

BARTONELLA (ROCHALIMAEA) QUINTANA ENDOCARDITIS IN THREE HOMELESS MEN

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Abstract Background. *Bartonella (Rochalimaea) quintana* is the agent of trench fever and is transmitted by the body louse. We searched for this organism in three alcoholic homeless men with endocarditis.

Methods. Blood samples were cultured on a human endothelial cell line and on blood agar. Bacteria were identified by sequencing the amplified 16S ribosomal RNA gene. The presence of bartonella in tissue was assessed by Gram's staining, immunostaining, and polymerase-chain-reaction amplification. Serologic studies for antibodies to bartonella species were performed by indirect immunofluorescence and Western immunoblotting.

Results. *B. quintana* was isolated from one patient in the blood-agar culture and from the other two patients in

the endothelial-cell culture. The organism was also identified by both immunostaining and molecular techniques in the valvular vegetations from the three patients and in a cervical lymph node from one patient. The 16S ribosomal RNA gene sequences of the three isolates were almost identical to that of the prototype strain of *B. quintana*. High titers of antibodies to *B. quintana* were detected in all three patients, but so were cross-reacting antibodies to chlamydia species. In all three patients studies were repeatedly negative for antibodies to the human immunodeficiency virus.

Conclusions. *B. quintana* is a cause of endocarditis in homeless patients and may be serologically misdiagnosed as a chlamydial infection. (N Engl J Med 1995;332:419-23.)

BARTONELLA QUINTANA, the agent of trench fever, affected about 1 million soldiers during World War I and a smaller number during World War II. Sporadic reports have suggested that infection with this agent, which is transmitted by the body louse, is not restricted to wartime.¹ Over the past few years, *B. quintana* (formerly known as *Rochalimaea quintana*) has been reported in North America in 5 patients infected with the human immunodeficiency virus (HIV),^{2,3} including 1 with endocarditis,⁴ and in 10 HIV-negative patients, including 1 with subacute endocarditis⁵ (and Spach DH: personal communication). We recently described two additional strains isolated from blood specimens obtained from patients with bacillary angiomatosis⁶ and one from a patient with chronic adenopathy.⁷ We now report on three homeless men with *B. quintana* endocarditis.

CASE REPORTS

Patient 1

Patient 1 was a 47-year-old man who presented with fatigue, fever, a weight loss of 12 kg, and purpura of the lower limbs. His medical history included a heart murmur and chronic alcoholism. He had been homeless for one year and had been in contact with a dog but reported no dog bites or scratches. The weight loss, fatigue, and fever had started six months before admission.

At the time of admission, physical examination revealed a temperature of 38.5°C, bilateral epistaxis, and purpura of the lower limbs. The pulse was 110 beats per minute and the blood pressure was 105/60 mm Hg, with a grade 4/6 systolic murmur of mitral regurgitation and a gallop rhythm. An echocardiogram showed severe mitral regurgitation, perforation of the mitral valve with ruptured chordae, and an echogenic mass on the mitral valve. There was also an echogenic mass on the posterior cusp of the aortic valve and moderate aortic regurgitation.

Pertinent laboratory data are presented in Table 1. Eight sets of

blood cultures were performed. Enzyme-linked immunosorbent assays and Western blot immunoassays for HIV-1 antibody, performed at the time of admission and repeated two months later, were negative.

Therapy was begun with a continuous intravenous infusion of amoxicillin (12 g per day) and gentamicin (180 mg per day). The mitral insufficiency became more marked, and on day 4 the patient underwent mitral-valve replacement with a Starr prosthesis. Pathological examination of the excised valve disclosed fibrin vegetation. Numerous minute coccobacillary organisms were observed after Gram's staining, but the culture of tissue from the valve remained sterile.

The same antibiotic treatment was continued for six weeks. The patient reported dysphonia, and an enlarged cervical lymph node was excised for diagnostic purposes. Five of the eight blood cultures grew a gram-negative bacillus subsequently identified as *B. quintana*, and the patient's serum had high titers of antibody to *B. quintana* (Table 2). The patient remained well 10 months after surgery.

