

## Detection of *Bartonella quintana* by Direct Immunofluorescence Examination of Blood Smears of a Patient with Acute Trench Fever

C. Foucault,\* J. M. Rolain, D. Raoult, and P. Brouqui

Unité des Rickettsies, CNRS UMR 6020, IFR48, Faculté de Médecine, Université de la Méditerranée, Marseille Cedex, France

Received 4 February 2004/Returned for modification 28 March 2004/Accepted 26 June 2004

**We report a case of *Bartonella quintana* acute symptomatic infection in a homeless man, presenting as a typical trench fever. *B. quintana* has been retrieved in erythrocytes in large clusters and in erythroblasts. Direct immunofluorescence of blood smears allows a rapid diagnosis.**

### CASE REPORT

A 54-year-old human immunodeficiency virus (HIV)-negative, homeless, alcoholic male usually living in shelters in Marseilles, France, with a history of previous undiagnosed pneumonia was admitted in June 2002 for diarrhea, fever, and cutaneous lesions. On admission at the emergency room, he was febrile at 38°C, and scratching of lesions on both thighs and arms were observed. Three erythematous lesions, diagnosed as ecthyma in the scratching area, and a few maculopapulous lesions were noted. As one of the areas of ecthyma was suspected to be an eschar, Mediterranean spotted fever was suggested and the patient was given two tablets of doxycycline as a single dose (1). He was then hospitalized in the infectious diseases ward for survey. On day 2, the fever and diarrhea resolved. He was kept hospitalized to investigate the etiology of the past pneumonia and for dental care. After 3 weeks of hospitalization, he presented high-grade fever (40°C), chills, headaches, and dizziness. He reported having pain in the shins, as though someone had pulled on the shin. This was so painful that he had difficulty walking during the attack. Dizziness and pain in the shins led him to lose his balance and fall down. All of these signs lasted for 72 h and disappeared spontaneously. He relapsed 5 days later with similar symptoms and attacks that lasted for 2 days. Between the attacks, he felt asthenic, but he recovered partially. The diagnosis of *B. quintana* infection was made by isolation of the bacterium from nine blood cultures and one bone marrow culture performed on day 31, using methods described elsewhere (7). Briefly, each sample of blood and bone marrow was cultivated on blood culture broth, on sheep blood agar, and inoculated into shell vials containing ECV 304 human endothelial cell monolayers. Samples were incubated for 3 months before being considered negative (7). Identification was achieved by a PCR-based method (15). *B. quintana* was also observed by direct immunofluorescence in blood in six out of nine tested samples that

were positive in culture, using a mouse monoclonal antibody as described previously for other patients (13). *B. quintana* was also detected in bone marrow smear samples on day 31, as previously described in other patients (12). The intraerythrocytic location of the bacterium was confirmed by examination of sections of positive smears, taken in 0.5- $\mu$ m increments, with a laser confocal microscope (Fig. 1). Infected erythrocytes were found to be clustered, and up to 1.5% of the erythrocytes were found to be infected. Main clinical symptoms and laboratory data are presented chronologically in Fig. 2. At this time, there were no available established guidelines for the treatment of *B. quintana* bacteremia. As the patient recovered spontaneously, no further treatment was prescribed.

The patient presented with a typical case of trench fever, as reported for natural (6) or experimental infection of volunteer soldiers (10). Relapsing fever or “quintan fever,” also known as “shinbone fever,” was characterized by attacks of fever lasting for 1 to 3 days and recurring at every 4 to 6 days, associated with headaches, shin pain, and dizziness (11). This patient is the first description of the natural course of acute trench fever completely documented with follow-up of the bacteremia. Several aspects should be pointed out. Clinical signs of acute *B. quintana* infection (trench fever) appeared only 17 days after the first positive blood culture was collected (Fig. 2), this being in agreement with the fact that incubation periods between inoculation and occurrence of clinical signs varied from 6 to 22 days in experimental infection of human volunteers (10). We also noticed that blood cultures remained sterile when clinical signs were present. The second important point is the good sensitivity of direct immunofluorescence of blood smears for the detection of *B. quintana*, data that confirm previous reports (13). Immunofluorescence is thus an interesting diagnostic tool and allows a rapid diagnosis compared to blood cultures, which require up to 45 days of incubation (9). As we described previously in chronically infected homeless subjects (13), this intraerythrocytic presence of *B. quintana* was not associated in our patient with biological signs of hemolysis (normal serum bilirubin concentration and normal lactate dehydrogenase concentration), although hemoglobin level and erythrocyte count

\* Corresponding author. Mailing address: Unité des Rickettsies, CNRS UMR 6020, IFR48, Faculté de Médecine, Université de la Méditerranée, 27, Boulevard Jean Moulin, 13385 Marseille Cedex 05, France. Phone: 33 (0)4.91.38.55.17. Fax: 33 (0)4.91.38.77.72. E-mail: cedric.foucault@medecine.univ-mrs.fr.

