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Rapid detection of *Brucella* by an automated blood culture system at a tertiary care hospital of north India

Sir,

Brucellosis is a significant veterinary and public health problem in endemic areas including the Mediterranean countries, the Middle East, the African subcontinent, the Latin America and parts of Asia[1]. Species prevalent in India include *Brucella melitensis* and *B. abortus*[2]. *B. melitensis* is the most virulent and the commonest species and it causes severe and prolonged disease with a risk of disability in man. Goats, reared for meat, constitute the main source of infection. Goat and sheep milk is used for adulteration when there is a shortage of cows and buffalos milk in summer months[3]. *B. abortus* is the main species in cattle[4], and bovine brucellosis is widespread in India[5]. *Brucella* species are capable of evading host defense mechanisms, surviving as intracellular organisms, and are able to cause prolonged morbidity, relapses, and long-term sequelae. It has very low mortality (1%) but high morbidity[6]. It may affect any organ of the body with clinical manifestations that include fever, joint pains, loss of weight, sweating, cough, sciatica, splenic enlargement, liver enlargement, orchitis, *etc*[7]. The non-specific and protean clinical presentation mimics other infectious and non-infectious conditions and consequently, the diagnosis of the disease is often missed or delayed[8].

The present study was conducted in a tertiary care hospital in Chandigarh, north India. The culture confirmed cases of *Brucella* infection admitted to or attending various outpatient clinics of the Post-graduate Institute of Medical Education and Research (PGIMER), Chandigarh, during March 2010 to June 2012 were retrospectively studied. Blood culture was performed in the clinical bacteriology laboratory in the department of Medical Microbiology as a part of routine diagnostic services using BACTEC 9240 (BD Diagnostics, USA) system. Standard aerobic/F and Peds plus/F media (BD Diagnostics, USA) were used for the adult and the paediatric patients, respectively. Inoculated bottles were monitored up to five days or until those became positive[9]. The flagged positive vials were subjected to Gram stained smear microscopy and subcultured on sheep blood agar and MacConkey agar, incubated at 37°C. *Brucella* was identified and differentiated from other Gram negative genera on the basis of small, translucent, soft and easily emulsifiable colonies on MacConkey and blood agar (non-pigmented and non-haemolytic) with absence of X and V factor dependence; Gram-negative tiny coccobacilli, non-encapsulated, non-motile, oxidase, catalase and urease positive, producing acid from xylose in oxidative fermentative medium[6]. The results of culture were compared with standard agglutination tube test (SAT) for *Brucella* with or without pre-treatment of serum with 0.05M 2-mercaptoethanol (2-ME) using antigen obtained from Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh. During the study period, *Brucella* spp. was isolated from 13 blood and one CSF specimens culture. Clinical details were available in seven cases. The patients in general, had one to three weeks history of fever associated with chills and rigour, malaise, anorexia, headache and vomiting. Two patients had hepatosplenomegaly and three had only hepatomegaly and history of weight loss. Associated risk factors includes intake of nonpasteurized milk or milk products, rearing cattle, veterinarian as profession, and laboratory exposure. In all the cases *Brucella* serology using SAT without 2ME pre-treatment demonstrated a titre of 2560 IU. The quantity of specific IgG as determined by treatment of serum with 2-ME was 2560 IU in six cases and 1280 IU in one case (Table).

The only confirmatory evidence of brucellosis is the recovery of the bacterium from the patient. Therefore, culture is considered the gold standard in the laboratory diagnosis of brucellosis[6]. Various blood culture

Table. Clinical profile and outcome of confirmed cases with *Brucella* infection

Patient	Age (yr)	Sex	Risk factor	Sign and symptoms (duration in days)	Serology titre in IU		Treatment for 6 wk duration	Outcome
					BSAT	2ME		
1	50	M	Domestic animal handling	Loss of appetite (15) Fever (12) Tiredness (10) Weight loss (10) Mild hepatomegaly	2560	2560	Doxycycline +streptomycin combination	Recovered
2	33	M	Veterinarian	Dry cough (4) Headache (3) Fever (2) No organomegaly	2560	2560	Doxycycline+ rifampicin combination	Recovered
3	30	M	Unpasteurized milk and milk products intake	Fever (15) Loss of appetite (7) Weight loss (7) Lymphadenopathy (juglodigastic) Mild hepatosplenomegaly	2560	1280	Doxycycline +streptomycin combination	Recovered
4	07	M	Unpasteurized milk and milk products intake	Fever (13) Headache and vomiting (5) Lymphadenopathy at multiple sites Hepatosplenomegaly	2560	2560	Doxycycline +rifampicin combination	Recovered
5	58	M	Laboratory exposure	Fever (25) Headache and malaise (15) Joints pain (7) Hepatomegaly	2560	2560	Tetracycline +rifampicin combination	Recovered
6	2.5	F	Unpasteurized milk and milk products intake	Fever (30) Loss of appetite (15) Right hip swelling (10) Right leg limping (7)	2560	2560	Cotrimoxazole +rifampicin combination	Recovered
7	10	M	Unpasteurized milk and milk products intake	Fever (15) Headache (13) Redness and cracking of lips with oral ulcers (13) Mild hepatomegaly	2560	2560	Tetracycline +rifampicin combination	Recovered

BSAT, *Brucella* standard agglutination test without 0.05M 2-mercaptoethanol; 2 ME, BSAT with 0.05M 2-mercaptoethanol; M, male; F, female; IU, international units

methods are available but the newer semiautomatic methods such as BACTEC 9240 has shortened the time taken for detection; the presence of *Brucella* can be detected with these methods within five days of incubation[10]. In the present study, nine cultures became positive by the third day and all were detected within five days. In one patient, blood and CSF samples were received in BACTEC bottles and both were positive on the third day (though CSF inoculation in these vials is not routinely recommended) while the same CSF was negative by conventional culture. Direct invasion of the central nervous system occurs in less than 5 per cent cases of brucellosis[11]. Therefore, rapid detection of scanty load of bacteria in CSF by automated culture system is an added advantage for sterile body fluids.

All the proven cases received proper treatment[11,12] because of rapid and accurate aetiological diagnosis aided by use of automated blood culture system, and recovered.

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