Patient 2

Patient 2 was a 41-year-old man who presented with a temperature of 38.5°C and an erythematous lesion of the right foot. He was homeless and lived in close contact with two dogs, a cat, kittens, and a goat. He had a history of alcoholism and cigarette smoking. Aortic insufficiency was diagnosed clinically at the time of admission, and echocardiography revealed abnormal aortic cusps with one vegetation and dilatation of the left ventricle. The cutaneous lesions were clinically diagnosed as septic emboli but were not examined by biopsy. Blood cultures performed according to routine procedures were negative.

The patient was treated for three weeks with intravenous vancomycin combined with ofloxacin and netilmicin, followed by oral rifampin plus ofloxacin and pristinamycin for three additional weeks. The patient, then afebrile, was admitted to the Hôpital Louis Pradel (Lyons, France) for aortic-valve replacement. Pertinent laboratory data are shown in Table 1. Enzyme-linked immunosorbent assays and Western blot immunoassays, performed at the time of surgery and repeated three months later, were negative for HIV-1 antibody. Examination of the aortic valve at the time of surgery revealed numerous perforations of the cusps and multiple vegetations of the aortic ring. The valve was excised, and a mechanical prosthetic valve was implanted. Histologic examination of the valve showed vegetations consisting of fibrin and platelets. The patient received intravenous antibiotic treatment with vancomycin plus ofloxacin for two weeks after surgery, followed by doxycycline for four weeks. He was well six months after surgery.

Patient 3

Patient 3 was a 43-year-old man who presented with congestive heart failure and fever. He was a former soldier in the French For-

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Table 1. Demographic, Clinical, and Serologic Characteristics of Three Men with *B. quintana* Endocarditis.

CHARACTERISTIC	PATIENT 1	PATIENT 2	PATIENT 3
Age (yr)	47	41	43
Homeless	Yes	Yes	Yes
History of alcoholism	Yes	Yes	Yes
Contact with a cat	No	Yes	No
Contact with a dog	Yes	Yes	No
History of valvulopathy	Yes	No	No
Involved valve	Mitral	Aortic	Aortic and mitral
Hemoglobin (g/dl)	9.2	8.1	9.1
Leukocytes (per mm ³)	4,900	13,000	7,000
Polymorphonuclear cells (per mm ³)	2,100	9,500	4,300
Platelets (per mm ³)	15,000	250,000	52,000
Erythrocyte sedimentation rate (mm/hr)	100	100	32
Bilirubin (mg/dl)*	Normal	Normal	1.7
Aspartate aminotransferase (U/liter)	Normal	Normal	51
Alanine aminotransferase (U/liter)	Normal	Normal	150
Lactate dehydrogenase (U/liter)	Normal	Normal	895
Blood culture			
On agar	Positive	Negative	Negative
On human endothelial cell line	Not done	Positive	Positive
Valve culture†			
On agar	Negative	Negative	Negative
On human endothelial cell line	Negative	Negative	Negative
Immunodetection on valve	Positive	Positive	Positive
Genomic amplification on valve	Positive	Positive	Positive

*To convert values for bilirubin to micromoles per liter, multiply by 17.1.

†Performed after antibiotic therapy.

eign Legion, had been back in France for 10 years, and was living in poor sanitary conditions in a seedy hotel close to the harbor in Marseilles. He had no contact with animals. He was an alcoholic and a cigarette smoker. His medical history was unremarkable with the exception of an episode of undiagnosed microscopic hematuria in 1983. Progressive dyspnea and cough developed one month before his hospitalization.

