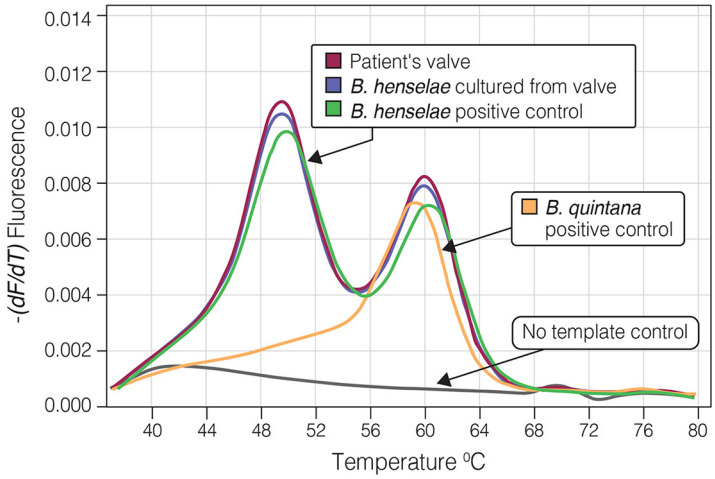

FIG 5 Multiplex real-time PCR using 2 SimpleProbe probes, *B. henselae*-specific and genus-specific probes. PCR was performed on valvular tissue of a 56-year-old patient with native mitral valve *Bartonella henselae* endocarditis (patient number 13 [Table 2]). Two fluorescent peaks corresponding to T_ms of 49.3°C and 59.8°C are the result of detachment of the *B. henselae*-specific and genus-specific probes from the PCR product, respectively. Plasmids pMR-ribC185-Bq and pMR-ribC185-Bh were used as positive controls for *B. quintana* and *B. henselae*, respectively. The peak corresponding to the *B. quintana*-specific probe is not seen since the *B. quintana*-specific probe was not used in this specific assay.

IFA, either as a "homemade" or a commercial product, is the most commonly used serological method for the diagnosis of *Bartonella* infections, including *Bartonella* endocarditis, utilized among others by the national reference center for Q fever and *Bartonella* infection in France and the Centers for Disease Control and Prevention (CDC) in the United States (1, 34). In comparison with EIA, IFA has several limitations. Preparation of antigen requires cultivation of *Bartonella* spp. in Vero cells or other cell lines. Attempts to use agar-derived antigens have resulted in autoagglutination of the *Bartonella* bacilli, which interferes with the IFA (34). In addition, IFAs in general suffer from interobserver variation and are also not suitable for automation or for screening large numbers of specimens. We found that anti-*Bartonella* IgG titers of ≥1:800 were recorded for 94% of patients with *Bartonella* endocarditis, while the remaining patients, though PCR positive on cardiac infected tissue, demonstrated lower titers. A previous study from France utilizing an IFA on 106 patients with *Bartonella* endocarditis also demonstrated that IgG titers of ≥1:800 correlate with endocarditis in 94% of cases (1). Clinicians should be aware, however, that relatively low IgG titers, obtained by either IFA or EIA, may represent rare cases of *B. henselae* or *B. quintana* endocarditis but may also be attributable to non-*quintana*, non-*henselae Bartonella* spp., as was demonstrated in our *B. koehlerae* endocarditis patient who had IgG titer of 1:400 when tested using *B. henselae* antigen, compared with a titer of 1:1,600 when *B. koehlerae* antigen was incorporated in the EIA. Similarly, an IgG titer of <1:800 was reported for patients with *B. alsatica* endocarditis who were tested with IFA using either *B. henselae* or *B. quintana* antigens (1).

