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CASE REPORT

Bartonella clarridgeiae Bacteremia Detected in an Asymptomatic Blood Donor

Gislaine Vieira-Damiani,[a] Pedro Paulo Vissotto de Paiva Diniz,[b] Luiza Helena Urso Pitassi,[a] Stanley Sowy,[b] Diana Gerardi Scorpio,[c]* Bruno Grosselli Lania,[a] Marina Rovani Drummond,[a] Tânia Cristina Benetti Soares,[a] Maria de Lourdes Barjas-Castro,[d] Edward B. Breitschwerdt,[e] William L. Nicholson,[f] Paulo Eduardo Neves Ferreira Velho[a]

Division of Dermatology, Department of Medicine, State University of Campinas (UNICAMP) Medical School, Campinas, SP, Brazil[a]; Western University of Health Sciences, College of Veterinary Medicine, Pomona, California, USA[b]; Department of Molecular & Comparative Pathobiology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA[c]; Division of Hematology, Department of Medicine, State University of Campinas (UNICAMP) Medical School, Campinas, SP, Brazil[d]; Intracellular Pathogens Research Laboratory, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, USA[e]; Rickettsial Zoonoses Branch, Centers for Disease Control and Prevention, Atlanta, Georgia, USA[f]

Human exposure to *Bartonella clarridgeiae* has been reported only on the basis of antibody detection. We report for the first time an asymptomatic human blood donor infected with *B. clarridgeiae*, as documented by enrichment blood culture, PCR, and DNA sequencing.

CASE REPORT

During a study designed to determine the prevalence of *Bartonella* bacteremia in samples from 500 blood donors from the Blood Bank of State University of Campinas (UNICAMP—HEMOCENTRO), Sao Paulo State, Brazil (L. H. U. Pitassi, P. P. V. de Paiva Diniz, D. G. Scorpio, M. R. Drummond, B. G. Lania, M. L. Barjas-Castro, S. Colombo, S. Sowy, E. B. Breithschwerdt, W. L. Nicholson, and P. E. N. F. Velho, submitted for publication), we identified a blood donor infected with *Bartonella clarridgeiae* on the basis of specific PCR amplification and DNA sequencing of an amplicon obtained from the 14-day liquid enrichment blood culture.

The donor was a 39-year-old male from Santo Antonio de Posse (22°36′22″S, 46°55′10″W), a city with approximately 20,000 inhabitants located 88 miles north of Sao Paulo City, the capital of Sao Paulo state, southeastern Brazil. The man self-reported donating blood from 6 to 10 times previously. For more than 10 years, the man shared his home with a cat, and he had sustained a bite 2 years earlier. At that time, he had no symptoms. In addition, the man had domiciliary contact with two pet dogs for 4 years. He reported that these animals occasionally had flea and tick infestation. He had no tattoos and had never traveled outside Sao Paulo state, Brazil.

Immediately after the collection of a blood unit, 5 ml of blood was collected into an EDTA tube, with other 5 ml collected into a serum separator tube via accessory port. After serum centrifugation, samples were stored at −20°C until analysis. Two milliliters of the donor's whole EDTA blood was thawed and added into 8 ml of liquid *Bartonella* Alpha-Proteobacteria Growth (BAPGM) medium (1), and the reaction mixture was incubated at 37°C in 5% CO_2, in a water-saturated atmosphere. The flask was maintained with a constant shaking motion for 14 days. A negative-control flask containing only BAPGM medium was added to each batch of samples tested and subjected to the same laboratory procedures and culture conditions. After a 14-day incubation period, a 1-ml aliquot of liquid culture medium was used for DNA extraction and another 1-ml aliquot was plated onto a blood agar plate and incubated for an additional 42 days. Blood agar plates were prepared with 30% of sheep blood confirmed to be free of *Bartonella* DNA by PCR (2). The liquid culture negative-control blood-agar plate was incubated under the same conditions. All culture methods were carried out in a class 2 biosafety cabinet in order to minimize the risk of specimen DNA or bacterial contamination and to protect laboratory personnel.