Clinical examination at the time of admission disclosed a 15-kg weight loss, a rectal temperature of 38.5°C, jaundice, cough, peripheral edema, hepatomegaly, splenomegaly, and loud systolic aortic and mitral murmurs. A chest radiograph showed marked cardiomegaly and a diffuse interstitial infiltrate. Pertinent laboratory results are shown in Table 1. Enzyme-linked immunosorbent assays and Western-blot tests for the presence of HIV-1 antibodies, performed at the time of admission and repeated 40 days later, were negative. Six blood cultures were performed, and empirical treatment with intravenous ceftazidime (3 g per day) plus ofloxacin (400 mg per day) was prescribed. Transthoracic echocardiography disclosed an aortic vegetation and a left ventricular ejection fraction of 30 percent. A diagnosis of infectious endocarditis with severe cardiac failure was made.

Table 2. IgG Antibodies against *B. quintana* Grown on 5 Percent Sheep-Blood Agar (Agar) and Human Endothelial Cells (Cells), *B. henselae*, *C. pneumoniae*, *C. psittaci*, and *C. trachomatis* in the Three Patients with *B. quintana* Endocarditis.*

ANTIGEN	PATIENT 1	PATIENT 2	PATIENT 3
<i>B. quintana</i>			
Agar	400	800	800
Cells	12,800	12,800	6400
<i>B. henselae</i>	0	100	0
<i>C. pneumoniae</i>	512	256	256
<i>C. psittaci</i>	64	64	32
<i>C. trachomatis</i>	64	64	32

*Data are expressed as the reciprocals of the antibody titers at the time of admission.

The fever resolved after two days of antibiotic therapy. Netilmicin (300 mg per day) was added to the regimen when infection with *B. quintana* was suspected.

The aortic and mitral valves were excised five weeks after admission and replaced by a Carpentier-type bioprosthesis and a Hancock II-type bioprosthesis, respectively. Antibiotic therapy was continued for three weeks. Vegetations were observed on both valves, and microscopical examination confirmed the presence of infectious endocarditis. All microbiologic studies were negative, with the exception of serologic tests for chlamydia and bartonella species (Table 2). The patient died four months after surgery; no autopsy was performed.

METHODS

Bacterial Strains

The following bacterial strains were obtained from the American Type Culture Collection (ATCC, Rockville, Md.): *B. quintana* (Fuller strain, ATCC VR358), *B. henselae* (Houston, ATCC 49882), *Chlamydia pneumoniae* (ATCC VR1310), *C. psittaci* (ATCC VR601), and *C. trachomatis* (ATCC VR878). *B. quintana*, Oklahoma strain, was provided by D.F. Welch; *B. quintana*, Toulouse strain, is a local isolate.⁶

Isolation Procedures

The blood samples, obtained in Bactec blood-culture bottles, and the excised cardiac valves were plated on 5 percent sheep-blood agar incubated at 37°C in an atmosphere of 5 percent carbon dioxide and on human endothelial-cell-line ECV 304,³ cultivated as previously described.³

Identification of the Isolates

For the antigenic characterization, the isolates were allowed to react with serial dilutions of polyclonal mouse anti-*B. quintana* serum and with a monoclonal antibody specific for *B. henselae*, with the use of an indirect immunofluorescence technique described previously.⁶ Analysis of cell-wall fatty acids was carried out as previously reported⁹ in the first isolate only. Restriction-fragment-length polymorphisms (RFLPs) were analyzed in a portion of the citrate-synthase gene amplified by the polymerase chain reaction (PCR).¹⁰ 16S ribosomal RNA (rRNA) gene sequencing was performed on amplified products with the use of the direct, solid-phase method.¹¹ For PCR amplifications, quick DNA extractions were performed with bacteria grown in agar (the first isolate) or cell culture (the second and third isolates), with the use of the Chelex 100 method.¹² A fragment of the 16S rRNA gene (approximately 1400 base pairs [bp]) was amplified by PCR with the fD1 primer described by Weisburg et al.,¹³ with 5' biotin labeling and primer roc4 (5' CACCCAGTCTGACCCTA3'). After immobilization of the biotinylated amplicon with streptavidin-coupled magnetic beads (Dynabeads M-280, Dynal, Oslo, Norway) and alkaline denaturation, the immobilized products were sequenced with an automatic sequencer (ALF DNA sequencer, Pharmacia Biotech, Uppsala, Sweden) and the Autored sequencing kit supplied by the same manufacturer. 5'-Fluorescein-labeled primers roc1 (5' AGGCACGAAGTTAGCCGGGGC3'), roc2 (5' ATCGTTTACGGCGTGGACTAC3'), roc3 (5' GAGGGTTGCGCTCGTTGCGGG3'), and roc4 were used for sequencing the direct DNA strand. For each isolate, the sequence was determined four times. The sequences for the isolates were compared with those of *B. quintana*, *B. henselae*, *B. elizabethae*, and *B. vinsonii* in GenBank through Bisance,¹⁴ with the use of the Clustal package.¹⁵ The Fuller and Oklahoma strains of *B. quintana* were used as controls.

