

Isolation and Characterization of *Bartonella quintana* from the Parotid Gland of an Immunocompetent Man[∇]

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We describe a case of the isolation of *Bartonella quintana* from the parotid gland of an apparently healthy man. Pathological examination showed intraparotid granulomatous abscessual lymphadenitis. Diagnosis was made on the basis of high titers of immunoglobulin G (IgG) and IgM antibodies and of culture isolation of a causative agent from parotid aspirate.

CASE REPORT

An apparently healthy 58-year-old man presented in April 2001 to the emergency room of our Dipartimento di Scienze Otorinolaringoiatriche of the University of Palermo with a swollen, indolent mass in the right retromandibular region that had appeared 3 days earlier. He did not report other relevant clinical data and had no cats or other pets at home.

His general condition was satisfactory, and the patient was afebrile. Clinical investigation revealed a palpable, not pulsating, indolent mass (approximately 3 by 2 cm) behind the mandibular angle in the region of the right parotid, not adhering with the overlying erythematous skin. The cervical lymph nodes were enlarged only on the right side. The physical examinations of the other systems were essentially normal. In addition, 2 days after admission, the patient reported the appearance of a little (0.4- by 0.4-cm), painful phlogistic ulceration on the skin of the right cheek.

For differential diagnosis, we considered infectious causes, collagen vascular diseases, sarcoidosis, neoplasms, sialolithiasis, alcoholism, and congenital masses. Particularly, we considered viral (i.e., cytomegalovirus, Epstein-Barr virus, and human immunodeficiency virus infection), bacterial (*Brucella*), tuberculosis, and protozoan (*Leishmania infantum* and *Toxoplasma gondii*) etiology, using both in vivo (i.e., Mantoux test) and in vitro (i.e., serologic assays) tests and direct examination of parotid mass aspirate (see below).

Table 1 shows the results of the routine hematochemistry and serologic assays performed during hospitalization. Ultrasonography and computed tomography of the neck showed a mass (3.5 by 3 cm), attributable to the right parotid gland, with swelling of the intraparotid lymph nodes. Chest X ray, thoracic echocardiography, and ultrasonography of the abdomen were normal.

Aspirate from the right parotid mass showed an inflammatory infiltration of lymphocytes in different phases of follicular nodulation together with polymorphonuclear leukocyte and

epithelioid histiocyte populations—pathological findings that are compatible with the diagnosis of intraparotid granulomatous abscessual lymphadenitis. The approximate relative percentages of cells in the parotid aspirate were as follows: lymphocytes, 30%; polymorphonuclear leukocytes, 30%; and epithelioid histiocytes, 40%.

The serum sample was examined by the indirect fluorescent antibody test for the presence of immunoglobulin G (IgG) and IgM class antibodies to *Bartonella quintana* and *Bartonella henselae*. For antigens, we used either our prepared in-house slides or commercial slides (Focus Technologies, Cypress, California, distributed in Italy by Alifax). We prepared the in-house slides by using *B. henselae* strain Houston-1 and *B. quintana* strain Oklahoma as antigens, obtained from the collection of the National Reference Center of Rickettsiosis, Marseille, France, and grown in Vero cells for 2 weeks. An IgG titer of $\geq 1:64$ and IgM titer of $\geq 1:20$ were used as cutoffs (18).

The final diagnosis of *B. quintana* infection was first suggested by the finding of high titers of IgG and IgM antibodies for this agent, 1:256 and 1:40, respectively (serology for *B. henselae* was negative), and then was confirmed by the isolation and characterization of *B. quintana* from the parotid aspirate.

In particular, the isolation of *B. quintana* was performed on Columbia 5% sheep blood agar plates (BioMerieux, Marcy-l'Etoile, France). Inoculated plates were incubated at 37°C under 5% CO₂ for 30 to 40 days and controlled weekly for evidence of bacterial growth. Four weeks after some *Bartonella*-like colonies became visible, and 2 weeks after that, they were inoculated in a Vero cell monolayer and incubated at 37°C under 5% CO₂ for 2 weeks. Then, infected Vero cells were examined by the indirect fluorescent antibody test, using human *B. henselae* and *B. quintana* hyperimmune serum from our collection, and *B. quintana*, the only demonstrated microorganism, was characterized by using PCR as previously reported (17). The amplicon size was 619 bp, and the sequence was 100% identical to that of the *B. quintana* intergenic transcribed sequence (GenBank accession no. 897700).