Although, similarly to IFA, our EIA cannot distinguish between endocarditis due to *B. henselae* and *B. quintana* as a result of cross-seroreactivity between these two species, the clinical significance of this finding is limited since the medical approaches to *B. henselae* and *B. quintana* endocarditis are quite similar (17, 31). More concerning is the cross-reactivity between *Bartonella* spp. and *Coxiella burnetii*, since they are important etiologic agents of culture-negative endocarditis with very similar clinical characteristics yet with dissimilar practices for antimicrobial treatment and serological follow-up (17, 18). Of 34 patients with serology consistent with chronic Q fever, we found that 13 patients (38%) showed cross-seroreactivity with *Bartonella* spp.; however, all had IgG titers of <1:800, except 1 patient (3%) with anti-*Coxiella burnetii* phase I IgG titer of

1:6,400 who tested positive for *Bartonella* with an IgG titer of 1:800. Cross-reaction between *C. burnetii* antibodies and *Bartonella* antigen have been reported before (17, 35). La Scola and Raoult (17) have found that using IFA, more than 50% of chronic Q fever patients had sera which reacted to a significant level with *B. henselae* and/or *B. quintana*. Although cross-adsorption studies and Western blot analysis have been shown to assist identification of the true culprit in cases with cross-reactivity while also increasing the diagnostic sensitivity (1), these assays are not currently available in most clinical microbiology laboratories (1, 17). However, *Bartonella-Coxiella* cross-seroreactivity infrequently poses a clinical quandary since in the majority of cases, IgG titers against the true pathogen are several dilutions higher than those of the pathogen causing the false-positive serology and thus allowing for the correct diagnosis. To further explore the specificity of our EIA, we tested sera from several control groups. We have also demonstrated low-level cross-reactivity in cases of *Chlamydia pneumoniae* infections similarly to a previous study using an IFA (19), in cases infected with *Brucella melitensis*, a pathogen phylogenetically close to *Bartonella*, and in cases with culture-positive bacterial endocarditis. Cross-reactivity, however, was uncommon in these cases and always in low titers, supporting an anti-*Bartonella* IgG titer of $\geq$1:800 as a cutoff for *Bartonella* endocarditis diagnosis even in the presence of cross-reactivity. Such low-level cross-reactivity does not necessarily indicate antigenic similarity but may also be the result of polyclonal B cell activation, known to occur in endocarditis (27, 36). Of particular interest is a subgroup of patients with *E. faecalis* endocarditis, since a recent publication demonstrated cross-seroreactivity with this pathogen in *Bartonella*-infected endocarditis patients tested by Western immunoblotting (28). We have tested serum samples from 31 patients with *E. faecalis* endocarditis, and although 10% were *Bartonella* seropositive, none demonstrated high-level seropositivity ($>$1:100) that might interfere with the interpretation of *Bartonella* EIA. Further studies are needed to evaluate the added value of Western blotting, which may increase diagnostic sensitivity (at the expense of lower specificity). The potential benefit, however, must be weighed against the increased burden placed on the microbiology laboratory that does not perform Western immunoblotting or cross-adsorption studies on a routine basis.

We also showed that the multiplex real-time PCR assay performed on cardiac tissue accurately identified *B. quintana* and *B. henselae*, which are responsible for almost all *Bartonella* endocarditis cases in humans, without the need for DNA sequencing. The unique melting curves generated by the interaction of the genus-specific and species-specific probes with the PCR product results in 2 distinct T_ms, 9 to 11°C apart, which are easily recognized. Melting peaks of clinical specimens were compared to those of known standards (plasmids pMR-ribC185-Bh and pMR-ribC185-Bq). This comparison to standards is essential since T_m is not a constant value and a significant T_m shift may occur due to multiple factors, such as oligonucleotide concentration, cation concentration, particularly free magnesium (which by itself is inversely related to the amount of deoxynucleoside triphosphates [dNTPs], number of probes and primers, or amount and length of the target DNA), and other components of the reaction. We have observed a T_m shift of 1 to 4°C (Tables 1 and 2); however, all probe-generated peaks of a tested specimen and positive controls are shifted in the same direction and magnitude, resulting in a fixed interval between