

BARTONELLA (ROCHALIMAEA) QUINTANA BACTEREMIA IN INNER-CITY PATIENTS WITH CHRONIC ALCOHOLISM

DAVID H. SPACH, M.D., ANDREW S. KANTER, M.D., M.P.H., MOLLY J. DOUGHERTY, B.S.,
ANN M. LARSON, M.S., MARIE B. COYLE, PH.D., DON J. BRENNER, PH.D., BALA SWAMINATHAN, PH.D.,
GHASSAN M. MATAR, PH.D., DAVID F. WELCH, PH.D., RICHARD K. ROOT, M.D., AND WALTER E. STAMM, M.D.

Abstract *Background.* *Bartonella (Rochalimaea) quintana* is a fastidious gram-negative bacterium known to cause trench fever, cutaneous bacillary angiomatosis, and endocarditis. Between January and June 1993 in Seattle, we isolated *B. quintana* from 34 blood cultures obtained from 10 patients not known to be infected with the human immunodeficiency virus (HIV).

Methods. After identifying the isolates as *B. quintana* by direct immunofluorescence and DNA-hybridization studies, we determined strain hybridization with studies of restriction-fragment-length polymorphisms (RFLPs) of the intergenic spacer (noncoding) region of ribosomal DNA amplified by the polymerase chain reaction (PCR). To characterize the epidemiologic and clinical features of bartonella infections in these patients, we performed a retrospective case-control study using as controls 20 patients from whom blood was obtained for culture at approximately the same time as from the index patients.

Results. *B. quintana* isolates from the 10 patients were indistinguishable by PCR-RFLP typing. All 10 patients had chronic alcoholism, and 8 were homeless ($P=0.001$ for both comparisons with controls). The six patients who underwent HIV testing were seronegative. At the time of their initial presentation, seven patients had temperatures of at least 38.5°C. Six patients had three or more blood cultures that were positive for *B. quintana*, and in four of these patients *B. quintana* was isolated from blood cultures obtained 10 or more days apart. Subacute endocarditis developed in two patients and required surgical removal of the infected aortic valve in one of them. Nine patients recovered; one died of sepsis from *Streptococcus pneumoniae* infection.

Conclusions. *B. quintana* is a cause of fever, bacteremia, and endocarditis in HIV-seronegative, homeless, inner-city patients with chronic alcoholism. (N Engl J Med 1995;332:424-8.)

BARTONELLA QUINTANA (formerly known as *Rochalimaea quintana*¹) is a fastidious gram-negative bacterium first identified as the cause of louse-borne epidemic trench fever in Europe during World War I.² *B. quintana* was subsequently recognized as the cause of trench fever in Africa and Mexico.³ Descriptions of the clinical manifestations of this disease have varied, with an insidious onset of nonspecific symptoms reported in some patients and a more sudden illness characterized by fever, malaise, headache, bone pain, and a transient macular rash reported in others.⁴ In addition, several patterns of fever have been observed, including a single episode of fever, continuous fever for five to seven days, and recurrent febrile episodes occurring every four to five days.⁴ Studies involving inoculation of humans with *B. quintana* from infected louse feces suggest that the incubation period ranges from 5 to 20 days, depending on the size of the inoculum.⁴ Once humans become infected, *B. quintana* may circulate in the blood for weeks to months or even, in some cases, for longer than a year.⁴

Until recent years, bartonella species have not been isolated from humans in the United States. Several recent studies, all involving patients infected with the human immunodeficiency virus (HIV), have shown that *B. quintana* can cause cutaneous bacillary angiomatosis, bacteremia, endocarditis, and chronic lymphadenopa-

thy.⁵⁻⁹ Thus far, however, *B. quintana* has not been reported in HIV-negative people in the United States. *B. henselae*, a closely related organism that has also recently been reported in HIV-infected patients, causes cutaneous bacillary angiomatosis, peliosis hepatis, and bacteremia.^{5,10-15} In addition, *B. henselae* has been shown to infect immunocompetent persons and cause cat scratch disease, cutaneous bacillary angiomatosis, bacillary splenitis, bacteremia, and endocarditis.¹⁶⁻²⁰

Between January and June 1993, the microbiology laboratory at the Harborview Medical Center in Seattle isolated organisms presumptively identified as bartonella species in blood cultures from 10 patients.²¹ Subsequently, we conducted a retrospective case-control study to characterize the epidemiologic and clinical features of bartonella infections in these patients. In addition, we performed studies to identify the species and to determine the strain relatedness of these bartonella isolates.