Meanwhile, antibiotics (clarithromycin, 500 mg orally twice a day [b.i.d.], and ceftazidime, 1 g intramuscularly b.i.d.), together with nonsteroidal anti-inflammatory drugs

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TABLE 1. Laboratory findings

Laboratory testing	Result	Normal range or result
Red blood cell count	5,440,000 cells/ μ l	4,000,000–6,000,000 cells/ μ l
Hemoglobin	15.2 g/dl	12–17 g/dl
Hematocrit	46.3%	40.7–50.3%
Mean corpuscular volume	83.6 μ m ³	80–99 μ m ³
Mean corpuscular hemoglobin	27.4 pg/cell	26–38 pg/cell
Platelet count	319,000 cells/ μ l	150,000–450,000 cells/ μ l
White blood cell count	9,470 cells/ μ l	5,000–9,000 cells/ μ l
Differential leukocyte count		
Neutrophils	57.4%	
Lymphocytes	27.6%	
Monocytes	9.0%	
Eosinophils	5.5%	
Prothrombin activity	100%	70–120%
International normalized ratio	1.18	0.90–1.20
Activated partial thromboplastin time	31.0 s	18–35 s
Serum fibrinogen	450 mg/dl	200–400 mg/dl
Glycemia	98.0 mg/dl	70–105 mg/dl
Serum urea nitrogen	23.0 mg/dl	10–50 mg/dl
Serum creatinine	1.22 mg/dl	0.60–1.20 mg/dl
Serum sodium	140 meq/liter	136–146 meq/liter
Serum potassium	4.90 meq/liter	3.50–5.10 meq/liter
Total serum protein	6.8 g/liter	6.6–8.3 g/liter
Serum protein fractions		
Albumin	3.09 g/dl	3.48–5.39 g/dl
α ₁ -Globulin	0.25 g/dl	0.20–0.50 g/dl
α ₂ -Globulin	0.84 g/dl	0.45–0.97 g/dl
β ₁ -Globulin	0.63 g/dl	0.32–0.51 g/dl
β ₂ -Globulin	0.29 g/dl	0.20–0.53 g/dl
Gamma globulin	1.00 g/dl	0.67–1.56 g/dl
Aspartate transaminase	22 units/liter	<40 units/liter
Alanine transaminase	12 units/liter	<40 units/liter
Pseudocholesterase	8,890 units/liter	5,400–13,200 units/liter
Total bilirubin	0.50 mg/dl	<1.0 mg/dl
Direct bilirubin	0.15 mg/dl	<0.25 mg/dl
Erythrocyte sedimentation rate	15 mm/h	<14 mm/h
Urinary analysis	Normal	Normal
Cytomegalovirus serology test	Negative	Negative
Epstein-Barr virus serology test	Negative	Negative
Hepatitis B virus serology test	Negative	Negative
Hepatitis C virus serology test	Negative	Negative
Human immunodeficiency virus serology test	Negative	Negative
<i>Brucella</i> serology test	Negative	Negative
Mantoux test	Negative	Negative
<i>Mycobacterium tuberculosis</i> serology test	Negative	Negative
<i>Leishmania infantum</i> serology test	Negative	Negative
Direct examination of parotid mass aspirate	Negative	Negative
<i>Toxoplasma gondii</i> serology test	Negative	Negative

(nimesulide, 100 mg orally three times a day) and steroids (deflazacort, 6 mg orally b.i.d., and betamethasone, 4 mg intramuscularly once daily), were administered for 14 days. The mass completely disappeared within 3 months, and the 6-, 12-, and 24-month and 3-year follow-up visits were completely normal. Serology for *B. quintana*, performed at the 6-month follow-up visit, demonstrated an IgG titer of 1:256 and an IgM titer of 1:20. At 24 months, serology for IgM was negative, whereas serology for IgG was at the cutoff titer (1:64). After 3 years, serology was repeated, and the results were negative for both IgM and IgG.