The liquid culture sample used for molecular analysis was centrifuged, and the pellet was tested for the presence of *Bartonella* DNA. Genomic DNA from the liquid culture was extracted using a QIAamp DNA minikit (Qiagen Inc., Valencia, CA, USA) following the manufacturer's instructions and tested by a real-time PCR using primers manually designed to amplify a fragment of the 16S-23S rRNA intergenic transcribed spacer (ITS) region of all *Bartonella* species. The 25-μl reaction mixture contained 1× PCR mix (SYBR Premix *Ex Taq*; TaKaRa Bio Inc., Shiga, Japan), 7.5 pmol of primer 314A-s (5′-CGTTTCTCTTTCTTCMGATGATG ATCCC-3′), 7.5 pmol of primer 314B-s (5′-CGTTTCTCTTTCT TCRGATGATGATCCC-3′), 10.5 pmol of primer 382-as (5′-AC TTSAACCTCCGACCTCACGCTTAT-3′), 1× ROX reference dye, and 5 μl of DNA template. Amplifications were performed under the following conditions: one hot-start cycle at 95°C for 1 min followed by 50 cycles of denaturing at 94°C for 10 s, annealing at 58°C for 20 s, and extension at 72°C for 20 s. Amplification was detected by SYBR green I fluorescence with emission at 522 nm. The expected melting curves were obtained. Serial dilutions of *B.*

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Address correspondence to Pedro Paulo Vissotto de Paiva Diniz, diniz@westernu.edu.

* Present address: Diana Gerardi Scorpio, Department of Biomedical Sciences, Ross University School of Veterinary Medicine, Basseterre, St. Kitts, West Indies.

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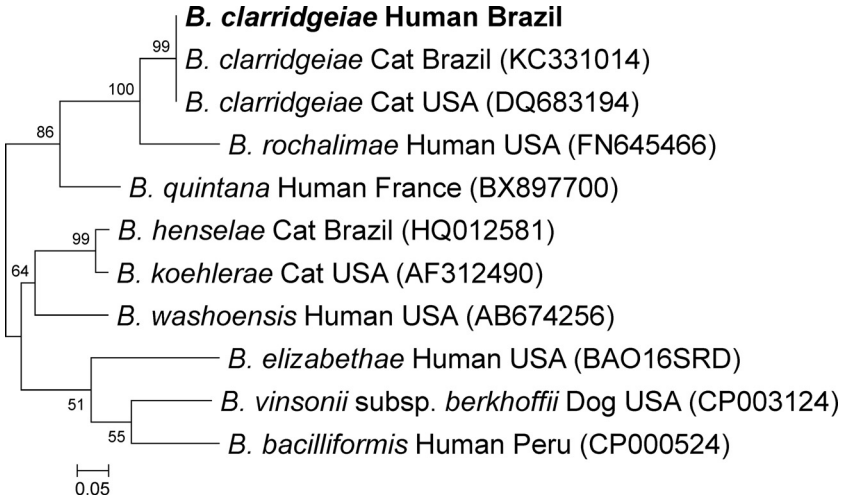

FIG 1 Phylogenetic tree based on 136 bp from the 16S-23S rRNA intergenic transcribed spacer (ITS) sequences of *Bartonella clarridgeiae* obtained from a blood donor (in boldface) and closely related organisms constructed by using the maximum-likelihood method on the basis of the Kimura 2-parameter model. Each bacterial name is followed by isolation source and geographic-origin data, and the GenBank accession number is provided in parentheses. The numbers at the nodes indicate percentages of bootstrap support based on 1,000 replicates. Percentages corresponding to partitions reproduced in fewer than 50% of bootstrap replicates are collapsed. The scale bar indicates 0.05 substitutions per nucleotide position.

henselae DNA from 10^9 genome equivalents (GE) per microliter to 1 GE/μl were used as a positive control, for the determination of the limit of detection of this assay (5 copies per reaction). A Mastermix reagent negative control was included in each batch of samples tested. The amplicons generated were purified (MiniElute kit; Qiagen, Valencia, CA, USA) according to the manufacturer's instructions and sequenced with a fluorescence-based automated sequencing system (Eurofins MWG Operon, Huntsville, AL, USA). Chromatogram evaluation, primer deletion, and sequence alignment were performed using Contig Express software and AlignX software (Vector NTI Suite 10.1; Invitrogen Corp., Carlsbad, CA, USA). The bacterial species was defined by comparing similarities with other sequences deposited in the GenBank database using the Basic Local Alignment Search Tool (3). A phylogenetic tree (Fig. 1) was generated based on 136-bp sequences of the ITS of *B. clarridgeiae* from the blood donor and closely related organisms by using the maximum-likelihood method on the basis of the Kimura 2-parameter model, with bootstrap support based on 1,000 replicates (Molecular Evolutionary Genetics Analysis [MEGA] software, version 5.2).