METHODS

Patients

All 10 patients were seen at Harborview Medical Center. Case patients were defined as those with bartonella species isolated from blood cultures at the hospital's microbiology laboratory from January through June 1993. For each case patient, we selected two control patients: one with blood specimens obtained for culture just before those obtained from the case patient and one with specimens obtained just after those from the case patient. Thus, there were 20 controls. Epidemiologic, clinical, and routine laboratory data were obtained from the medical records of the case and control patients.

Isolation of Bartonella in Blood Cultures

Routine blood samples from each patient were inoculated into one Bactec aerobic Plus 26 bottle (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.) and one Bactec anaerobic NR 7 bottle, as previously described.²¹ Before the blood cultures were discarded on day 8, a small aliquot of blood-culture broth was removed from the aerobic bottles for staining with acridine orange.²¹ This procedure had been routinely performed with all blood cultures since May

From the Division of Infectious Diseases and the Department of Medicine (D.H.S., R.K.R., W.E.S.) and the Department of Laboratory Medicine (M.J.D., A.M.L., M.B.C.), Harborview Medical Center and the University of Washington Medical Center, Seattle; the Department of Medicine, University of Washington Medical Center, Seattle (A.S.K.); the Emerging Bacterial and Mycotic Diseases Branch (D.J.B.) and the Foodborne and Diarrheal Diseases Branch (B.S., G.M.M.), Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, Atlanta; and the Department of Pediatrics and the Clinical Microbiology Laboratories, University of Oklahoma Health Sciences Center, Oklahoma City (D.F.W.). Address reprint requests to Dr. Spach at Madison Clinic, ZA-09, 1001 Broadway, Suite 206, Seattle, WA 98122.

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1988. When bacteria were stained with acridine orange, subcultures were performed as previously described.²¹ Isolates were identified at the genus level by biochemical testing and analysis of cellular fatty acids.²¹

Identification of Bartonella Species by Direct Immunofluorescence

The immunofluorescence assays were performed on one blood-culture isolate from each of the 10 patients, as described previously.²² In brief, murine polyclonal antibodies specific for *B. henselae* or *B. quintana* were used to distinguish the two species. The fluorescent antibody label was fluorescein-conjugated antimouse IgG (Organon Teknika, West Chester, Pa.).

DNA-Hybridization Studies

DNA relatedness was determined by comparing at least one blood-culture isolate from each of the 10 patients with type strains of all known bartonella species. The methods used to extract and purify DNA and the hydroxyapatite hybridization method for determining DNA relatedness were performed as described previously.²³ All studies were performed twice. The change in the thermal-stability midpoint of the heterologous DNA reaction was compared with that of the homologous DNA reaction. Divergence (unpaired bases in a re-associated DNA sequence), which is approximately 1 percent for each degree of decreased thermal stability, was calculated to the nearest 0.5 percent.

Studies of Restriction-Fragment–Length Polymorphisms

Restriction-fragment–length polymorphisms (RFLPs) of *B. quintana* DNA amplified by the polymerase chain reaction (PCR) were studied in one blood-culture isolate from each of the 10 patients, as previously described,²⁴ except that *DdeI* and *RsaI* were used to restrict the PCR-amplified ribosomal intergenic spacer region.

RESULTS

Isolation and Characterization of *B. quintana* Isolates

B. quintana was isolated from a total of 34 Bactec nonradiometric aerobic resin bottles containing blood samples from the 10 patients.²¹ All 34 bottles were monitored with the Bactec NR-660 detection system for the first five days of incubation, and none produced sufficient levels of carbon dioxide to indicate growth. For 9 of the 10 patients, acridine-orange staining of the Bactec medium resulted in the first evidence of bacterial growth.²¹ After the isolates had been subcultured on chocolate agar, visible growth appeared in three to eight days. Representative isolates were negative for catalase, oxidase, and urease. The following major fatty acids, expressed as the percentage of total fatty acids, were detected in isolates from each of the 10 patients: C18:1 (60 to 65 percent), C18:0 (13 to 18 percent), and C16:0 (16 to 19 percent).