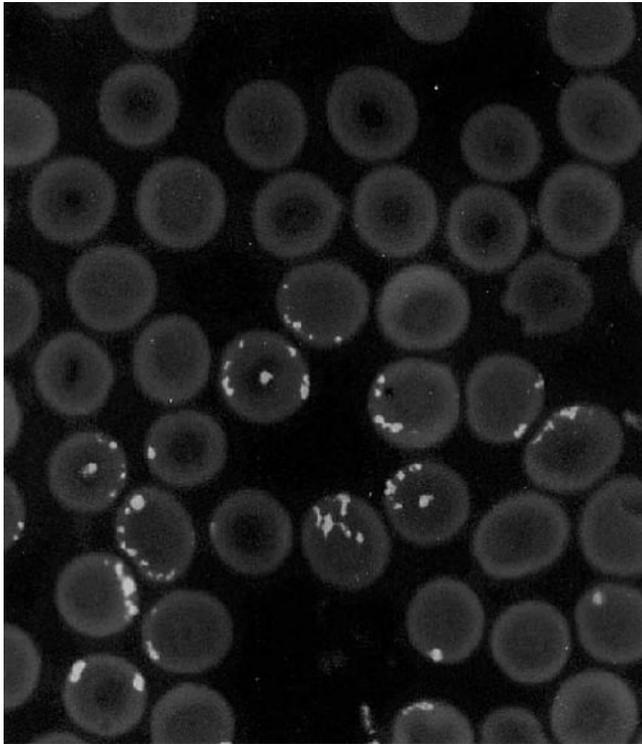


FIG. 1. Infected erythrocytes as shown by confocal microscopy. The infected erythrocytes are clustering. Original magnification,  $\times 400$ .

fell under the normal value following acute infection (data not shown). Our patient did not develop specific antibodies to *B. quintana* during and after acute infection, displaying negative serology more than 6 weeks after the first positive blood culture. One may speculate that, during the acute stage of the infection, *B. quintana* rapidly multiplies into erythrocytes and that this intraerythrocytic location protects the pathogen from the host immune response (3, 14).

We believe that this observation corresponds to the primary symptomatic infection. This assertion is supported by the high number of infected erythrocytes for this patient (up to 1.5%), compared with chronic bacteremic patients, who presented with a maximum of 0.005% infected erythrocytes (13), suggesting that, after this primary infection, some people may develop a specific immunity to control the infection and leading to chronic asymptomatic infection. This is closely related to Carrion's disease caused by *B. bacilliformis*. During primary infection, some patients develop Oroya fever when organisms enter erythrocytes. Parasitism of erythrocytes can reach 100%, while it is much lower during the chronic stage of the disease (5). During *B. quintana* infection, it has been demonstrated that the persistence of *B. quintana* in chronic bacteremic patients is associated with oversecretion of interleukin-10 (2), leading to the absence of control of the infection. In our patient, presenting a typical episode of trench fever, clustered erythrocytes were observed. The reason for clustering is unknown and has never been observed either in *B. quintana* chronic bacteremia among homeless people or during *B. henselae* infection in cats, but we cannot exclude that this may be based on microscopic artifacts.