The donor serum sample was analyzed for the presence of IgG antibodies to *B. henselae* and *B. quintana* antigens grown in Vero cell cultures by an indirect immunofluorescence assay (IFA) according to a previously described procedure (4). The bacterial antigens were supplied by the Centers for Disease Control and Prevention (CDC, Atlanta, GA, USA). The serum sample was diluted 1/64 in phosphate-buffered saline (PBS), and 25 μl was applied to *Bartonella*-infected cells fixed to a glass slide and incubated for 30 min at 37°C. The slides were washed three times (for 5 min each time) in PBS, overlaid with fluorescein isothiocyanate-conjugated goat anti-human immunoglobulin IgG (Sigma, St. Louis, MO, USA) at a dilution of 1/150, incubated at 37°C for 30 min, washed, and dried. Slides were examined on a Labophot fluorescence microscope (Nikon, Melville, NY, USA). The cutoff titer of the IFA was 1/64. Endpoint titers were not determined. A positive test was warranted if brightly stained bacteria could be detected by fluorescence microscopy at ×400 magnification. Previously IFA-negative serum samples were used as negative controls.

By real-time PCR, amplicons of 190 bp in size were obtained from the liquid culture aliquot. After primer deletion, the remaining 136-bp sequence was 100% similar to *B. clarridgeiae* DNA sequences from samples collected from cats in the United States and Brazil (Fig. 1). Following subculture, no isolate was obtained from this subject and he was not seroreactive to *B. henselae* and *B. quintana* antigens.

This report describes the molecular documentation of *B. clarridgeiae* DNA in human blood. The subject was an asymptomatic blood donor who had previously donated blood multiple times. *Bartonella* spp. are the only known Gram-negative bacilli that can live inside erythrocytes while often causing persistent infections in the host (5). To date, 15 species, subspecies, or *Candidatus* species of *Bartonella* have been known to infect humans (Table 1). Asymptomatic infection with *Bartonella bacilliformis* in humans is well established (6), and numerous outwardly healthy animals support persistent *Bartonella* sp. bacteremia (5). However, a spectrum of disease manifestations associated with *Bartonella* spp. has gained importance within the last decades. The genus *Bartonella* comprises a group of reemerging and neglected zoonotic pathogens that have been associated with a broad range of serious human diseases (Table 1).

Worldwide, *Bartonella henselae* is a causative agent of cat scratch disease (CSD), bacillary angiomatosis, peliosis hepatis, bacteremia, endocarditis, chronic lymphadenopathy, and neurological disorders (7–9). *B. clarridgeiae* has also been suggested to cause fever, headaches, malaise, lymphadenopathy (10, 11), and thoracopulmonary manifestations (12) in humans; endocarditis (13) and hepatic diseases (14) in dogs; and blindness (15) and neuritis (16) in cats. The diversity of the clinical manifestations

TABLE 1 Species, subspecies, or *Candidatus* species of *Bartonella* confirmed by molecular methods to infect humans

Species	Clinical outcome(s)	Reference(s) or source
B. bacilliformis	Oroya fever, verruga peruana	6
B. quintana	Bacillary angiomatosis, trench fever, bacteremia	34
B. henselae	Cat scratch disease, endocarditis, bacillary angiomatosis, peliosis hepatis, bacteremia	7–9
B. elizabethae	Fever, endocarditis	35
B. grahamii	Cat scratch disease (lymphadenopathy, reddish papule, abscess)	36
B. vinsonii subsp. *berkhoffii*	Endocarditis, arthritis, neurological disease, and epithelioid hemangioendothelioma	33
B. koehlerae	Anxiety, severe headaches, muscle spasms, decreased peripheral vision, and hallucinations	37
B. alsatica	Fever, lymphadenitis, and endocarditis	38
B. vinsonii subsp. *arupensis*	Fatigue, headache, and myalgias	39
B. washoensis	Meningitis	40
B. rochalimae	Bacteremia, fever, and splenomegaly	24
B. tamiae	Fever, headache, myalgia, maculopapular rash, petechial rash in arms and legs, abnormalities in liver function	41
B. melophagi[a]	Dry cough, fatigue, muscle pain, and severe chills	42
B. mayotimonensis[a]	Endocarditis	43
B. volans (including *volans*-like)	Symptoms uncertain	44
B. clarridgeiae	Asymptomatic	This report

