A rare case of seronegative culture-proven infection with Brucella suis

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CASE REPORT

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Abstract

Brucellosis is a chronic infection produced by members of the *Brucella* family. Diagnosis of this condition requires either isolation of the organism in culture or positive serological tests.

We describe a 27-year-old male admitted as a case of pyrexia of unknown origin (PUO), who tested negative for *Brucella* IgM ELISA test on preliminary evaluation but was subsequently diagnosed on the strength of positive blood and bone marrow cultures to be a case of brucellosis secondary to *Brucella suis* infection. In addition to highlighting the pathogenic potential of an unusual organism, this case demonstrates the unreliability of standard serological tests based on the *Brucella melitensis* antigen for infection with other species of *Brucella*.

Key Words

Brucella suis, pyrexia of unknown origin, serological tests

Implications for Practice

1. *Brucella suis* infection is extremely rare, incidence estimates for most areas are unavailable. Sensitivity and specificity of serological tests for this organism have not been determined.
2. Serological tests may be unreliable for diagnosis of *B. Suis* infection, and should not be used to definitively rule out this condition.
3. Blood cultures should be performed in all patients with suspected brucellosis even if serological tests are persistently negative.

Background

Brucellae are small, gram-negative coccobacilli with a worldwide distribution. Six species have been identified, of which four (*Brucella melitensis*, *Brucella abortus*, *Brucella suis* and *Brucella canis*) are known to be human pathogens. While brucellosis as a whole is widely prevalent in the developing world, infections by the *B. suis* organism are rare. An epidemiological study by Guerrier et al[1] yielded a mean annual incidence for *B. suis* of just 19 per 100,000 individuals in Polynesia, an area considered to have a relatively heavy burden of the disease. There are no estimates available for other regions, emphasising the rarity of infection.

Case details

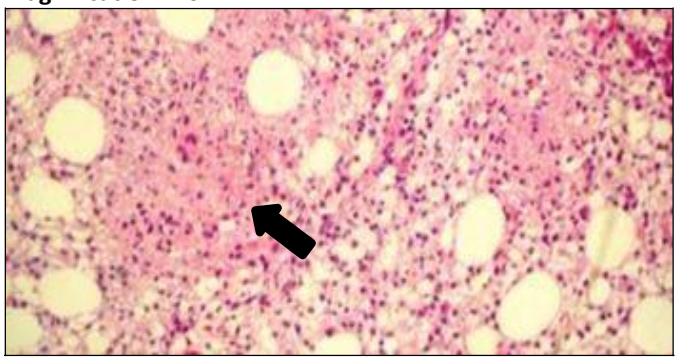
A 27-year-old male railway security guard presented with low grade remittent fever over the previous 40 days associated with profuse sweating, fatigue and weight loss of 12kg over one year. He had been treated at a local clinic with anti-malarials despite blood smears being persistently negative for malarial parasites, and was referred to us when his symptoms failed to respond. Detailed questioning failed to elicit any localising symptoms including musculoskeletal pain. His past medical history was unremarkable. The patient also denied any history of substance abuse.

General physical examination revealed a fever of 102°F, tachycardia and tachypnoea. There was no significant lymphadenopathy. Systemic examination revealed a palpable liver two centimetres below the right costal margin in the mid-clavicular line, as well as an enlarged spleen with the tip just palpable below the left costal margin.

Complete blood counts showed relative lymphocytosis and an elevated erythrocyte sedimentation rate (ESR) of 75 mm/hr; other laboratory parameters were normal. Preliminary evaluation for causes of fever prevalent in the south-western region of India, including enteric fever and malaria, was negative. This included an IgM ELISA test for

Brucella, as well as the standard tube agglutination (STA) test. Abdominal ultrasonography confirmed the presence of hepato-splenomegaly but was otherwise unremarkable and a screening thoracic CT imaging was also normal. Pending blood culture reports, bone marrow aspiration and biopsy were performed. Smear preparation of the aspirate provided the first positive finding of the case with the report of a single granuloma. Thereafter staining of the biopsy also revealed the presence of a granuloma (Figure 1). Subsequently aerobic cultures of both blood and bone marrow by BacT/ALERT®3D (BioMerieux) technique yielded growth of gram negative coccobacilli, identified on further analysis by agglutination with a monospecific antisera as *B. suis*. Retrospective questioning of the patient revealed a history of occupational exposure to pigs.

Figure 1: Bone marrow biopsy showing the presence of a granuloma (black arrow). Haematoxylin-eosin stain, magnification x20



The patient was immediately initiated on a six-week course of rifampicin and doxycycline along with intramuscular streptomycin for the first 14 days, a regimen shown in multiple studies to be the most effective in brucellosis.[2] On follow-up, the patient reported steady improvement in weight and sustained absence of fever. Complete regression of splenomegaly was documented by palpation as well as by abdominal ultrasonography. Further blood tests showed the lymphocytosis and elevated ESR had also resolved.

Patient consent

Signed informed consent was given by the patient for publication of material pertaining to this case.

