Case Report

PCR confirmation of cutaneous manifestation due to *Brucella melitensis*

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Human brucellosis, a zoonotic disease, is endemic in the Belgaum district, Karnataka, India. A male patient presented with a generalized itchy rash. Blood was sent for venereal disease research laboratory testing. Screening was carried out for *Brucella* antibodies following hospital policy and diagnosis was confirmed by PCR.

Case report

A 50-year-old male patient presented himself at the Skin and Venereal Diseases Outpatient Department, Karnataka Lingayat Education Society's Hospital and Medical Research Center, India, with the sole complaint of itchy macular lesions (Fig. 1). No other symptoms of note were found. The clinical diagnosis was made as syphilis, with no significant history of exposure. A serum sample was sent to the Department of Microbiology, Jawaharlal Nehru Medical College, India, for venereal disease research laboratory testing, with the suspicion of secondary syphilis. We also screened the serum sample for *Brucella*-specific antibodies, following the policy of screening all serum samples received in the Department of Microbiology for various other investigations; this policy has been adopted because of *Brucella* endemicity in the Belgaum district, Karnataka, India. The specimen was strongly positive for *Brucella*-specific antibodies using a slide agglutination test. A standard tube agglutination (SAT) test was performed and titres were noted after incubation for 48 h. The SAT showed an antibody titre of 320 IU ml⁻¹, and a modified SAT with 2-mercaptoethanol showed a titre of 20 IU ml⁻¹. This diagnosis was conveyed to the physician at this juncture. A detailed history of the patient was then taken with specific questions pertaining to contact with cattle. It was found that he was a farmer by occupation and that a relevant history of contact with cattle was present. A fresh serum specimen was obtained and stored at -20 °C. In parallel with this, a blood culture was also established in the biphasic medium brain heart infusion broth and incubated at 37 °C. The patient was put on standard *Brucella*-specific therapy.

An attempt to isolate *Brucella* was carried out without success; the biphasic medium was observed for 6 weeks before reporting it as negative for *Brucella* growth. A PCR

specific for *Brucella melitensis* was performed. DNA was extracted from the serum sample using a DNA extraction kit (Bangalore Genei) with certain modifications and stored at -20 °C in 200 µl TE buffer [10 mM Tris/HCl (pH 8.0), 1 mM EDTA]. A PCR assay targeting an IS711 element downstream of BMEI1162 (GenBank accession no. NC_003317) was set up using primer sequences described by Probert *et al.* (2004) and an amplicon of 279 bp was detected by agarose gel electrophoresis.

The PCR mixture (25 µl) consisted of 20 µl PCR master mix, 3 µl template and 1 µl each of the forward and reverse primers (Bangalore Genei). Amplification was performed using a Biometra T90 thermal cycler for 35 cycles, with initial denaturation at 95 °C for 30 s, annealing at 65 °C for 30 s and extension at 72 °C for 30 s. This was preceded by initial incubation at 95 °C and followed by a final extension step at 72 °C for 5 min. A known strain of *B. melitensis* was used as a positive control, and PCR master mix without template was used as a negative control to monitor for contamination and non-specific amplification.

Following DNA amplification, the products were run on 1.5% agarose gels in the presence of ethidium bromide. Amplified products were visualized under a UV transilluminator.

The serum sample tested was positive for a DNA band of 279 bp corresponding to the known strain of *B. melitensis* and no non-specific amplification was observed (Fig. 2). The patient was treated with 100 mg doxycycline twice a day for 45 days and 1 g streptomycin daily for 15 days, following the recommended World Health Organization regimen. The cutaneous lesions disappeared within 1 week of commencement of the therapy.

Brucellosis continues to be of great health significance and economic importance in many countries. There are no available data about its prevalence in India, but various studies conducted by different authors have reported

Abbreviation: SAT, standard tube agglutination.

Fig. 1. Photographs showing generalized macular rashes.

seropositivity ranging from 0.8 % in the general population to 58–72 % in occupationally exposed personnel such as veterinarians (Corbel, 1997). *Brucella* spp. are able to cause prolonged morbidity due to their ability to evade host defence mechanisms by surviving as intracellular organisms. Diagnosis of the disease is challenging and is frequently delayed or missed because the clinical picture may mimic other infectious and non-infectious conditions (Al Dahouk *et al.*, 2003; World Health Organization, 1997). It is often missed because of its non-specific symptoms unless the clinician is aware of the organism and maintains a high degree of suspicion. Diagnosis can be established by laboratory methods such as serology and blood cultures. A prolonged incubation period, special growth media and subcultures are required for the isolation of these fastidious,

slow-growing bacteria. However, cultures are not always positive when other tests are positive (Yagupsky, 1999). Traditionally, serology is the mainstay of diagnosing human brucellosis, although interpretation of titres requires that baseline titres are discounted, as these are present in the population without actually harbouring the organism and without any illness (Orduna *et al.*, 2000).

Multiple cutaneous findings may be observed in brucellosis due to direct inoculation, hypersensitivity phenomena, deposition of immune complexes and direct invasion by the organism reaching the skin haematogenously. The prevalence of skin involvement in brucellosis is reported to range from 1 to 12 % (Metin *et al.*, 2001). Skin lesions in brucellosis are known to occur in the form of macules, papules and nodules, along with the characteristic fever and joint pain, but itchy lesions as the sole complaint of the patient, to the best of our knowledge, has not been reported previously. This finding reaffirms the unusual clinical presentation in brucellosis and points towards the need to be aware of this organism and to screen for brucellosis in all serum samples from cases of pyrexia of unknown origin, especially in *Brucella*-endemic areas.

Although isolation of *Brucella* is considered to be the gold standard, it is problematic, with yields ranging from 15 to 60 % and considerable delay in diagnosis, as the medium needs to be observed for a minimum of 4 weeks before it can be considered negative due to the long doubling time and slow-growing nature of brucellae (Yagupsky, 1999).

Serological methods are the mainstay of diagnosis, but need clinical correlation, as single titres are not diagnostic, especially in *Brucella*-endemic areas. Paired sera showing a considerable rise in titre are desirable, but this is not usually possible due to practical difficulties (Al Dahouk *et al.*, 2003; Kumar *et al.*, 1997; Orduna *et al.*, 2000). As on-and-off fever is typical of brucellosis, coinciding with the waves of

Fig. 2. PCR for *B. melitensis*. Lanes: 1, *B. melitensis* (positive control); 2, patient serum; 3, negative control (no template); M, 100 bp molecular mass marker (Bangalore Genei).

bacteraemia, clinical correlation is highly equivocal in symptom-free periods. Molecular techniques, if available, play an important role in clinching the confirmatory evidence (Morata *et al.*, 2001; Romero *et al.*, 1995), as in this case. This is particularly true in cases that are culture negative, but serologically positive without significant titres. This is especially relevant in *Brucella*-endemic areas, where high base-line titres commonly occur and pose a significant problem in interpretation.

The pathogenic nucleic acids in serum samples are most probably released into the circulation as breakdown products during bacteraemia. Several studies have documented the presence of circulating pathogen DNA in serum samples (Zerva *et al.*, 2001). Patients often approach their physician after taking non-specific antibiotics, with subsequent effects on isolation of the bacterium. In a study conducted by Morata *et al.* (2001), no significant difference was found in the sensitivity of PCR assays between individuals in the treated group and those who had not received antimicrobial therapy prior to collection of the sample, indicating the superiority of PCR assays over culture methods.

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