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Development and validation of a real-time PCR test to detect *Bartonella quintana* in clinical samples

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Abstract

This study reports on the validation of a real-time polymerase chain reaction test targeting the *vomp* region of *Bartonella quintana*. The assay displayed 100% sensitivity and specificity for the 52 bloods and 159 cultures tested. Molecular diagnosis of *Bartonella quintana* can aid clinical treatment during acute infection.

Keywords

Real-time PCR; Clinical diagnostics; Laboratory method

Human *Bartonella quintana* infection, transmitted via body lice, causes trench fever, endocarditis, bacillary angiomatosis, lymphadenitis, and peliosis hepatis [1]. Throughout World War I, *B. quintana* infected an estimated 800,000 Allied soldiers on the Western Front and accounted for at least one-fifth of illness in the British and Central Powers' armies [2]. Infections today commonly occur in areas of high population density and poor sanitation, with persons experiencing homelessness at increased risk [3–6].

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Authors' contributions

Jamie Choat: conceptualization, methodology, validation, formal analysis, investigation, writing − original draft, visualization. Brook Yockey: investigation, resources, writing − review and editing. Sarah Sheldon: validation, investigation, writing − review and editing. Ryan Pappert: methodology, resources, writing − review and editing. Jeannine Petersen: conceptualization, methodology, resources, writing − review and editing, supervision, project administration. Elizabeth Dietrich: conceptualization, methodology, resources, data curation, writing − review and editing, supervision, project administration.

Declaration of Competing Interest

The authors report no conflicts of interest relevant to this article.

Disclosures

Residual diagnostic specimens were made available as part of a CDC TickNet Emerging Infections Program study.

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Humans are the main reservoir for *B. quintana*. The bacteria infect the bloodstream, causing fevers that last 2 to 4 days and relapse after 5 days intervals for several weeks [2,7]. Symptoms of *B. quintana* infection include headache, muscle and joint pain, chills, sweating, frequent urination, dizziness, nausea, and diarrhea. Although infection is typically not fatal, it can lead to significant morbidity, most commonly endocarditis, if untreated [1,7]. Diagnosis rests on clinical suspicion, as symptoms may be nonspecific [6,8]. Techniques for diagnosis include serology, culturing, and/or PCR. Serologic testing is not species-specific, may cross react with other pathogens, requires the host to develop a detectable antibody response to *B. quintana*, and is interpreted subjectively. Furthermore, persons infected with *B. quintana* can remain seroreactive for years after treatment [4,9,10]. Blood cultures are often negative, due to *B. quintana*'s fastidious nature and prolonged incubation time [10,11].

To help improve direct detection of *B. quintana* infection, this study focuses on the validation of a specific real-time PCR test for detection of *B. quintana* in blood or culture samples. We chose the *vomp* (variably expressed outer membrane protein) region as the DNA amplification target. The Vomp proteins (*vomp*A-D) assist in escaping immune response through antigenic and phase variation [12]. Although the *vomp* region varies among *B. quintana* isolates, we designed oligonucleotides that bind to a conserved sequence that is present in at least 2 copies in all publicly available *B. quintana* genome sequences. They were confirmed to be specific to *B. quintana* by NCBI Primer BLAST [13].

Oligonucleotides consisted of a forward primer (5'CATCGCTCTGGTTATACTCTTATCGA3'), reverse primer (5'GATCCAAAATAACTTCCTGGGTCAT3'), and PrimeTime probe (5'/56-FAM/TGTATCGGCTGTTTTTGCCTCGACTTTACC/3BHQ_1/3') (Integrated DNA Technologies; Coralville, Iowa). Each 20-μL PCR reaction included 750 nM concentrations of each primer and 250 nM of the probe, with PerfeCTa Multiplex Supermix (Quantabio; Beverly, MA). The run conditions included an initial denaturation at 95°C for 2.5 minutes, followed by 40 cycles of denaturation at 95°C for 12 seconds and annealing at 60°C for 45 seconds. We used the human endogenous retrovirus ERV3 as an endogenous control with previously described oligonucleotide sequences [14]. All DNA extractions were performed using the QIAamp DNA Mini Kit (Qiagen; Germantown, MD), unless otherwise specified.

We obtained all bacterial culture samples (Table 1) in house or from the American Type Culture Collection (ATCC). We performed PCR using 10 pg/reaction to assess analytic sensitivity and specificity. Among 18 different isolates of *B. quintana*, the average Ct was 26.80 (SD 1.26). 141/141 (100%) isolates for 40 non-*B. quintana* bacteria, including 15 other *Bartonella* species, were undetected.

To assess the analytical limit of detection (LOD), we tested DNA from *B. quintana* ATCC 51694 in quantities ranging from 20 fg to 0.625 fg per reaction in 2-fold serial dilutions, with 8 replicates per dilution. All replicates down to 5 fg were detected, whereas 7/8 replicates of both the 2.5 fg and 1.25 fg were detected, and 3/8 replicates of the 0.625 fg were detected. We analyzed LOD by probit regression within the MedCalc software (MedCalc Software Ltd; Ostend, Belgium). The resulting LOD was 2.750 ± 0.859 fg/reaction (1.6 genome equivalents based on *B. quintana*'s median genome size in GenBank).

