Case Report and Seeded Blood Culture Study of
Brucella Bacteremia

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Received 26 February 1990/Accepted 12 June 1990

Diagnosis of brucellosis requires prompt detection and identification of the cocobacillus for appropriate patient management, as the organism is associated with a potentially severe outcome. In a recent experience, an 18-year-old migrant farm worker presented at a local hospital with nonspecific symptoms. A significant Brucella titer of 2,560 was followed by the recovery of a gram-negative cocobacillus, subsequently identified as Brucella abortus, from subcultured 5-day-old BACTEC NR730 negative blood cultures. The organism proved to be susceptible to a variety of antimicrobial agents and resistant to nitrofurantoin. The patient was administered antimicrobial therapy for Brucella spp. consisting of tetracycline and streptomycin for 21 days. During the course of therapy the patient experienced defervescence and was discharged with the recommendation for periodic follow-up examinations. Seeded culture studies of this isolate with fresh human blood and target inocula of 5 and 500 CFU/ml indicated that the larger (500-CFU/ml) inoculum produced positive instrument detection within 2 days, whereas the smaller (5-CFU/ml) inoculum required 5.5 to 7.5 days for detection, depending on the medium used. These findings underscore the potential for Brucella bacteremia to escape instrument detection given a low bacterial inoculum.

Brucellosis is a significant occupationally associated disease in many parts of the world. Several countries have successfully eradicated brucellosis in domestic animals and thus decreased the incidence of human infection. In the United States, there has been a dramatic decrease in the prevalence of human brucellosis over the past 40 years due in part to the surveillance and eradication programs initiated to reduce bovine outbreaks (8). In 1947, from a peak of 6,321 cases (8), there has been a marked downturn to 232 cases in 1977, with continued progressive diminution to 83 cases in 1989 (1a). However, it is clear that infection with Brucella spp. has been and continues to be both underdiagnosed and underreported; for each case reported in the United States, it is estimated that 26 cases go undetected or unreported (8).

Symptoms of brucellosis are often nonspecific, yet infection with this organism is frequently associated with a severe or even fatal outcome (9). Laboratory diagnosis requires prompt detection and identification for appropriate patient management, although routine cultures of blood are often negative. Current recommendations for the isolation and identification of Brucella spp. from blood cultures require incubation periods longer (up to 30 days) than those routinely used. Thus, the potential exists for Brucella bacteremia to go undetected.

Case report. An 18-year-old Mexican male emigrated to the United States in the first week of June 1988. He was employed as a farm field worker picking vegetables in the Rochester area of New York State. On 9 June 1988, he was taken to the emergency room of a local hospital complaining of chills, weakness, and dizziness. He had an oral temperature of 105°F (ca. 40.5°C). The patient was transferred to the Erie County Medical Center on 15 July 1988. Upon admission, he had a temperature of 104°F (40°C), lower left quadrant pain, paravertebral tenderness (T4 to T8), diarrhea, weakness, and chills. He denied recent vaccination, ingestion of well water or spoiled food, exposure to chemicals, drug usage, or travel outside Mexico or the United States. Several days later, the patient related exposure to sheep, goats, cattle, and chickens while in Mexico. He also stated that his brother, who had been sick in Mexico prior to their arrival in the United States, had similar symptoms and referred to the illness as "Malta fever."

Blood was collected on the day of admission for culturing and antimicrobial susceptibility testing. Preliminary blood culture results were reported as negative 2 days later. Urine and fecal specimens were collected on the day of admission and 5 days after admission, respectively. They were cultured using BAP (5% sheep blood in Trypticase soy agar), colistin-nalidixic acid (CNA), chocolate, MacConkey, and campylobacter agars (BBL Microbiology Systems, Cockeysville, Md.) and xylene-lactose-Desoxycholate, Hektoen enteric, and yersinia agars (Difco Laboratories, Detroit, Mich.). All cultures were incubated for 24 to 48 h at 35°C, except for the campylobacter cultures, which were incubated at 42°C. The urine culture demonstrated no growth after 5 days; the fecal culture was negative for enteric pathogens (i.e., Salmonella, Shigella, Yersinia, Campylobacter, and Vibrio spp.). Six days after admission, blood sent for a Brucella serologic agglutination test (Wellcome Diagnostics, Dartford, England) had a titer of 2,560, which is indicative of current or recent infection (2).

A Brucella sp. was isolated, 14 and 18 days after admission, when 5-day-old instrument-negative blood bottles and a 7-day-old culture of bone marrow aspirate, respectively, were subcultured to chocolate agar. The Brucella isolates were sent to the New York State Department of Health Division of Laboratories and Research in Albany for confr-
mation and species identification. On the basis of dye inhibition studies, the isolate was identified as *Brucella abortus*.

The patient was started on antimicrobial therapy for *Brucella* spp. 7 days after admission. The regimen consisted of tetracycline (2 g/day) for 21 days and streptomycin (1 g/day) for 14 days, during which time the patient's fever subsided. He was discharged 27 days after admission with the recommendation that follow-up examinations be performed at 2-week, 2-month, 6-month, and 1-year intervals.