Discussion

Brucellosis in humans derives from exposure to infected animals through the ingestion of unpasteurised dairy products, inhalation of aerosolised bacteria, or from direct contact with infected animals through contaminated skin or mucosal surfaces.[3] Infection is initiated by rapid replication of the organism within regional lymph nodes followed by haematogenous dissemination, seeding the reticulo-endothelial system including the liver, spleen, and bone marrow with bacteria.[4] This feature of the disease is important because a biopsy from any of these organs can often permit diagnosis in suspected cases with persistently negative blood cultures.[5]

Clinical features of brucellosis are variable and frequently non-specific, hampering early diagnosis and treatment. Gastrointestinal and hepato-biliary involvement can afflict up to 70% of patients.[6] Endocarditis is encountered in less than 2% of cases, but accounts for the majority of brucellosis-related deaths.[7] Interestingly, Andriopoulos et al[8] describe such diverse manifestations as splenomegaly (51%), osteo-articular involvement (42%), cervical lymphadenitis (31%), hepatomegaly (25%), genitourinary involvement (13% of male cases), cholecystitis (2%) and breast abscess (0.7%) as occurring in cases of brucellosis. Infected patients had a relevant occupational history in fewer than 20% of cases. Mantur et al,[9] reported urinary tract infections and Stevens-Johnson syndrome as presentations of the disease. Respiratory involvement can also occur in brucellosis.[10] Kocher et al[11] in a study of neuro-brucellosis, reported cases of meningoencephalitis, myelitis leading to spastic paraparesis, polyradiculoneuropathy and polyneuroradiculomyelo-encephalopathy.

Laboratory tests often reveal only subtle abnormalities such as mild elevation in inflammatory markers, with occasionally elevated liver enzymes.[12] Radiographic changes can be non-specific, often mimicking slow growing neoplasms such as giant-cell tumours and multiple myeloma[6].

Such a wide range of clinical manifestations coupled with non-specific results on routine laboratory parameters can pose a significant diagnostic challenge to physicians. In such situations, evaluation can proceed by either attempted isolation of the organism in culture, or serological evidence of infection, or as in our case, by a combination of both methods.

While isolation from tissue or blood culture can yield a definitive diagnosis, there are a number of pitfalls to this approach. Brucellosis is an important cause of laboratory acquired infection among health-workers to the extent that the CDC now recommends biosafety level 3 (BSL-3) practices, equipment and facilities in all laboratories handling specimens from suspected cases of *Brucella.*[13] Unfortunately, such facilities are often unavailable in the developing world; the risk of infection can be a deterrent to attempts to isolate the organism in culture. Partial treatment with empirical broad-spectrum antibiotics can suppress bacteremia[14] without eradicating the infection, rendering blood cultures sterile. In such situations, bone

marrow cultures can still detect the organism, and are therefore considered the gold standard of diagnosis.[15]

Nonetheless, bone marrow aspiration and biopsy is a technically cumbersome, invasive and painful procedure, and is often relegated in favour of other easier techniques, principally serological. Moreover, isolation in culture is possible in 50 to 80% of patients with acute brucellosis, with the yield rate falling to less than 5% for individuals with chronic brucellosis.[16] Finally, even in the presence of bacteraemia, conventional culture in broth media can take up to six weeks, which is an extremely long period of time in regions such as India where durable patient follow-up is difficult to achieve. To some extent, this problem can be overcome by utilising automated blood culture systems such as the one utilised in this case. This can accelerate growth producing positive results from blood cultures within seven days and bone marrow cultures within four days, thus providing a relatively quick diagnosis[17] within the constraints of a limited yield rate as noted above.

This combination of drawbacks to culturing the organism has spurred the development of alternative serology-based tests for brucellosis, with the aim of achieving rapid diagnosis and cost-effectiveness.

Amongst these tests, the first-generation standard tube agglutination (STA) test and the indirect fluorescent antibody (IFA) test utilise whole cell preparations of *B. melitensis* and *B. abortus* containing A and B antigenic epitopes shared by the various species of *Brucella*. In contrast, newer ELISA-based tests employ purified lipopolysaccharide extracts of *B. melitensis* and *B. abortus*. While the older STA and IFA tests are comparable in reliability to ELISA with regard to acute brucellosis, they are of lesser value in cases of chronic brucellosis.[16] Nevertheless, their low cost and simplicity have ensured their continued application in developing countries, where the burden of brucellosis is the greatest.

In contrast, the IgM and IgG ELISA tests are considered extremely reliable with a sensitivity of 100% and a specificity of 96%.[18] Moreover, of the two, the IgM ELISA is considered superior being positive in both acute as well as chronic cases of brucellosis.[19] In our case, an IgM ELISA test was performed and was negative. Furthermore, an STA test was also negative; a negative result in a combination of two different serological tests is usually considered sufficient to rule out false-negative results.[20]

Conclusion

This case clearly demonstrates the importance of isolation of the organism in culture, despite the high specificity and sensitivity of serological tests, especially in areas where brucellosis is known to be prevalent. It is pertinent to note that serology-based investigations are only indirect indicators of infection with an inherent short-coming in respect to rare diseases like *B. suis* infection. Until the development and widespread availability of more specific PCR-based technology[21] for diagnosis of brucellosis, isolation by blood and bone-marrow culture should constitute the mainstay of diagnosis.

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PEER REVIEW

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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CONSENT

The authors declare that

1. They have obtained informed consent for the publication of the details relating to the patient in this report.
2. All possible steps have been taken to safeguard the identity of the patient.
3. This submission is compliant with the requirements of local research ethics committees.