Isolation of *Brucella* spp. from Clinical Specimens

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Received 27 September 1983/Accepted 21 May 1984


Blood-broth cultures are frequently negative when specimens are drawn from patients with symptoms and histories suggesting brucellosis (4, 6). Braun and Kelsh (2) showed that recovery of brucellae from experimentally infected rabbits was favored by an alternative procedure, lysis-filtration, and Finegold et al. (5) tested this procedure for human blood specimens, with favorable results. As an alternative to filtration and using rabbits experimentally infected with brucellae, Smith (M.S. thesis, University of California at Los Angeles, Los Angeles, 1953) compared a lysis-concentration procedure with the procedure of Castañeda (3); the lysis-concentration procedure yielded more positive cultures and yielded them earlier. The rationale for this procedure has been presented previously (1). We report here an evaluation of this procedure for the processing of specimens obtained from patients in Iran diagnosed clinically by one of us (A.R.) as having brucellosis.

For the Castañeda procedure, 10 ml of blood or 2 to 3 ml of cerebrospinal fluid or bone marrow was added directly to a bottle containing Castañeda medium (BBL Microbiology Systems, Cockeysville, Md.) For the lysis-concentration procedure, 5 to 10 ml of blood was collected in a 50-ml screw-capped sterile centrifuge tube containing 1.5 ml of 4% sodium citrate. Approximately 40 ml of sterile distilled water was added to the tube, the contents were mixed, and the tube was centrifuged (2,000 × g, 30 min). The supernatant fluid was then discarded, and 0.5 ml of the sediment was transferred to duplicate sheep blood agar plates. Cerebrospinal fluid or bone marrow (2 to 3 ml) was transferred to a 20-ml centrifuge tube containing citrate, ca. 15 ml of sterile water was added, the contents were mixed, the tube was centrifuged, and the sediment was transferred to blood agar plates.

All cultures were incubated at 35 to 37°C under elevated carbon dioxide (candle jar) and were examined after 48 h. Blood agar plates showing no growth at 48 h were incubated for an additional 6 days. Castañeda bottles were incubated for 21 days before being discarded as negative. For those plates showing growth, identification as *Brucella* species was based on colony and microscopic morphologies, agglutination by antibrucella serum (Difco Laboratories, Detroit, Mich.), and biochemical features (1, 4).

The recovery of brucellae by the two procedures was as follows. Of 142 specimens examined, 14 of 126 blood, 2 of 9 bone marrow, and 2 of 7 cerebrospinal fluid specimens were culture positive within 48 h. With the exception of one EF-4 strain (Centers for Disease Control, Atlanta, Ga.) recovered from blood, all organisms were *Brucella* species. The same specimens were culture negative when tested by the Castañeda procedure. We conclude that these results, as well as those obtained earlier with experimentally infected animals (D. F. Smith, M.S. thesis, University of California at Los Angeles, Los Angeles, 1953), recommend the lysis-concentration procedure for the recovery of brucellae from clinical specimens.

We thank F. Siavoshi for reading and reorganizing this article.

**LITERATURE CITED**