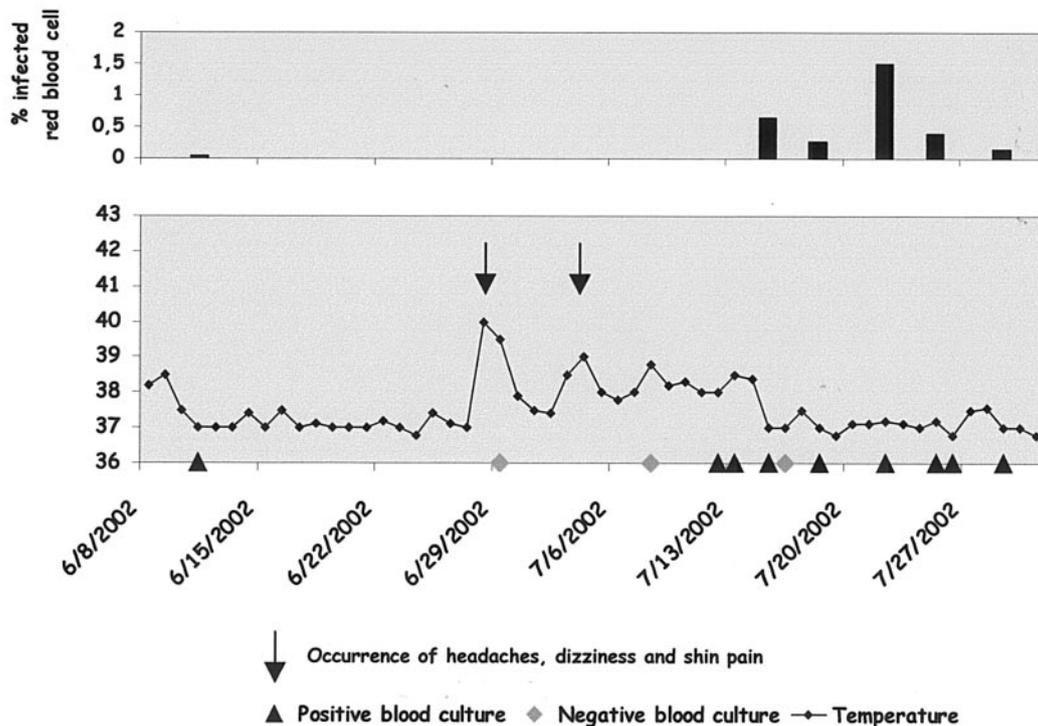


FIG. 2. Main clinical symptoms and percentage of infected erythrocytes during the course of the disease. Blood smears were observed by epifluorescence microscopy. Smears were stained with mouse monoclonal antibody as previously described (8). The percentage of infected erythrocytes was determined after counting at least 10,000 erythrocytes.

*B. quintana* has been retrieved in the bone marrow, in erythroblasts. Besides erythrocytes, this location may constitute a reservoir of the bacterium and may explain that *B. quintana* bacteremia can be as prolonged as 78 weeks and that recurrent waves of bacteremias have been observed in some patients (4, 12).

Our case report highlights the facts that, during trench fever, which is the acute stage of *B. quintana* infection, (i) the bacterium is retrieved in erythrocytes and erythroblasts, (ii) the infected erythrocytes are clustered for unknown reasons, and (iii) the proportion of infected erythrocytes is much higher than in the chronic stage of the infection, as in the course of *B. bacilliformis* infection.

## REFERENCES

1. Bella-Cueto, F., B. Font-Creus, F. Segura-Porta, E. Espejo-Arenas, P. Lopez-Pares, and T. Munoz-Espin. 1987. Comparative, randomized trial of one-day doxycycline versus 10-day tetracycline therapy for Mediterranean spotted fever. *J. Infect. Dis.* **155**:1056–1058.
2. Capo, C., N. Amirayan-Chevillard, P. Brouqui, D. Raoult, and J. L. Mege. 2003. *Bartonella quintana* bacteremia and overproduction of interleukin-10: model of bacterial persistence in homeless people. *J. Infect. Dis.* **187**:837–844.
3. Dehio, C. 2001. *Bartonella* interactions with endothelial cells and erythrocytes. *Trends Microbiol.* **9**:279–285.
4. Foucault, C., K. Barrau, P. Brouqui, and D. Raoult. 2002. *Bartonella quintana* bacteremia among homeless people. *Clin. Infect. Dis.* **35**:684–689.
5. Jacomo, V., P. J. Kelly, and D. Raoult. 2002. Natural history of *Bartonella* infections (an exception to Koch's postulate). *Clin. Diagn. Lab. Immunol.* **9**:8–18.
6. Kostrzewski, J. 1949. The epidemiology of trench fever. *Bull. Acad. Pol. Sci. (Med.)* **7**:233–263.
7. La Scola, B., and D. Raoult. 1999. Culture of *Bartonella quintana* and *Bartonella henselae* from human samples: a 5-year experience (1993 to 1998). *J. Clin. Microbiol.* **37**:1899–1905.
8. Liang, Z., B. La Scola, H. Lepidi, and D. Raoult. 2001. Production of *Bartonella* genus-specific monoclonal antibodies. *Clin. Diagn. Lab. Immunol.* **8**:847–849.
9. Maurin, M., and D. Raoult. 1996. *Bartonella (Rochalimaea) quintana* infections. *Clin. Microbiol. Rev.* **9**:273–292.
10. McNee, J. W., A. Renshaw, and E. H. Brunt. 1916. "Trench fever": a relapsing fever occurring with the British forces in France. *Br. Med. J.* **12**:225–234.
11. Ohl, M. E., and D. H. Spach. 2000. *Bartonella quintana* and urban trench fever. *Clin. Infect. Dis.* **31**:131–135.
12. Rolain, J. M., C. Foucault, P. Brouqui, and D. Raoult. 2003. Erythroblast cells as a target for *Bartonella quintana* in homeless people. *Ann. N. Y. Acad. Sci.* **990**:485–487.
13. Rolain, J. M., C. Foucault, R. Guieu, B. La Scola, P. Brouqui, and D. Raoult. 2002. *Bartonella quintana* in human erythrocytes. *Lancet* **360**:226–228.
14. Schulein, R., A. Seubert, C. Gille, C. Lanz, Y. Hansmann, Y. Piemont, and C. Dehio. 2001. Invasion and persistent intracellular colonization of erythrocytes. A unique parasitic strategy of the emerging pathogen *Bartonella*. *J. Exp. Med.* **193**:1077–1086.
15. Zeaiter, Z., Z. Liang, and D. Raoult. 2002. Genetic classification and differentiation of *Bartonella* species based on comparison of partial *ftsZ* gene sequences. *J. Clin. Microbiol.* **40**:3641–3647.