The isolates from all 10 patients were identified as *B. quintana* on the basis of immunofluorescence assays with species-specific polyclonal antibodies and DNA-hybridization studies (Table 1). Repeat isolates from the four patients who had positive cultures of blood obtained 10 or more days apart were also identified as *B. quintana* (data not shown). The isolates from the 10 patients were indistinguishable from each other by PCR–RFLP typing (Fig. 1).

Demographic and Clinical Characteristics of the Case Patients and Controls

All cases of bartonella bacteremia occurred from January through June 1993 (Fig. 2). The group of 10 case patients and the group of 20 control patients did

Table 1. DNA Relatedness of Isolates of Bartonella Species from the 10 Patients.*

SOURCE OF UNLABELED DNA (STRAIN NO.)	LABELED DNA FROM		
	<i>B. QUINTANA</i> (ATCC VR358)	<i>B. HENSELAE</i> (ATCC 49882)	
	55°C	70°C	55°C
	% relatedness (% divergence)		
<i>B. quintana</i> (ATCC VR358)	100 (0.0)	100	55 (9.0)
Isolate from Patient 1 (CDC G8377)	100 (1.0)	100	55 (9.5)
Isolate from Patient 2 (CDC G8379)	100 (0.5)	99	60 (7.0)
Isolate from Patient 3 (CDC G8381)	100 (0.5)	99	56 (8.5)
Isolate from Patient 4 (CDC G8701)	100 (2.0)	99	ND
Isolate from Patient 5 (CDC G8528)	97 (1.5)	97	ND
Isolate from Patient 6 (CDC G8700)	97 (3.0)	94	ND
Isolate from Patient 7 (CDC G8380)	96 (0.5)	97	52 (8.5)
Isolate from Patient 8 (CDC G8529)	96 (2.5)	95	ND
Isolate from Patient 9 (CDC G8523)	96 (0.5)	93	50 (9.0)
Isolate from Patient 10 (CDC G8406)	96 (2.5)	93	ND
<i>B. henselae</i> (ATCC 49882)	56 (9.5)	36	100 (0.0)
<i>B. vinsonii</i> (ATCC VR142)	52 (10.5)	28	63 (8.5)
<i>B. elizabethae</i> (ATCC 49927)	45 (13.0)	21	54 (11.5)
<i>B. bacilliformis</i> (ATCC 35685)	28 (13.0)	14	35 (12.5)

*ATCC denotes American Type Culture Collection, CDC Centers for Disease Control and Prevention, and ND not done.

not differ significantly in terms of age, sex, or history of injection-drug use (Table 2). A univariate analysis showed that the patients with bartonella bacteremia were significantly more likely than the controls to be homeless (8 of 10 vs. 3 of 20, $P=0.001$), to have a history of chronic alcohol abuse (10 of 10 vs. 7 of 20, $P=0.001$), and to be nonwhite (9 of 10 vs. 7 of 20, $P=0.007$). All 10 patients with bartonella bacteremia either resided or spent most of their time in downtown Seattle. Because 8 of these 10 patients were homeless, we could not determine precisely where in downtown Seattle they spent most of their time.

Six of the 10 patients with bartonella bacteremia underwent HIV testing, and all 6 were seronegative. Information on the HIV status of the other four patients was not available, but none had risk factors for HIV infection. Of the seven control patients whose HIV status was known, two were seropositive.

Clinical Presentation of Patients with *B. quintana* Bacteremia

At the time of their initial presentation, the 10 patients with *B. quintana* bacteremia had a median temperature of 38.5°C; 7 of the 10 had a temperature of 38.5°C or higher, and 1 had hypothermia (35.5°C). Three patients reported a history of recent weight loss that exceeded 9.1 kg (20 lb). Other than fever, no consistent symptoms or abnormalities were noted on physical examination. Two patients had splenomegaly. Three patients reported a recent cat scratch, five were

presumptively diagnosed as having scabies at the time of their initial presentation, and lice were detected on one patient. The mean white-cell count was 9600 per cubic millimeter.