[a] Candidate species in the genus *Bartonella*.

associated with bartonellosis depends mainly on the virulence of the *Bartonella* sp. or strain, the level of evolutionary host adaptation of the bacterium, and the immune response of the host, which is a critical determinant of disease expression (5). In some patients, and especially in immunocompromised subjects, the disease can be fatal (17). The long-term consequence of chronic subclinical infection with *Bartonella* spp. in immunocompetent humans is currently unknown.

Previously, serological evidence of anti-*B. clarridgeiae* antibodies supported hypotheses of exposure in humans (10, 12). However, to our knowledge, *B. clarridgeiae* was never isolated or documented by molecular techniques in blood or tissue samples from a human. *B. clarridgeiae* DNA was detected from fleas (18), and bacteremic infection has been reported in sick dogs (13, 14) and in the healthy pet cat of a sick veterinarian with CSD (11). Experimentally, *B. clarridgeiae* can be transmitted by blood transfusion and be detected by PCR from blood, brain, lymph node, myocardium, liver, and kidney tissues not only from blood culture-positive cats but also from blood culture-negative cats (19). Furthermore, natural concomitant infection with *B. clarridgeiae* and *B. henselae* has been reported in domestic cats (20), because both *Bartonella* spp. are considered adapted to this host and are likely transmitted by the common cat flea (*Ctenocephalides felis*). *In vitro*, both of these *Bartonella* species can infect feline erythrocytes (21) and are capable of entering human endothelial cells (22).

The DNA fragment of *B. clarridgeiae* sequenced from this blood donor was 100% similar to DNA sequences obtained from samples collected from cats in Brazil (23) and from a cat from the United States (24). Cats are considered the natural reservoir of *B. clarridgeiae*, being capable of sustaining high levels of bacteremia (25). The blood donor infected with *B. clarridgeiae* reported long-term daily contact with two dogs and a cat for years, and he reported a cat bite 2 years earlier. Despite the fact that these animals were not tested for *Bartonella* sp. bacteremia, the fleas and ticks that occasionally infested his cat and dogs may have transmitted the pathogen to the owner. In a previous study of 14 immunocompetent people infected with *Bartonella henselae* or *B. vinsonii* subsp. *berkhoffii*, all participants reported frequent exposure to fleas and ticks from domestic cats and dogs (26).

Annually, about 15 million units of red cells are transfused in the United States and about 85 million worldwide (27). The overall rate of any bacterial contamination of red blood cells (RBCs) in blood units reportedly ranges from 0.03% to 0.3% (28). Bacterial contamination is the third most common cause of death from transfusion in the United States (29). *Bartonella* transmission by blood transfusion has not yet been described; however, in light of our findings, this possibility should be considered. We previously described the presence of *B. henselae* in a RBC unit from an asymptomatic donor (30) and noted that *B. henselae* is also able to survive in stored red blood cell units for 35 days (31). Additionally, suspected iatrogenic transmission by needle pricking of *B. vinsonii* subsp. *berkhoffii* and *B. henselae* to two veterinarians who subsequently developed different clinical manifestations (32, 33) further supports the hypothesis that *Bartonella* transmission by blood transfusion or blood products is feasible.

Our findings demonstrate that *B. clarridgeiae* can be present in healthy blood donors, and may pose a risk to blood recipients, in particular immunocompromised patients receiving blood transfusions. The relevance of the genus *Bartonella* to transfusion medicine in Brazil and other countries should be further investigated.

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E.B.B. has a patent (US patent 7,115,385) for media and methods for cultivation of microorganisms (issued to E.B.B., S. Sontakke, and North

Carolina State University.) E.B.B. is the chief scientific officer for Galaxy Diagnostics, a company that provides diagnostic testing for the detection of *Bartonella* species infection in animals and human patient samples. All other authors have no conflict of interest to declare.

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