Detection of *B. quintana* in Tissues

A formalin-fixed, paraffin-embedded cervical lymph node from Patient 1 and formalin-fixed, paraffin-embedded cardiac valves from all three patients were examined after Gram's and Giménez staining.¹⁶ Immunofluorescent staining was performed with mouse polyclonal anti-*B. quintana* serum. The immunohistochemical analysis was a modification of that described by Dumler et al.¹⁷ Alkaline phosphatase and fast red were used to stain the tissue specimens after incubation with mouse polyclonal anti-*B. quintana* serum. PCR ampli-

fication of a portion of the bartonella citrate-synthase gene was also used to detect these organisms in the tissue specimens.¹⁰

Serologic Studies

Serum samples were reacted with two *B. quintana*, Oklahoma strain, antigen preparations. In one preparation, the bacteria were grown on 5 percent sheep-blood agar and resuspended in formaldehyde. In the other preparation, the bacteria were propagated on the ECV 304 cell line, purified by low-speed centrifugation of the cell-culture supernatant, and resuspended in formaldehyde. Antibodies were detected by a microimmunofluorescence technique.⁶ A Western blot immunoassay was performed as previously reported,⁶ with bacteria grown on 5 percent sheep-blood agar used as antigens, both boiled and not boiled. Mouse polyclonal anti-*B. quintana* serum was used as a positive control. The presence of IgG, IgA, and IgM antibodies against *C. pneumoniae*, *C. trachomatis*, and *C. psittaci* was determined by microimmunofluorescence. To detect cross-reactivity, one serum sample from Patient 1 was adsorbed with *C. pneumoniae* or blood-agar-grown *B. quintana*, Oklahoma strain, and tested against *B. quintana* and *C. pneumoniae*.

RESULTS

Isolation of Bacteria

The first strain, isolated from a blood sample from Patient 1, was grown on sheep-blood agar after 27 days of incubation and was subsequently subcultured on sheep-blood agar and on endothelial cells (Table 1). The second and third isolates, obtained from blood samples from Patients 2 and 3, were grown only on endothelial cells after 20 and 15 days of incubation, respectively. Subsequent attempts to culture these organisms on sheep-blood agar were unsuccessful. Cultures of the excised valves remained sterile.

Identification of the Isolates

After Giménez staining, the three isolates appeared as thin bacilli or coccobacilli. They reacted with mouse polyclonal antibody against *B. quintana* but not with a monoclonal antibody directed against *B. henselae*. The fatty-acid composition of the cell wall was comparable to that previously reported for bartonella species.¹⁸ Insufficient material was available for phenotypic characterization of the second and third isolates. For all three isolates, PCR-RFLP analysis of the citrate synthase amplicons resulted in profiles identical to those reported for *B. quintana*.¹⁰ For each isolate, a total of 1300 nucleotide positions of the 16S rRNA gene were determined, representing 92 percent of the gene in *B. quintana*. In this overlapping region of 1300 nucleotides in the sequences for *B. henselae*, *B. quintana*, and the three clinical isolates, no difference was found between *B. quintana* and the three isolates at the 16 positions that differentiate *B. quintana* from *B. henselae*. However, one discrepancy was noted between the sequence in the Fuller strain of *B. quintana* from GenBank and the sequences in the three isolates and our Fuller and Oklahoma strains: a thymidine residue at position 1150 (*Escherichia coli* standard numbering system) appeared as a guanosine residue in the GenBank sequence.