Six patients had three or more positive blood cultures for *B. quintana*, and four of these patients had positive blood cultures 10 or more days apart. One patient, who did not comply with antimicrobial therapy, had positive blood cultures on five separate occasions over a period of eight weeks. One patient with *B. quintana* bacteremia also had a positive blood culture for *Streptococcus pneumoniae*.

Five patients underwent echocardiographic studies, and the results were normal in four of the five. However, one patient had echocardiographic evidence of an aortic-valve vegetation.²⁵ After 21 days of antimicrobial therapy, surgical removal of the infected aortic valve was required; *B. quintana* was detected in the valve tissue by PCR techniques.²⁵

Clinical Outcome

Nine of the 10 patients with *B. quintana* bacteremia survived; 1 patient died, presumably from overwhelming sepsis associated with *S. pneumoniae* infection. Follow-up of the surviving patients was limited, mainly because they were homeless (Table 3).

Five patients were treated with ceftriaxone (given intravenously or intramuscularly) for seven days plus either oral erythromycin or oral azithromycin for at least three weeks. Three of these five patients returned for blood-culture testing after completing the antimicrobial therapy, and all the cultures were negative. The patient with endocarditis received more than four months of oral antimicrobial therapy after valve surgery and had negative blood cultures one year later. Another patient, who was lost to follow-up after an initial blood culture

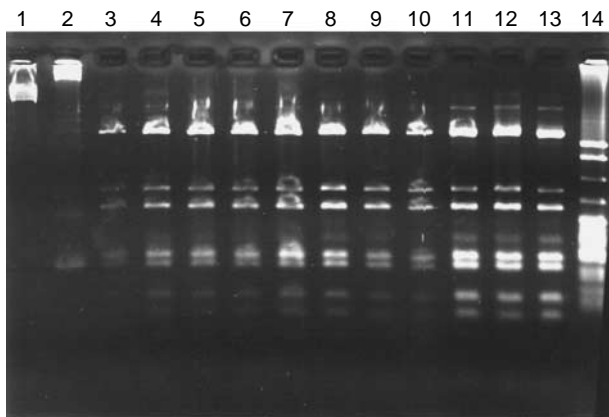


Figure 1. PCR-RFLP Analysis of the Ribosomal DNA Intergenic Spacer Region in *B. quintana* Isolates.

PCR-amplified DNA was restricted with *DdeI*, and the fragments were separated by agarose-gel electrophoresis. Lane 1 shows undigested DNA from *B. quintana* type strain ATCC (American Type Culture Collection) VR358, lane 2 a 123-base-pair ladder (molecular size marker), lanes 3 through 7 and 9 through 13 *B. quintana* isolates from the 10 study patients, lane 8 *B. quintana* type strain ATCC VR358, and lane 14 a digest of pBR322 (molecular size marker).

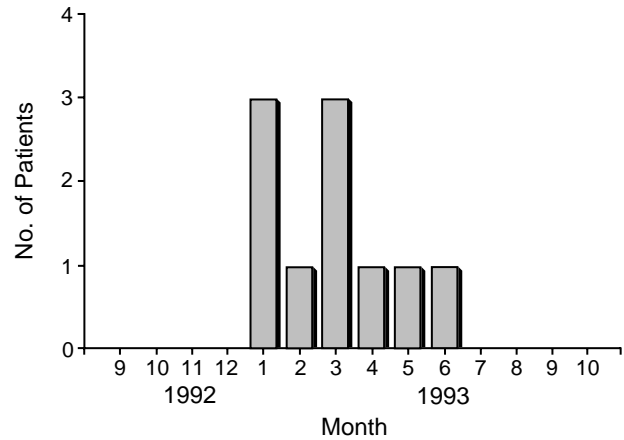


Figure 2. Number of Patients with *B. quintana* Bacteremia during a 14-Month Period at Harborview Medical Center in Seattle.

had been found to be positive for *B. quintana*, returned nine months later with fever, echocardiographic evidence of endocarditis, and multiple splenic lesions noted on computed tomography. Organisms were not reisolated from blood cultures, but blind subculturing was not performed. He did not require valve surgery.

