Shell Vial Culture as a Tool for Isolation of *Brucella melitensis* in Chronic Hepatic Abscess

Clarisse Rovery,¹ Jean Marc Rolain,² Didier Raoult,² and Philippe Brouqui¹,2*

Service de Maladies Infectieuses et de Médecine Tropicale, Hôpital Nord, AHPM,¹ and Unité des Rickettsies, IFR 48, CNRS UMR 6620, Faculté de Médecine, Université de la Méditerranée,² Marseille, France

Received 24 April 2003/Accepted 22 June 2003

A 50-year-old man was admitted with a liver abscess and positive serology for *Brucella* spp. Liver pus and blood cultures remained sterile on conventional culture. Inoculation of liver pus onto eukaryotic cells by the centrifugation-shell vial technique yielded *Brucella melitensis*, identified by 16S rRNA gene amplification and sequencing.

Hepatic involvement in acute brucellosis occurs frequently and primarily manifests as liver enlargement and/or mild elevations of liver enzyme levels and granulomatous hepatitis (1). Hepatic abscess as a complication of acute brucellosis has seldom been reported (16). More common is the chronic form renamed “chronic hepatosplenic suppurating brucellosis” (CHSB) (2). *Brucella* spp. have been cultivated from hepatic abscesses only rarely in such situations. By using a tissue cell culture system designed for rickettsial culture (9), we have been able to isolate *Brucella melitensis* from a liver abscess.

A 50-year-old man was admitted to the hospital for persistent fever and night sweats over 6 weeks. He was a sheep breeder in Corsica, an area of endemicity for brucellosis until the early 1980s. He stated that he had been given a diagnosis of acute brucellosis in 1978 when his sheep were infected by *Brucella* spp. and had numerous abortions. He was treated for 6 months. He was apparently cured with no relapse. In August of 2001, he presented to his general practitioner with fever and a 6-kg weight loss over 3 weeks. A Wright’s seroagglutination test showed a titer of 480. A diagnosis of reinfection or relapse was suspected, and rifampin (900 mg/day) and doxycycline (200 mg/day) were prescribed. Three weeks later, fever and night sweats persisted, and the patient was admitted for further investigation. At examination, the patient appeared to be in good health. The temperature was 38.5°C. The liver was enlarged, but splenomegaly was absent. Antibiotics were discontinued. The laboratory findings were as follows. The leukocyte count was 13.4 g/liter, with 82% polymorphonuclear neutrophils; the erythrocytic sedimentation rate was 89 mm; the protein C reactive level was 188 mg/liter; and the fibrinogen level was 9.48 g/liter. The gamma glutamyl transferase level was 240 IU/liter, and the transaminase and total bilirubin levels were normal. Polyclonal hypergammaglobulinemia, circulating anticoagulant, and anticardiolipin antibody were noted. A second Wright agglutination test revealed a titer of 2,560. Three blood cultures (BACTEC 9240) and cultures of bone marrow did not yield any bacteria despite a prolonged incubation time (30 days). An abdominal computed tomography (CT) scan noted a hypodense zone with a necrotic or cystic appearance centered around a 1-cm calcified density. An ultrasonography-guided percutaneous aspiration yielded 30 ml of thick purulent fluid. Few leukocytes and no organisms were seen on Gram and acid-fast smear staining of the aspiration fluid. The pus was inoculated onto 5% sheep blood agar (bio-Mérieux, Marcy l’Etoile, France) and incubated in a 5% CO₂ atmosphere for 30 days at 37°C; no organisms grew. In parallel, an aliquot was inoculated onto human embryonic lung (HEL) fibroblasts in shell vials by a method described previously (5, 9). Briefly, the pus was homogenized in 1 ml of sterile brain heart infusion broth and was aspirated into a 1-ml syringe through a 27-gauge needle to filter out coarse material. The sample was inoculated onto three shell vials (3.7 ml; Sterlin, Feltham, England) containing monolayers of confluent HEL fibroblasts on 1-cm² coverslips (9) and centrifuged at 700 × g for 1 h at 22°C. Supernatant was discarded and replaced with fresh culture medium (Eagle’s minimal essential medium with 4% fetal bovine serum and 2 mM l-glutamine). After incubation for 21 days at 37°C, a coverslip from one shell vial was stained by the Gimenez method and showed small extracellular and apparently intracellular coccobacilli (Fig. 1). The patient’s own serum reacted to these bacteria by immunofluorescence (Fig. 2). To identify the organism, DNA was extracted from both liver pus and tissue cell culture-grown bacteria with the QIAmp tissue kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer’s recommendations. The extracted DNA was subjected to a PCR assay that incorporated the broad-range eubacterial primers fD1 and rP2, derived from the 16S rRNA-encoding gene sequences (3). Amplification was successful in both samples, and the PCR product was sequenced as previously described and found to be 100% similar to *B. melitensis* (15). Liver pus was stored at −80°C. One year later, liver pus was thawed and reinculated on HEL in shell vials and again yielded *B. melitensis*. The patient was treated with rifampin (900 mg daily) and doxycycline (200 mg daily). His clinical state quickly improved, and 3 months later, the CT scan was normal.

In 35 published CHSB cases suspected to be due to *B. melitensis*, bacteria have been isolated from liver or splenic abscesses in only 7 cases (2, 10, 16, 17). The centrifugation-
shell vial system is a method of cell culture initially used for virus isolation that we have adapted for isolation of rickettsiae (9). We use this technique routinely and successfully to isolate Rickettsia spp. (8), Coxiella burnetii (12), and Bartonella spp. (14) from blood and tissue biopsy samples. We have also used this approach for the isolation of Francisella tularensis from inoculation eschar biopsy samples (5), Legionella pneumophila from liver and lung biopsy specimens (7), Chlamydia trachomatis from lymph node (11), Tropheryma whipplei from a cardiac valve (13), and Mycobacteria spp. from different clinical samples, while the BACTEC automated liquid culture system yielded no organisms (6). After inoculation and incubation in shell vials, detection of bacteria can be assessed with acridine orange, Gimenez and Giemsa staining of the shell vial supernatant, or immunofluorescence staining of the cell monolayer.

When bacterial growth is detected, identification can be achieved by PCR amplification and sequencing of the 16S rRNA gene. Because the use of the shell vial technique requires specialized equipment and trained personnel, it is not suitable for use in most clinical conditions. This procedure, however, provides a means for isolation of a wide range of intracellular bacteria, even when only very little material is available. To our knowledge, this is the first isolation of Brucella spp. by the shell vial culture system. Work on the recovery of Bartonella spp. in our laboratory and others has shown that, in some instances, cell culture is successful while conventional cultures are not (4). The present report reemphasizes the usefulness of the centrifugation-shell vial system as a versatile culture system that allows isolation of fastidious facultative or obligate intracellular pathogens.

We thank J. S. Dumler (Johns Hopkins University, Baltimore, Md.) for review of the English in the manuscript.

REFERENCES