peaks. We demonstrated that the genus-specific probe also identified 5 non-*henselae*, non-*quintana Bartonella* spp. known to cause endocarditis. *B. alsatica* and "*Candidatus* Bartonella mayotimonensis," also reported to rarely cause endocarditis, were not tested with our real-time PCR assay; however, due to the use of degenerate primers designated to encompass a large number of *Bartonella* spp. and the characteristics of the genus-specific probe, we predict that they will be recognized by this probe. To further support this prediction, we tested 21 additional *Bartonella* spp. isolated from various hosts, including ruminants, rodents, felines, squirrels, a kangaroo, and a bat, from different continents, including North America, Africa, Europe, Asia, and Australia, and all were recognized by the genus-specific probe, resulting in a single peak with variable T_ms (Giladi, unpublished). Along these lines, we

expect that if a new *Bartonella* sp. is implicated in endocarditis, it will be recognized by the real-time PCR assay. Contrary to the case with *B. quintana* and *B. henselae*, which are accurately identified by specific probes, the identity of other species recognized by the genus probe alone needs to be corroborated by either DNA sequencing or a second PCR to distinguish between members of the same melting group. As non-*henselae*, non-*quintana Bartonella* spp. are extremely rare causes of endocarditis, a decision to add additional specific probes for the identification of these species is unlikely to be supported by cost-benefit analysis. However, if future epidemiological studies demonstrate that the incidence of another endocarditis-associated *Bartonella* sp. is increased, new species-specific probes can be incorporated into this assay. The high specificity of the real-time PCR system is achieved by using *Bartonella*-specific degenerate primers to amplify part of the *ribC* gene and 3 *Bartonella*-specific probes. This was demonstrated by testing a large number of bacterial pathogens known to be associated with culture-negative endocarditis, including members of the HACEK group (a group of Gram-negative bacilli consisting of *Haemophilus* spp., *A. actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens*, and *Kingella* spp.), nutritionally variant streptococci (e.g., *Abiotrophia* spp.), *Coxiella burnetii*, *Legionella pneumophila*, and *Tropheryma whipplei*, none of which produced false-positive results. The high positivity rate of PCR performed on infected cardiac tissue is in agreement with previous studies reporting 93 to 98% positivity rates of PCR performed on valvular specimens in spite of the fact that in one of these studies, 62% of the specimens were obtained from patients receiving antibiotic therapy. To summarize, the current study highlights the SimpleProbe real-time PCR as a sensitive and specific method which is also rapid and high throughput for the detection and differentiation of *Bartonella* spp. causing endocarditis.

In conclusion, we have demonstrated that EIA coupled with a novel real-time PCR assay can play an important role in the laboratory diagnosis of *Bartonella* endocarditis and expand the diagnostic arsenal at the disposal of the clinical microbiologist. Since serology remains the major diagnostic tool in the absence of tissue suitable for PCR, recognizing its many pitfalls is essential to avoid incorrect diagnosis.

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REFERENCES

1. Edouard S, Nabet C, Lepidi H, Fournier PE, Raoult D. 2015. *Bartonella*, a common cause of endocarditis: a report on 106 cases and review. J Clin Microbiol 53:824–829. https://doi.org/10.1128/JCM.02827-14.
2. Fournier PE, Thuny F, Richet H, Lepidi H, Casalta JP, Arzouni JP, Maurin M, Celard M, Mainardi JL, Caus T, Collart F, Habib G, Raoult D. 2010. Comprehensive diagnostic strategy for blood culture-negative endocarditis: a prospective study of 819 new cases. Clin Infect Dis 51:131–140. https://doi.org/10.1086/653675.
3. Houpikian P, Raoult D. 2005. Blood culture-negative endocarditis in a reference center: etiologic diagnosis of 348 cases. Medicine (Baltimore) 84:162–173. https://doi.org/10.1097/01.md.0000165658.82869.17.
4. Daly JS, Worthington MG, Brenner DJ, Moss CW, Hollis DG, Weyant RS, Steigerwalt AG, Weaver RE, Daneshvar MI, O'Connor SP. 1993. *Rochalimaea elizabethae* sp. nov. isolated from a patient with endocarditis. J Clin Microbiol 31:872–881. https://doi.org/10.1128/JCM.31.4.872-881.1993.
5. Roux V, Eykyn SJ, Wyllie S, Raoult D. 2000. *Bartonella vinsonii* subsp. *berkhoffii* as an agent of afebrile blood culture-negative endocarditis in a human. J Clin Microbiol 38:1698–1700. https://doi.org/10.1128/JCM.38.4.1698-1700.2000.