DISCUSSION

We studied 10 patients in Seattle with bacteremia caused by *B. quintana*, the agent of trench fever. None were known to be infected with HIV, and six had a documented seronegative status. It is highly unlikely that the *B. quintana* isolates in these patients represent contamination of blood cultures, since six of the patients had three or more positive blood cultures, some of which were performed 10 or more days apart. One patient had positive blood cultures on five separate occasions over an eight-week period.

Our patients with *B. quintana* typically had a symptomatic febrile illness resembling that reported by Slater et al. in five patients with *B. henselae* bacteremia.¹⁴ In addition, the *B. quintana* bacteremia in most of our patients was clinically similar to previous descriptions of trench fever — a disease also caused by *B. quintana*.^{2,4} Some of the clinical manifestations of trench fever that have occasionally been observed, such as rash, headache, and bone pain, were not observed in any of our patients.

Because the follow-up of our patients was limited, clear treatment recommendations cannot be given. Although the optimal type and duration of therapy for this and other bartonella infections are not clear, such infections appear to have a tendency to persist or recur,^{14,19,26,27} especially if antimicrobial therapy is given for less than 14 days. Indeed, the one patient in our study who did not comply with antimicrobial therapy had persistent (or relapsing) bacteremia until he finally completed a course of azithromycin. Careful follow-up to detect relapsing infection is essential.

Although the retrospective nature of our investigation did not permit us to obtain definitive information about how these 10 patients became infected with

Table 2. Demographic and Clinical Characteristics of the Case Patients with *B. quintana* Bacteremia and the Controls.

CHARACTERISTIC	CASE PATIENTS (N = 10)	CONTROLS (N = 20)	P VALUE*
Median age — yr	38.4	37.8	NS
Sex — no. of patients (%)			NS
Male	9 (90)	16 (80)	
Female	1 (10)	4 (20)	
Race or ethnic group — no. of patients (%)			0.007†
White	1 (10)	13 (65)	
Native American	5 (50)	0	
Black	2 (20)	6 (30)	
Hispanic	2 (20)	0	
Other	0	1 (5)	
Homeless — no. of patients (%)	8 (80)	3 (15)	0.001
Chronic alcohol abuse — no. of patients (%)	10 (100)	7 (35)	0.001
Injection-drug use — no. of patients (%)	4 (40)	3 (15)	NS

*P values were calculated with Fisher's exact test. NS denotes not significant.

†For the comparison between white and nonwhite patients.

B. quintana, there are several possibilities. In classic cases of trench fever, *B. quintana* is transmitted by lice among persons in close contact with one another under poor sanitary conditions, most notably the crowded trenches in World Wars I and II.^{2,4} The *B. quintana* infections we describe occurred predominantly among homeless people with chronic alcoholism — conditions that may have resulted in close contact and poor hygiene. Although these conditions may have led to infestation with lice, only one patient was actually observed to have lice at the time of his presentation to the hospital.

Koehler and colleagues have recently shown that the domestic cat serves as a reservoir for *B. henselae*; in addition, they found *B. henselae* in fleas from an infected cat.²⁸ Three of our patients reported a recent cat scratch, but none reported flea bites. Five of our patients had scabies infestation. The scabies mite, *Sarcoptes scabiei*, although it has never been shown to harbor either *B. quintana* or *B. henselae*, theoretically could have transmitted *B. quintana* to these patients. Further study is clearly warranted to determine whether one or more of the above-mentioned vectors transmit *B. quintana*.

There are several possible explanations for this sudden cluster of 10 cases of *B. quintana* bacteremia. Increased detection of infection could have occurred as a result of our laboratory's use of acridine-orange staining and rigorous subculturing

of presumptively positive isolates. The microbiology laboratory, however, had routinely performed acridine-orange staining on all blood cultures for approximately five years before January 1993 without identifying other cases of bartonella bacteremia. In addition, during the 12-month period after these 10 cases of infection were diagnosed, we isolated *B. quintana* from only 1 patient, despite use of the same blood-culture techniques and vigilant surveillance. Thus, it seems unlikely that increased laboratory detection of *B. quintana* could be the sole explanation for the sudden occurrence of these 10 cases.

