

Letters to Editor

Utility of Bone Marrow Culture in the Definitive Diagnosis of Human Brucellosis

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Dear Editor,

Brucellosis still continues to remain a zoonosis of worldwide public health & economic importance. Half a million new cases are reported worldwide each year, but according to WHO these numbers greatly underestimate the true incidence of human brucellosis. Clinical picture of brucellosis is very nonspecific hence diagnosis requires laboratory confirmation.¹ Although a presumptive diagnosis of brucellosis can be made by demonstrating high or rising antibody titres, isolation of brucellae from blood, bonemarrow or other tissue remains the only irrefutable evidence for the disease.²

Because of suboptimal recovery rate of brucellae from blood, it has been suggested that culture of bone marrow, liver tissue or lymph node may improve recovery rate of the organism. Rationale for this alternative approaches is that *Brucella* organisms survive intracellular killing by phagocytes & localize in reticuloendothelial system.³ Hence the

present study was undertaken to determine the utility of bone marrow culture in the definitive diagnosis of human brucellosis.

A total of 1948 serum samples obtained from patients with fever of unknown origin between April 2004 to June 2006 were screened for *Brucella* agglutinins by Rose Bengal plate test. The positive serum samples were subjected to standard tube agglutination test employing *Brucella abortus* plain antigen (Indian Veterinary Research Institute, Izatnagar) to determine titre of antibodies. The diagnosis of brucellosis was established in 25 patients based on diagnostic titres of $\geq 1 :160$. Detailed clinical history including demographic data occupational history and history of ingestion of unpasteurized milk was obtained from each patient.

Blood and bone marrow aspirate were collected simultaneously for culture from all the 25 cases. The samples were inoculated into Castaneda biphasic media consisting of

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brain heart infusion agar and broth in duplicate.⁴ The Castaneda biphasic media were incubated at 37°C under aerobic conditions and subcultured daily till colonies appeared on agar phase or for 30 days if negative. The date of appearance of first colony in either bone marrow or blood was recorded for comparison of growth rates.³ Following isolation of *Brucella* organisms they were identified by standard laboratory techniques.⁵ All the isolates were sent to Indian Veterinary Research Institute, Izatnagar for confirmation.

The age of patients ranged from 16 to 75 years. Out of 25 cases, 23 were males and 2 were females. Majority of cases were associated with agriculture (24%) while shepherd formed the next largest group (20%). History of ingestion of unpasteurized milk was present in 4 cases. Cultures of bone marrow were positive in 9 of 25 (36%) cases, whereas blood cultures were positive in only 6 cases (24%). Gotuzzo et al³ also found cultures of bone marrow (92%) to be more sensitive than blood cultures (70%). All the isolates were confirmed as *B. melitensis* biotype 1 at Indian Veterinary Research Institute, Izatnagar. Detection of bacterial growth was faster in bone marrow cultures than cultures of blood i.e. 5.55 days V/s 9.33 days respectively which is consistent with Gotuzzo et al³ i.e. 4.32 days V/s 6.65 days respectively. Despite the small volume of bone marrow samples (0.5- 1ml) compared with those of blood samples (5-10ml), *Brucella* grew more rapidly from bone marrow aspirates, a result suggesting that the inoculum of bacteria in the marrow may be more concentrated than that in the blood. This is consistent with the hypothesis that brucellae are localized within the phagocytic cells of organs of the reticuloendothelial system including liver, spleen, lymph nodes

and bone marrow. All the patients received a standard regimen of two drugs consisting of Doxycycline orally 200 mg per day in two divided doses for 6-12 weeks together with Streptomycin 0.75-1 gm per day intramuscularly for the first 2-4 weeks depending on the resolution of signs and symptoms.

Combination of cultures of blood and bone marrow yielded a diagnosis of brucellosis in 36% of cases; since an additional 12% of cases were diagnosed exclusively by bone marrow culture, we recommend this procedure as an adjunct in special cases, such as patients with fever of unknown origin, especially in areas where brucellosis is endemic.

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