Brucella Isolated from Bone Marrow
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Abstract
A 40 year patient presented with pyrexia of one month duration. Routine work up for fever of unknown origin (FUO) was negative. Bone marrow aspiration and culture done yielded Brucella. Bone marrow cultures are recommended for patients for FUO for whom the routine workup turns out to be negative. Serological tests for brucellosis can be false-negative in some cases of brucellosis due to prozone phenomena.

INTRODUCTION
Brucellosis is one of the uncommon causes of FUO. The diagnosis of brucellosis is often not supplanted with the isolation of the organism. We describe below a case of 40 year old male who presented with fever of unknown origin. The diagnosis of brucellosis was established by bone marrow culture while the serological tests for brucella were negative.

CASE REPORT
A 40 year male presented in March 2000 with fever of one month duration. He had a history of travel to Rajasthan one month back. Fever was low grade without rigors, chills or periodicity. There was no history of myalgia, arthralgia, diarrhea, abdominal pain, dysuria, skin rash, cough, upper respiratory symptoms, headache, night sweats, anorexia, weight loss or bleeding manifestations. There was no lymphadenopathy, organomegaly and bone tenderness. All the other systems were within normal limits.

Patient was investigated for the pyrexia. Hb was 12 gm%, TC 9800/mm³ P58 L39 E5, ESR 35 mm end of first hour. The peripheral blood smear was normal. There was no albuminuria or casts in urine. Blood chemistry, chest X-ray and electrocardiogram were normal. Blood and urine cultures were sterile. IgM Elisa for leptospiral antibody, Widal for Salmonella, Paul Bunnel for infectious mononucleosis were negative. Brucella agglutination test was negative. Peripheral blood smear and buffy coat examination was negative for malarial parasite. Sonogram of abdomen was normal. A bone marrow aspiration and culture was performed as part of the work up for FUO as the diagnosis was not obvious till then. Culture grew oxidase positive Gram-negative pleomorphic bacilli identified as brucella (Fig. 1). The patient was treated with doxycycline and netilmicin for four weeks followed by doxycycline and rifampicin for another four weeks. He became afebrile and is now asymptomatic.

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**DISCUSSION**

Brucellosis is a zoonoses caused by one of four species, *B. melitensis*, *B. abortus*, *B. canis* and *B. suis*. It is a normal flora of genital and urinary tracts of many animals including goat, cow, pig and dog. Man gets infection from infected animal reservoirs. Brucella are nonencapsulated nonsporing nonmotile Gram-negative aerobic bacilli which can survive up to eight weeks in unpasteurised goat milk and up to 90 days in cheese.

The primary virulence factor for *Brucella* seems to be its ability to survive intracellularly. Animal contact through inhalation is the most frequent mode of transmission in endemic areas. Other modes are ingestion of animal products and penetration of intact or abraded skin. Transmission through breast feeding, transplacental route and sexual route can also occur.

Brucellosis has been arbitrarily classified into acute, subacute, chronic, bacteraemic, localized or mixed types. It is a disease of protean manifestations. Asymptomatic infection is ten times more common than symptomatic infection. The most common presenting symptom is fever without any localising signs. Migratory polyarthritis of large joints, spondylitis, sterile infective endocarditis, hepatitis, genitourinary involvement, neurobrucellosis with meningoencephalitis, multiple intracranial abscesses and cranial nerve lesion are other rarer manifestations.

Diagnosis depends on clinical features with positive blood or tissue culture and/or raised brucella agglutinins. Extended incubation of blood cultures of up to six weeks is needed. Other specimens used are bone marrow, CSF, lymph nodes, urine. Blood and bone marrow cultures are positive in 50-70% of cases. The efficacy of blood culture decreases significantly with subacute and chronic forms of infection whereas that of bone marrow culture decreases only in chronic forms. Prior use of antibiotics reduced the positivity of blood cultures but did not affect bone marrow culture. Bone marrow cultures are especially recommended for patients with FUO, negative serology and unexplained articular or hematologic manifestations. *Brucella* is commonly misidentified as *Moraxella* species. *Brucella* forms small smooth transparent slow growing colonies exhibiting a clear honey color in direct light and a bluish grey transparency in reflected light. The colonies of *Moraxella* are fast growing lacking the typical honey color of *Brucella*. *Brucella* shows a positive urease test within 30 minutes whereas it takes 18-24 hours to become positive for *Moraxella*.

Serological tests for brucellosis include standard agglutination test (SAT) and enzyme linked immunosorbent assay. The commonly used test is the standard agglutination test. A standard suspension of brucella is incubated with test sera and the highest dilution of the sera at which agglutination occurs is assessed. An agglutination titer of 1:60 in nonendemic areas and 1:320-1:640 in endemic areas are considered as significant. Since both IgG and IgM antibodies are detected an elevated titer does not differentiate between recent and past infection. So the serology should be repeated after three weeks and a further rise in titer sought. A positive test remains so for more than two years after the infection. Treatment with 2-mercaptoethanol removes the IgM alone. Thus an initial high titer changing to a low titer with mercaptoethanol suggests recent infection. Prozone phenomena due to IgG and IgA blocking antibodies can give a false-negative agglutination test. This can be overcome by testing sera at high dilutions (> 1:320). The blocking antibodies can be detected by a blocking antibody assay or by a Coomb's test. Our patient had a negative serology probably due to this phenomena. False-positive tests due to immunologic cross-reactivity have been associated with serum containing antibodies to *Vibrio cholerae*, *Francisella* or *Yersinia enterocolitica*. The enzyme linked immunosorbent assay which employs subcellular antigens is more sensitive.

Management of brucellosis involves combination therapy. Single agent therapy is associated with high incidence of resistance. The combination regime of doxycycline and aminoglycoside (streptomycin/gentamycin/netilmicin) for four weeks followed by doxycycline and rifampicin for 4-8 weeks is the most effective regimen. In cases of neurobrucellosis and cardiovascular manifestations all the three drugs are given in combination for 8-12 weeks.

This case report highlights the value of bone marrow culture to the practising physician in the case of FUO for which no diagnosis is obtained by other investigations.

**REFERENCES**