To assess the LOD in blood, we tested EDTA blood spiked with *B. quintana* OK90–268 at 3 concentrations in 15 to 20 replicates. We grew isolates on sheep blood agar for 48 to 72 hours at 37°C with 5% CO_2, and prepared standardized cell suspensions using a turbidity meter. We then spiked the suspensions into EDTA whole blood from healthy human donors (Innovative Research; Novi, MI) to the final concentrations of 1.2×10^2, 1.2×10^3, and 1.2×10^4 colony forming units (CFU/mL). *B. quintana* DNA was detected in 20/20 replicates at both higher concentrations and in 6/15 replicates of the 1.2×10^2 CFU/mL concentration, for an estimated LOD of 1.2×10^3 CFU/mL.We also tested EDTA whole blood from healthy human donors (Reprocell; Beltsville, MD) to ensure *B. quintana* was not detected in the blood of healthy individuals. All (10/10) samples were undetected.

As *B. quintana*-positive clinical blood samples were not available to assess diagnostic sensitivity, we spiked 5 isolates of *B. quintana* (CA15–0058, CA15–0053, CO20–0321, CO20–0297, and CO20–0256) into EDTA whole blood from healthy human donors to a final concentration approximately 10 times the LOD (1.3×10^4 CFU/mL). We froze the samples at ≤−65°C, extracted DNA, and ran PCR in duplicate. All (10/10) samples were detected with an average Ct of 31.46 (SD 0.74).

To evaluate reproducibility, 2 of these spiked blood samples were tested in 6 runs by 2 operators over 5 days. The coefficient of variation of these results was 5.5%. Additional extraction methods were evaluated by spiking blood with *B. quintana* OK90–268 and extracting five replicates separately using either the QIAamp DNA Mini Kit protocol, the Roche MagNA Pure 96 instrument, or the Roche MagNA Pure 24 instrument (Roche Diagnostics; Indianapolis, IN). The coefficients of variation were 0.6% when comparing samples extracted with the QIAamp kit to the MagNA Pure 96 instrument, and 2.4% when comparing the MagNA Pure 96 and MagNA Pure 24 instruments (Table 2).

The CDC Institutional Review Board (protocol #7102) approved the use of residual specimens for assay development and validation. We used residual EDTA blood previously identified positive for other bacterial pathogens to assess diagnostic specificity, including *Borrelia burgdorferi, Anaplasma phagocytophilum, Ehrlichia chaffeensis, Ehrlichia muris* subsp. *eauclairensis, Leptospira kirschneri, Staphylococcus aureus, Streptococcus pneumoniae, Legionella pneumophila*, and *Rickettsia rickettsii* [15,16]. All (42/42) bloods were undetected.

In conclusion, the *vomp* region of *B. quintana* targeted by real-time PCR in this study is highly specific and sensitive (Table 2), likely due to the presence of at least 2 gene copies per genome. This assay is specific to *B. quintana* and does not require additional testing to obtain a species-level diagnosis [6,10,17,18]. A limitation of this validation is the use of spiked samples to calculate diagnostic sensitivity. Real-time PCR targeting the *vomp* region provides a rapid and specific adjunct to blood culture for the diagnosis and clinical management of *B. quintana* bloodstream infections.

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Table 1

Bacterial isolates used for analytical sensitivity and specificity testing.

Species	Number of isolates
Sensitivity	
Bartonella quintana	18
Specificity	
Acinetobacter radioresistens	1
Afipia felis	1
Bartonella alsatica	1
Bartonella bacilliformis	30
Bartonella clarridgeiae	5
Bartonella doshiae	3
Bartonella elizabethae	3
Bartonella grahamii	2
Bartonella henselae	55
Bartonella koehlerae	2
Bartonella rochalimae	1
Bartonella silvicola	1
Bartonella tamiae	1
Bartonella tribocorum	1
Bartonella vinsonii	1
Bartonella vinsonii subsp. arupensis	1
Bartonella vinsonii subsp. berkhoffii	1
Bartonella volans	2
Bartonella washoensis	1
Borrelia burgdorferi	2
Borrelia hermsii	1
Cedecea neteri	1
Chlamydophila pneumoniae	1
Enterobacter cloacae	1
Escherichia coli	2
Francisella tularensis	2
Klebsiella oxytoca	1
Klebsiella pneumoniae	1
Legionella pneumophila subsp. pneumophila	1
Leptospira interrogans	1
Moraxella catarrhalis	1
Mycoplasma pneumoniae	1
Ochrobactrum anthropi	1
Pasteurella multocida	1
Proteus mirabilis	1
Pseudomonas aeruginosa	1

Species	Number of isolates
Salmonella enterica subsp. enterica serovar Typhi	1
Staphylococcus aureus	2
Staphylococcus epidermidis	1
Streptococcus gallolyticus subsp. gallolyticus	1
Streptococcus pyogenes	1
Yersinia pestis	2

Table 2

Summary of assay performance characteristics.

Analytical specificity	100% (0/141 non-*B. quintana* bacteria detected)
Analytical sensitivity (limit ofdetection)	2.750 ± 0.859 fg/reaction (1.6 genome equivalents/reaction)
Diagnostic specificity	100% (0/42 samples from patients with other pathogens detected; 0/10 samples from healthy donors detected)
Diagnostic sensitivity	100% (10/10 human blood samples spiked with *B. quintana* detected)
Reproducibility	5.5% coefficient of variation (2 separately spiked samples tested in 6 runs by 2 operators)
Extraction platforms: comparison of Qiagen QIAamp DNA Mini Kit and Roche MagNA Pure 96 instrument with DNA and Viral Nucleic Acid Small Volume Kit	0.6% coefficient ofvariation (5 spiked samples extracted in parallel and run in the same run)
Extraction platforms: comparison of Roche MagNA Pure 96 instrument with DNA and Viral Nucleic Acid Small Volume Kit and Roche MagNA Pure 24 instrument with Total NA Isolation Kit	2.4% coefficient ofvariation (5 spiked samples extracted in parallel and run in the same run)