6. Olarte L, Ampofo K, Thorell EA, Sanderson S, Doby E, Pavia AT, Rosado H, Raoult D, Socolovschi C, Hersh AL. 2012. Bartonella vinsonii endocarditis in an adolescent with congenital heart disease. Pediatr Infect Dis J 31:531–534. https://doi.org/10.1097/INF.0b013e31824ba95a.
7. Fenollar F, Sire S, Wilhelm N, Raoult D. 2005. *Bartonella vinsonii* subsp. *arupensis* as an agent of blood culture-negative endocarditis in a human. J Clin Microbiol 43:945–947. https://doi.org/10.1128/JCM.43.2.945-947.2005.
8. Avidor B, Graidy M, Efrat G, Leibowitz C, Shapira G, Schattner A, Zimhony O, Giladi M. 2004. *Bartonella koehlerae*, a new cat-associated agent of culture-negative human endocarditis. J Clin Microbiol 42:3462–3468. https://doi.org/10.1128/JCM.42.8.3462-3468.2004.
9. Raoult D, Roblot F, Rolain JM, Besnier JM, Loulergue J, Bastides F, Choutet P. 2006. First isolation of *Bartonella alsatica* from a valve of a patient with endocarditis. J Clin Microbiol 44:278–279. https://doi.org/10.1128/JCM.44.1.278-279.2006.
10. Jeanclaude D, Godmer P, Leveiller D, Pouedras P, Fournier PE, Raoult D, Rolain JM. 2009. Bartonella alsatica endocarditis in a French patient in close contact with rabbits. Clin Microbiol Infect 15(Suppl 2):110–111. https://doi.org/10.1111/j.1469-0691.2008.02187.x.
11. Lin EY, Tsigrelis C, Baddour LM, Lepidi H, Rolain JM, Patel R, Raoult D. 2010. Candidatus Bartonella mayotimonensis and endocarditis. Emerg Infect Dis 16:500–503. https://doi.org/10.3201/eid1603.081673.
12. Giladi M, Kletter Y, Avidor B, Metzkor-Cotter E, Varon M, Golan Y, Weinberg M, Riklis I, Ephros M, Slater L. 2001. Enzyme immunoassay for the diagnosis of cat-scratch disease defined by polymerase chain reaction. Clin Infect Dis 33:1852–1858. https://doi.org/10.1086/324162.
13. Tasher D, Raucher-Sternfeld A, Tamir A, Giladi M, Somekh E. 2017. Bartonella quintana, an unrecognized cause of infective endocarditis in children in Ethiopia. Emerg Infect Dis 23:1246–1252. https://doi.org/10.3201/eid2308.161037.
14. Metzkor-Cotter E, Kletter Y, Avidor B, Varon M, Golan Y, Ephros M, Giladi M. 2003. Long-term serological analysis and clinical follow-up of patients

with cat scratch disease. Clin Infect Dis 37:1149–1154. https://doi.org/10.1086/378738.
15. Habot-Wilner Z, Trivizki O, Goldstein M, Kesler A, Shulman S, Horowitz J, Amer R, David R, Ben-Arie-Weintrob Y, Bakshi E, Almog Y, Sartani G, Vishnevskia-Dai V, Kramer M, Bar A, Kehat R, Ephros M, Giladi M. 2018. Cat-scratch disease: ocular manifestations and treatment outcome. Acta Ophthalmol 96:e524–e532. https://doi.org/10.1111/aos.13684.
16. Jost M, Latz A, Ballhorn W, Kempf VAJ. 2018. Development of a specific and sensitive enzyme-linked immunosorbent assay as an in vitro diagnostic tool for detection of *Bartonella henselae* antibodies in human serum. J Clin Microbiol 56:e01329-18. https://doi.org/10.1128/JCM.01329-18.
17. La Scola B, Raoult D. 1996. Serological cross-reactions between *Bartonella quintana*, *Bartonella henselae*, and *Coxiella burnetii*. J Clin Microbiol 34:2270–2274. https://doi.org/10.1128/JCM.34.9.2270-2274.1996.