The finding that the isolates were indistinguishable from one another by PCR-RFLP analysis suggests that these cases are probably epidemiologically linked. Four PCR-RFLP patterns have previously been observed among 18 isolates of *B. quintana* from the San Francisco area (Koehler JE: personal communication). Our PCR-RFLP studies, however, do not clarify the mode of transmission of *B. quintana* in our patients. The outbreak could have occurred as a result of close contact among the patients, one of whom served as the initial source of infection. Because most of the patients were homeless, it is possible that they came into contact with each other on the street or at shelters. We do not, however, have epidemiologic evidence to validate this hypothesis. Whether *B. quintana* is endemic in our region or was imported is also not clear.

In summary, we have identified and characterized a cluster of 10 cases of symptomatic *B. quintana* bac-

Table 3. Antimicrobial Therapy and Outcome in the 10 Patients with *B. quintana* Bacteremia.

PATIENT No.	THERAPY	OUTCOME
1	Vancomycin (1 g intravenously every 12 hr) for 5 days	No follow-up
2	Cefazolin (1 g intravenously every 6 hr) for 1 day, then ampicillin-sulbactam (3 g intravenously every 6 hr) for 5 days, then ceftriaxone (2 g intravenously once a day) for 7 days, then azithromycin (500 mg orally once a day) for 28 days	Negative blood cultures at 2 and 4 months
3	Ceftriaxone (1 g intravenously once a day) for 7 days, then azithromycin (500 mg orally once a day) for 28 days	Negative blood cultures at 1 and 4 weeks
4	Ceftriaxone (2 g intravenously once a day) plus erythromycin (500 mg orally four times a day) for 7 days, then azithromycin (500 mg orally once a day) for 49 days	No follow-up
5	Cefotetan (1 g intravenously every 12 hr) plus gentamicin (65 mg intravenously every 8 hr) for 1 day, then ampicillin-sulbactam (3 g intravenously every 6 hr) for 13 days	Died of pneumococcal sepsis
6	Nafcillin (2 g intravenously every 4 hr) plus gentamicin (120 mg intravenously every 8 hr) for 3 days, then ampicillin (1 g intravenously every 6 hr) plus gentamicin (120 mg once a day) for 9 days, then ampicillin (1 g intravenously every 6 hr) for 9 days, then erythromycin (250 mg orally every 6 hr) for 42 days, then azithromycin (500 mg orally once a day) for 90 days	Valve resected, clinically well with negative blood cultures 1 year later
7	Penicillin (2.2 × 10 ⁶ U intravenously every 4 hr) plus cefazolin (1 g intravenously every 6 hr) for 5 days	No follow-up
8	Ceftriaxone (1 g intramuscularly every day) for 7 days, then erythromycin (500 mg orally four times a day) for 42 days	No follow-up
9	Nafcillin (2 g intravenously every 4 hr) plus gentamicin (120 mg intravenously every 8 hr) for 1 day, then clindamycin (900 mg intravenously every 8 hr) plus vancomycin (1 g intravenously every 12 hr) for 2 days, then ceftriaxone (2 g intravenously once a day) for 7 days, then azithromycin (500 mg orally once a day) for 15 days, then azithromycin (500 mg orally once a day) for 17 days	Positive blood cultures on five occasions during first 8 weeks, followed by negative cultures at 3, 5, and 6 months
10	Amoxicillin (500 mg orally three times a day) for 10 days	No follow-up for 9 months, then returned with endocarditis and negative blood cultures

teremia in Seattle. As compared with the controls, the infected patients were more likely to be homeless, alcoholic, and nonwhite. Because of the small sample size, a stratified or logistic-regression analysis of these characteristics was not possible. Further studies are needed to determine the prevalence, source, mode of transmission, and optimal management of *B. quintana* infections in populations with demographic and clinical characteristics similar to those of our 10 patients.

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