**Blood culture studies.** The BACTEC NR730 system (Becton-Dickinson Diagnostic Instrument Systems, Towson, Md.) was used for the detection of bacteremia. Over a period of 2 weeks, five blood specimen sets (15 bottles) were collected from the patient. Fifteen milliliters of the patient's blood had been inoculated into three media (5 ml per vial): NR6 (enriched soybean-casein digest broth with CO₂) for aerobic culturing, NR7 (prereduced enriched soybean-casein digest broth with CO₂) for anaerobic culturing, and NR8 (hypertonic enriched soybean-casein digest broth with CO₂) for aerobic culturing. Aerobic cultures were shaken for 24 h in accordance with the directions of the manufacturer and tested daily with the BACTEC instrument. Anaerobic cultures were not shaken. Each bottle was tested daily until a growth value of ≥30 (aerobic) or ≥20 (anaerobic) was achieved. When positivity was detected, the vial was subcultured to a variety of agar and liquid media (BBL). BAP, chocolate agar, MacConkey agar, CNA agar, vitamin K-enriched anaerobe blood agar, and thioglycolate broth were used to recover the microorganism. Blood cultures that remained instrument negative for 5 days were terminally subcultured to chocolate and enriched anaerobe blood agars.

The bone marrow aspirate was cultured by the Castaneda technique (biphasic media with Tryplicase soy agar) (1). Bottles were incubated at 35°C for up to 30 days. Of the 15 bottles received in the laboratory for blood culturing, 5 of 5 aerobic bottles were positive in 7 to 20 days, 4 of 5 hypertonic bottles were positive within 20 days, and 0 of 5 anaerobic bottles were positive within 20 days. The bone marrow aspirate culture was positive in 7 days. It is important to note that the initial two blood culture sets did not result in instrument detection but rather were positive on routine subculturing of 5-day-old instrument-negative blood cultures. Subsequent blood culture sets were detected by an instrument signal following an extended incubation time.

The microscopic morphology of the gram-negative *Brucella* bacillus was characteristic for *Brucella* spp. On blood agar after 72 h of incubation, translucent colonies 3 mm in diameter were round and moist. Identification of the *Brucella* sp. was performed by established procedures (3, 7). Biochemical reactions were typically negative for glucose, xylose, and citrate utilization but positive for nitrate, urease, and oxidase production.

Isolates were tested for antimicrobial susceptibility by standard agar (Mueller-Hinton [BBL]) disk diffusion (5) and microdilution susceptibility (6) methods. For each method, quality-control standards were run in parallel with clinical specimens. The MICs for the *Brucella* isolate were as follows: ≤2.0 µg/ml for ampicillin, cefoxitin, cephalothin, chloramphenicol, gentamicin, piperacillin, mezlocillin, tetracycline, and tobramycin (organisms interpreted as susceptible); ≤0.25/4.75 µg/ml for trimethoprim-sulfamethoxazole (organisms interpreted as susceptible); 8.0 µg/ml for amikacin (organisms interpreted as susceptible); and 64 µg/ml for nitrofurantoin (organisms interpreted as resistant). Except for amikacin, identical susceptibility interpretations resulted from Bauer-Kirby disk diffusion. The antimicrobial susceptibility profile of this organism was unanticipated in that the literature predominantly refers to streptomycin and tetracycline results.

BACTEC media, as described above, were seeded in duplicate with the *Brucella* isolate recovered from the patient at target concentrations of 5 and 500 CFU. A 20-ml sample of sterile blood was drawn from a healthy volunteer donor, and equal portions (3 ml) were promptly inoculated into each BACTEC bottle previously seeded with the appropriate bacterial concentration. Donor blood was monitored for sterility by directly inoculating 5% sheep blood agar with 1 ml and incubating it at 35°C in the presence of 5% CO₂-95% air for 24 h. The inocula were determined from previous population kinetics studies with a known spectrophotometric value to determine the concentration of microorganisms in a homogeneous suspension. Plate count CFU were used to verify the target concentrations at zero time.

Each blood-microorganism inoculum represented a mock bacteremic sample. Bottles were analyzed once daily for the detection of growth with the BACTEC NR730 system. Bottles were subcultured onto BAP in duplicate on day 0 and daily for 9 days. CFU were counted and averaged. Mean growth values were graphically plotted for each day that they were tested (Fig. 1).

Seeded *Brucella* blood cultures indicated that bottles containing ≥500 CFU/ml had the potential to signal BACTEC NR730 positivity in 1 to 2 days (Fig. 1). In contrast, cultures seeded with approximately 5 CFU/ml of blood were positive in 5.5 to 7.5 days in the aerobic media (Fig. 1). In the anaerobic medium, brucellae were undetectable during the 9-day observation period but were recoverable on subcultures.

The diagnosis of human brucellosis continues to be important despite the steady decline in the numbers of reported cases in the United States over the past several decades. The laboratory identification of *Brucella* infection remains difficult because of the slow growth of *Brucella* spp. in vitro and, hence, the inability to recover or the delay in recovering the organism from blood. In particular, given the nonspecific symptoms of this infection, a laboratory may not be alerted to the possibility of brucellosis and blood cultures may not
be kept for a sufficient time. The recommended incubation period for blood cultures in suspected cases of brucellosis is 30 days; the conventionally accepted incubation period for routine blood cultures is 5 to 7 days (3).

We studied the ability of a widely used blood culture system, the BACTEC NR730, to detect Brucella bacteremia with seeded cultures. On the basis of the results obtained with large (500-CFU/ml) and small (5-CFU/ml) inocula, we believe that the BACTEC NR730 does not have the capability to detect Brucella spp. in a routine 7-day incubation period if the bacterial load is small (≤5 CFU/ml of blood). However, if the bacterial load is larger (≥500 CFU/ml of blood), the instrument can detect the organism within the 1-week incubation period. Given the low number of bacteria commonly associated with this gram-negative infection (4), we conclude that there is a potential to miss Brucella bacteremia if the suspicion of brucellosis does not exist.

We thank Sarojini Misra and Kenneth Hamm for expert technical assistance.

LITERATURE CITED