18. Fournier PE, Mainardi JL, Raoult D. 2002. Value of microimmunofluorescence for diagnosis and follow-up of *Bartonella* endocarditis. Clin Diagn Lab Immunol 9:795–801. https://doi.org/10.1128/cdli.9.4.795-801.2002.
19. Drancourt M, Mainardi JL, Brouqui P, Vandenesch F, Carta A, Lehnert F, Etienne J, Goldstein F, Acar J, Raoult D. 1995. Bartonella (Rochalimaea) quintana endocarditis in three homeless men. N Engl J Med 332:419–423. https://doi.org/10.1056/NEJM199502163320702.
20. Avidor B, Kletter Y, Abulafia S, Golan Y, Ephros M, Giladi M. 1997. Molecular diagnosis of cat scratch disease: a two-step approach. J Clin Microbiol 35:1924–1930. https://doi.org/10.1128/JCM.35.8.1924-1930.1997.
21. Avidor B, Varon M, Marmor S, Lifschitz-Mercer B, Kletter Y, Ephros M, Giladi M. 2001. DNA amplification for the diagnosis of cat-scratch disease in small-quantity clinical specimens. Am J Clin Pathol 115:900–909. https://doi.org/10.1309/Y5WN-8DFD-WLVT-KKAD.
22. Giladi M, Maman E, Paran D, Bickels J, Comaneshter D, Avidor B, Varon-Graidy M, Ephros M, Wientroub S. 2005. Cat-scratch disease-associated arthropathy. Arthritis Rheum 52:3611–3617. https://doi.org/10.1002/art.21411.
23. Landes M, Maor Y, Mercer D, Habot-Wilner Z, Bilavsky E, Chazan B, Cohen R, Glikman D, Strahilevitz J, Katzir M, Litachevsky V, Melamed R, Guri A, Shaked H, Perets O, Wiener-Well Y, Stren A, Paul M, Zimhony O, Srugo I, Rahav G, Bishara J, Kuperman AA, Ben-Ami R, Ephros M, Giladi M. 23 November 2019. Cat scratch disease presenting as fever of unknown origin is a unique clinical syndrome. Clin Infect Dis https://doi.org/10.1093/cid/ciz1137.
24. Maman E, Bickels J, Ephros M, Paran D, Comaneshter D, Metzkor-Cotter E, Avidor B, Varon-Graidy M, Wientroub S, Giladi M. 2007. Musculoskeletal manifestations of cat scratch disease. Clin Infect Dis 45:1535–1540. https://doi.org/10.1086/523587.
25. Li JS, Sexton DJ, Mick N, Nettles R, Fowler VG, Jr, Ryan T, Bashore T, Corey GR. 2000. Proposed modifications to the Duke criteria for the diagnosis of infective endocarditis. Clin Infect Dis 30:633–638. https://doi.org/10.1086/313753.
26. Durack DT, Lukes AS, Bright DK. 1994. New criteria for diagnosis of infective endocarditis: utilization of specific echocardiographic findings. Duke Endocarditis Service. Am J Med 96:200–209. https://doi.org/10.1016/0002-9343(94)90143-0.
27. Holland TL, Bayer AS, Fowler VG. 2020. Endocarditis and intravascular infections, p 1068–1108. *In* Bennett JE, Dolin R, Blaser MJ (ed), Mandell, Douglas, and Bennett's principles and practice of infectious diseases, 9th ed, vol 1. Elsevier, Inc, Philadelphia, PA.
28. Arregle F, Gouriet F, Amphoux B, Edouard S, Chaudet H, Casalta JP, Habib G, Fournier PE, Raoult D. 2019. Western immunoblotting for the diagnosis of Enterococcus faecalis and Streptococcus gallolyticus infective endocarditis. Front Cell Infect Microbiol 9:314. https://doi.org/10.3389/fcimb.2019.00314.
29. Fournier PE, Lelievre H, Eykyn SJ, Mainardi JL, Marrie TJ, Bruneel F, Roure C, Nash J, Clave D, James E, Benoit-Lemercier C, Deforges L, Tissot-Dupont H, Raoult D. 2001. Epidemiologic and clinical characteristics of Bartonella quintana and Bartonella henselae endocarditis: a study of 48 patients. Medicine (Baltimore) 80:245–251. https://doi.org/10.1097/00005792-200107000-00003.