Detection of *B. quintana* in Tissue

Immunofluorescence and immunohistochemical techniques demonstrated the presence of *B. quintana* in the

cardiac valves from the three patients (Fig. 1) and in the cervical lymph node from Patient 1. The first isolate was also demonstrated by Gram's staining of the valve. Immunohistochemical techniques gave the best results. Also, PCR amplification of a portion of the citrate synthase gene was positive in the cardiac valves and in the lymph node.

Serologic Studies

Five serum samples from Patient 1, four from Patient 2, and five from Patient 3 were tested for IgG, IgA, and IgM antibodies. All the samples had IgG titers $\geq 1:400$ against agar-grown *B. quintana* antigen and IgG titers $\geq 1:1600$ against endothelial-cell-grown antigen, with almost no detectable IgM or IgA (Table 2). Only one sample had anti-*B. henselae* antibody, but all the samples had IgG titers $\geq 1:256$ against *C. pneumoniae* and IgG titers $\leq 1:64$ against *C. psittaci* and *C. trachomatis*. Adsorption of the serum samples with *C. pneumoniae* antigen did not alter the antibody titer against *B. quintana*, whereas adsorption with agar-grown *B. quintana* antigen eliminated antibody against *C. pneumoniae*. When analyzed by Western immunoblotting, the samples from the three patients had a slight reaction against *B. quintana* crude antigen, whereas they exhibited numerous bands against boiled *B. quintana* antigen (Fig. 2). There was a cross-reaction with *C. pneumoniae*, which decreased after adsorption of the serum with *B. quintana* antigen, with the persistence of one band (Fig. 3). Proteinase K digestion of the antigens suppressed their reactivity.

DISCUSSION

We report endocarditis due to *B. quintana* in three men who were not infected with HIV. All three were about 40 years old, had histories of chronic alcoholism and smoking, and were homeless.

Three isolates of *B. quintana* were identified on the basis of their antigenicity, RFLP analysis of the citrate synthase gene, the 16S rRNA gene sequence,¹⁹ and the cell-wall fatty-acid composition in one isolate. The se-

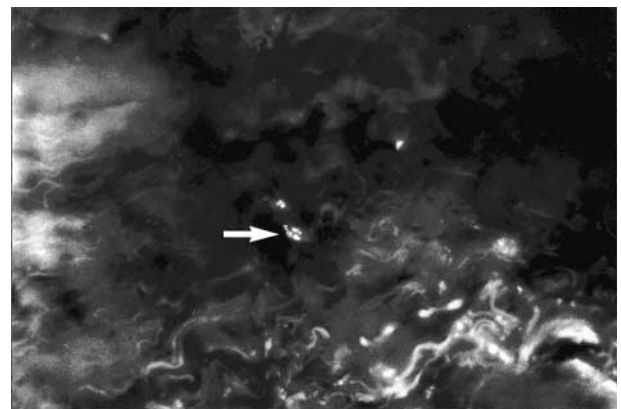


Figure 1. Immunofluorescence Staining of *B. quintana* in a Cardiac Valve from Patient 1 ($\times 2000$).

The pathogens appear as bright bacilli (arrow) against the dark field of the valve tissue.

quence of the 16S rRNA gene was determined with the use of rapid DNA preparation and solid-phase, automatic DNA sequencing. Solid-phase, manual sequencing has previously been used to identify *B. elizabethae*.¹⁸ Analysis of the 16S rRNA sequence allowed us to identify the three isolates as *B. quintana*, although a discrepancy in one base was noted between our sequence and that of GenBank. Also, differences in isolation were noted: whereas one strain was isolated in the blood-agar culture, the other two were isolated only in cell culture and could not be subcultured on other mediums. Such differences in the ease of isolation have previously been noted for *B. quintana*.³ Isolation from the cardiac valves was unsuccessful, despite immunologic and molecular detection of *B. quintana* in these specimens, but there had been antibiotic treatment before excision of the valves.²⁰