Since its first descriptions during World War I, trench fever has appeared on every continent except Australia and Antarc-

tica. In Europe and North America, infection with *B. quintana* is nowadays associated with poverty, alcoholism, and homelessness. Body lice seem to be the principal vector of *B. quintana* infection, although other ectoparasites, such as mites or cat fleas, might conceivably play a role in disease transmission (3, 8, 10, 13, 16). Unlike for most other *Bartonella* species, there is currently no clear evidence to support the existence of an animal reservoir for *B. quintana* (4, 11, 15).

Descriptions of the clinical manifestations of *B. quintana* infection in the immunocompetent host vary tremendously. Features of “classic” trench fever include fever, sometimes occurring at 5-day intervals with asymptomatic intervening periods (so-called quintan fever); debilitating and persistent typhoidal illness, often lasting many months; transient maculopapular rash; conjunctivitis; severe headache; myalgias; and, in chronic cases, splenomegaly. Progression to death is extremely rare (12).

B. quintana, together with *B. henselae*, is also associated with bacillary angiomatosis, a bacterial infection characterized by the proliferation of blood vessels, resulting in the appearance of tumorlike masses in the skin and other organs (liver, spleen, bone marrow). It most commonly manifests in people affected by acquired immune deficiency syndrome, rarely appearing in immunocompetent individuals. While curable, it is potentially fatal if not treated (5, 9, 19).

The recent descriptions of *B. quintana* bacteremia in urban homeless persons also reveal heterogeneous patterns of illness, especially chronic bacteremia and endocarditis, sometimes with severe and progressive valvular damage requiring valve replacement for the cure. These findings suggest that, although *B. quintana* clearly may cause acute and severe illness in homeless persons, it often produces a chronic and nonspecific illness that does not arouse clinical suspicion for *Bartonella* infection (2, 6).

Finally, *B. quintana* has also been reported to cause isolated chronic lymphadenopathy in both immunodepressed and immunocompetent individuals, with the pathological features of a granulomatous reaction and without fever and/or other symptoms/signs (7).

Confirming *B. quintana* infection requires serological assays (including indirect immunofluorescence-based techniques), specific cultures, and nucleic acid amplification procedures (1).

For therapy, treatment of uncomplicated *B. quintana* bacteremia with a 4- to 6-week course of doxycycline (100 mg orally b.i.d.), erythromycin (500 mg orally four times a day), or azithromycin (500 mg orally once daily) is recommended. Patients with endocarditis should receive 4 to 6 months of therapy, with close monitoring for indications of the need for valve replacement. Some authorities would recommend the addition of a bactericidal agent, such as a third-generation cephalosporin or an aminoglycoside, in the initial 2 to 3 weeks of therapy for treating endocarditis (7, 14).

To our knowledge, this is the first case of *B. quintana* infection characterized by parotid gland localization, without fever or other symptoms/signs suggesting trench fever or other *B. quintana* infection features, in an immunocompetent individual. We think that the phlogistic ulceration on the skin of the right cheek is the site of the body louse bite, even if the patient did not report anything about it. In addition, the pathological examination of the mass showed an intraparotid granuloma-

tous abscessual lymphadenitis more characteristic of *B. henselae* infection, i.e., cat scratch disease, than of *B. quintana* infection. It is thus possible to hypothesize a different specific *B. quintana* strain infection and/or different genetic characteristics for our patient, which is not easily demonstrated. With this subject, diagnosis was made on the basis of physical examination, high titers of the IgG and IgM antibodies against this agent, and especially, culture isolation from the mass. Therapy was successfully conducted by following the guidelines for complicated cases of *B. quintana* infection, i.e., endocarditis, with antibiotic association (macrolides plus cephalosporins) (7, 14).

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