30. Raoult D, Fournier PE, Drancourt M, Marrie TJ, Etienne J, Cosserat J, Cacoub P, Poinsignon Y, Leclercq P, Sefton AM. 1996. Diagnosis of 22 new cases of Bartonella endocarditis. Ann Intern Med 125:646–652. https://doi.org/10.7326/0003-4819-125-8-199610150-00004.
31. Chaloner GL, Harrison TG, Birtles RJ. 2013. Bartonella species as a cause of infective endocarditis in the UK. Epidemiol Infect 141:841–846. https://doi.org/10.1017/S0950268812001185.
32. Lam JC, Fonseca K, Pabbaraju K, Meatherall BL. 2019. Case report: Bartonella quintana endocarditis outside of the Europe-African gradient: comprehensive review of cases within North America. Am J Trop Med Hyg 100:1125–1129. https://doi.org/10.4269/ajtmh.18-0929.
33. Chambers HF, Bayer AS. 2020. Native-valve infective endocarditis. N Engl J Med 383:567–576. https://doi.org/10.1056/NEJMcp2000400.
34. Regnery RL, Olson JG, Perkins BA, Bibb W. 1992. Serological response to "Rochalimaea henselae" antigen in suspected cat-scratch disease. Lancet 339:1443–1445. https://doi.org/10.1016/0140-6736(92)92032-b.
35. Cooper MD, Hollingdale MR, Vinson JW, Costa J. 1976. A passive hemagglutination test for diagnosis of trench fever due to Rochalimaea quintana. J Infect Dis 134:605–609. https://doi.org/10.1093/infdis/134.6.605.
36. Kaell AT, Volkman DJ, Gorevic PD, Dattwyler RJ. 1990. Positive Lyme serology in subacute bacterial endocarditis. A study of four patients. JAMA 264:2916–2918. https://doi.org/10.1001/jama.1990.03450220082027.
37. Norman AF, Regnery R, Jameson P, Greene C, Krause DC. 1995. Differentiation of Bartonella-like isolates at the species level by PCR-restriction fragment length polymorphism in the citrate synthase gene. J Clin Microbiol 33:1797–1803. https://doi.org/10.1128/JCM.33.7.1797-1803.1995.
38. Bai Y, Malania L, Alvarez Castillo D, Moran D, Boonmar S, Chanlun A, Suksawat F, Maruyama S, Knobel D, Kosoy M. 2013. Global distribution of Bartonella infections in domestic bovine and characterization of Bartonella bovis strains using multi-locus sequence typing. PLoS One 8:e80894. https://doi.org/10.1371/journal.pone.0080894.
39. Johnson G, Ayers M, McClure SCC, Richardson SE, Tellier R. 2003. Detection and identification of Bartonella species pathogenic for humans by PCR amplification targeting the riboflavin synthase gene (ribC). J Clin Microbiol 41:1069–1072. https://doi.org/10.1128/jcm.41.3.1069-1072.2003.
40. Zeaiter Z, Fournier P-E, Raoult D. 2002. Genomic variation of Bartonella henselae strains detected in lymph nodes of patients with cat scratch disease. J Clin Microbiol 40:1023–1030. https://doi.org/10.1128/jcm.40.3.1023-1030.2002.
41. Harmsen D, Dostal S, Roth A, Niemann S, Rothganger J, Sammeth M, Albert J, Frosch M, Richter E. 2003. RIDOM: comprehensive and public sequence database for identification of Mycobacterium species. BMC Infect Dis 3:26. https://doi.org/10.1186/1471-2334-3-26.
42. Alsmark CM, Frank AC, Karlberg EO, Legault B-A, Ardell DH, Canbäck B, Eriksson A-S, Näslund AK, Handley SA, Huvet M, La Scola B, Holmberg M, Andersson SGE. 2004. The louse-borne human pathogen Bartonella quintana is a genomic derivative of the zoonotic agent Bartonella henselae. Proc Natl Acad Sci U S A 101:9716–9721. https://doi.org/10.1073/pnas.0305659101.