Knowledge of the clinical spectrum of human pathogens belonging to the bacterial genus bartonella (previously known as rochalimaea) has expanded dramatically over the past three years. Earlier, *B. quintana*, the agent of trench fever,²¹ and *B. vinsonii* were the only known members of the genus, and the latter was not associated with disease in humans.²² A third species, *B. henselae*, was subsequently identified in immunocompetent and immunocompromised patients, including HIV-infected patients with bacillary angiomatosis, visceral bacillary peliosis, relapsing fever with bacteremia, encephalitis, and endocarditis.^{2,10,23} More recently, *B. elizabethae* was isolated from an immunocompetent patient with endocarditis.¹⁸ A reclassification of these four species in the genus bartonella was proposed on the basis of their phenotypic and genotypic characteristics.²⁴

The body louse, *Pediculus humanus*, has been recognized as the vector of *B. quintana* causing trench fever.

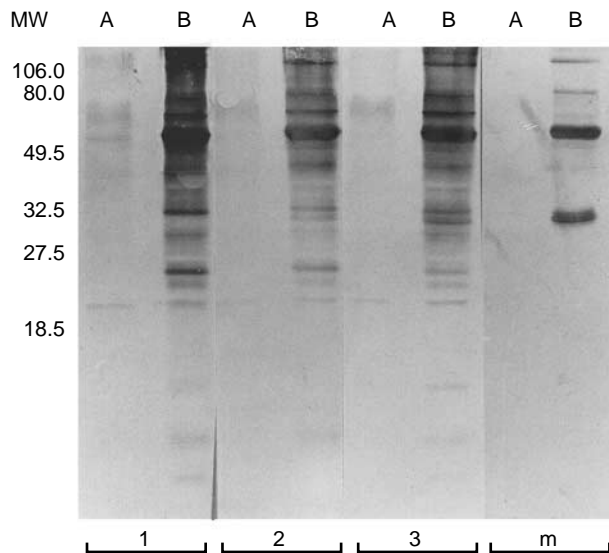


Figure 2. Western Blot Immunoassay of Serum Samples from Three Patients with *B. quintana* Endocarditis.

Lane 1 shows the results for Patient 1, lane 2 for Patient 2, lane 3 for Patient 3, and lane m for mouse anti-*B. quintana* serum (positive control). MW denotes molecular weight, A crude *B. quintana* antigen, and B boiled *B. quintana* antigen.

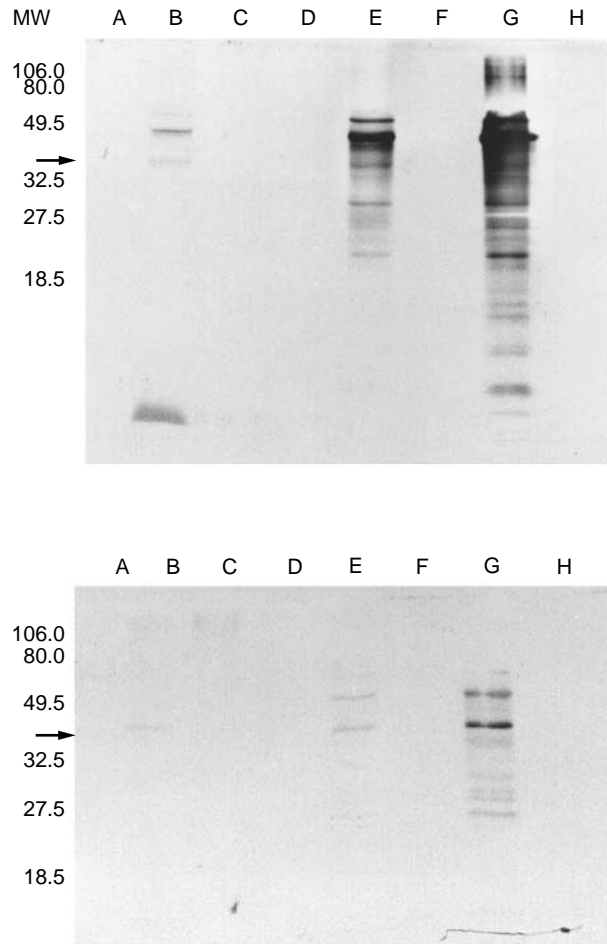


Figure 3. Western Blot Immunoassay with the Fourth Serum Sample from Patient 1.

The upper panel shows the results without adsorption, and the lower panel the results after adsorption with agar-grown *B. quintana*. Lane A shows HeLa cells (control), lane B *C. pneumoniae* antigen, lane C proteinase K-digested *C. pneumoniae* antigen, lane D endothelial-cell-line ECV 304 (control), lane E ECV 304-grown *B. quintana* antigen, lane F ECV 304-grown *B. quintana* antigen digested with proteinase K, lane G boiled, agar-grown *B. quintana* antigen, and lane H agar-grown *B. quintana* antigen digested with proteinase K. MW denotes molecular weight. The arrow in each panel indicates the band that may provide evidence of cross-reactivity between *B. quintana* infection and *C. pneumoniae* infection.

Although the American HIV-infected patients with *B. quintana* infection reported no contact with animals or arthropods, one of them had been treated for scabies eight months before the isolation of *B. quintana*,³ suggesting poor hygienic conditions. We isolated *B. quintana*, Toulouse strain, from a homeless patient with the acquired immunodeficiency syndrome and cutaneous bacillary angiomatosis.⁶ This suggests that ectoparasites, which are frequently associated with poor hygienic conditions, may be the vector of *B. quintana*. However, we also isolated two strains from patients who were not homeless.⁷

Several serologic methods have been proposed for the diagnosis of trench fever,²⁵⁻²⁷ and serotyping of bartonella species in mice has been reported.²⁸ The sero-

logic studies in our three patients were highly suggestive of infection due to *B. quintana*. Of 12,355 serum specimens tested in our laboratory for the presence of *B. quintana* antibodies over the past 17 months, 70 (0.57 percent) had IgG titers $\geq 1:100$, and 31 serum samples (0.25 percent) from 11 patients, including those described here, had IgG titers $\geq 1:400$. Thus, with the use of immunofluorescence and blood-agar-grown antigen, a cutoff value of 1:100 for the IgG titer allows detection of *B. quintana* infection. All tested samples had higher IgG titers when tested against endothelial-cell-grown antigen, which may be related to the endothelial-cell-associated expression of one or several antigens by *B. quintana*.

Our patients had substantial titers of IgG antibodies against *C. pneumoniae*, which may have been related either to dual infection with chlamydia species and *B. quintana* or to a serologic cross-reactivity between chlamydia species and *B. quintana*. To the best of our knowledge, there was no common source of exposure to chlamydia species and *B. quintana*, and there was no evidence of chlamydial infection in these patients, apart from the antibody titers. Therefore, the most likely explanation for the high titers of IgG antibodies against *C. pneumoniae* is cross-reactivity between *B. quintana* and chlamydia species. After adsorption of the serum samples by *B. quintana* antigen, the antibodies against *C. pneumoniae* disappeared, confirming a cross-reaction. A similar cross-reaction reported between *B. bacilliformis* and *C. psittaci* was attributed to a lipopolysaccharide epitope.²⁹

This cross-reactivity may be confusing, since both chlamydia and bartonella species are implicated in infective endocarditis.^{30,31} Serologic studies can support a presumptive diagnosis of endocarditis caused by bartonella or chlamydia species, but a definitive diagnosis requires demonstration of the microorganism in blood or a cardiac valve. We reviewed a series of 10 cases of endocarditis associated with chlamydia species.³¹ Serum samples from nine of these patients were tested for *B. quintana*, and all had high level of antibodies.

The studies in the three patients described here add to the evidence that bartonella species, including *B. quintana*, *B. henselae*, and *B. elizabethae*, are etiologic agents of endocarditis. These organisms should be included in the differential diagnosis of culture-negative endocarditis.

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