

Isolation of *Brucella melitensis* from a human case of chronic additive polyarthritis

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Abstract

Brucellar arthritis remains under diagnosed owing to non-specific clinical manifestations. Here, we report isolation of *Brucella melitensis* from synovial fluid of 5th metatarsophalangeal joint of a 39-year-old lady having unusually chronic asymmetric, additive, peripheral polyarthritis. This isolation was confirmed by Bruce-Ladder polymerase chain reaction (PCR). The patient had a history of contact with an aborted goat. Rose Bengal Plate Agglutination Test (RBPT) and Standard Tube Agglutination Test (SAT) were positive for *Brucella*-specific antibodies both for patient and in contact with sheep and goats. The patient was treated with doxycycline and rifampicin for 16 weeks and was recovered fully.

Key words: Arthritis, *Brucella melitensis*, synovial fluid

Introduction

Human brucellosis is a potentially life-threatening zoonotic disease caused by *Brucella* species. It is

characterized by systemic infection affecting multiple body organs or systems with wide range of the clinical symptoms.^[1] The disease transmission to humans usually occurs by direct contact with infected animals or by inhalation of infectious aerosols or through inoculation of cuts and abrasions or indirectly by the consuming unpasteurized milk or milk products. The most common clinical features of human brucellosis are undulant fever, sweating, arthralgias, myalgias, lymphadenopathy and hepatosplenomegaly.^[1] Arthritis is one of the common complications of human brucellosis. Brucellar arthritis in India may be under-reported or undiagnosed owing to non-specific clinical manifestations and failure to arrive

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on confirmatory diagnosis, since isolation of *Brucella* from affected joints is rarely attempted.^[2] In this correspondence, we report isolation of *Brucella melitensis* from synovial fluid of 5th metatarsophalangeal joint of an unusually chronic case having asymmetric, additive peripheral polyarthritis.

Case Report

A 39-year-old premenopausal lady was presented with multiple joint pains for the last 18 months. Firstly, there was involvement of right knee and right ankle followed 6 months later by left knee and left ankle. After 10 months, the 5th metatarsophalangeal joint was also involved [Figure 1a]. The patient was also having pain in both the elbows since last 2 months. There was no history of axial or hand joints involvement or early morning stiffness or any connective tissues disorder in the patient. Personal or family history of any joint disease or rheumatoid arthritis or tuberculosis or diabetes or hypertension was also absent. In addition, no history of weight loss, ocular involvement, skin rashes, oral/genital ulcers, urinary tract infection and diarrhoea was reported by the patient preceding to onset of the illness.

The patient was a resident of a place near Palampur, Himachal Pradesh and belonged to native 'Gaddi' community, traditionally involved in sheep and goat rearing. The studied patient was also rearing different livestock species and had a history of contact with an aborted goat about 18–20 months ago.

Systemic examination of the patient revealed unusual clinical findings. Swelling was present in right ankle and both the knees, more pronounced on right knee. The 5th metatarsophalangeal joint has large fluctuant, non-tender swelling with normal overlying skin [Figure 1a]. Painless tenosynovitis of flexor tendons of both the forearms, with no swelling or tenderness in wrist joints, was observed.

Clinically, patient was having body temperature of 38°C and was anaemic with haemoglobin 10.2 g/dl. Other blood/serum biochemical parameters in normal ranges were: Total leukocyte counts (TLC, 6000/mm³), erythrocyte sedimentation rate (ESR, 13 mm/h), serum creatinine (0.7 mg/dl), serum uric acid (3.3 mg/dl), serum glutamic oxaloacetic transaminase (SGOT, 34 unit/l) and serum glutamic pyruvic transaminase (SGPT, 36 unit/l). The differential leukocyte counts revealed 76% neutrophilia and 26% lymphocytes.

Initially, patient was considered to be a probable case of reactive arthritis in view of peripheral, asymmetric, arthritis mainly involving the lower limbs. She was treated with sulfasalazine (500 mg, every 12 h), rabeprazole (20 mg, every 12 h), aceclofenac (100 mg, every 12 h) and paracetamol (500 mg, every 12 h) for 2 weeks with no improvement. On the basis of the persistence of pain and swellings in different joints and keeping in view the occupational history, the patient was suspected for chronic

brucellosis. Blood, synovial fluid and serum samples were collected from the patient aseptically and were sent to Department of Veterinary Microbiology, DGCN College of Veterinary and Animal Sciences, CSK HPKV Palampur, Himachal Pradesh for diagnosis of brucellosis by microbiological and serological methods.

For sero-diagnosis, Rose Bengal Plate Agglutination Test (RBPT) and Standard Tube Agglutination Test (SAT) were used as primary screening tests for *Brucella* diagnosis using serum.^[3] *Brucella* antigens were obtained from Indian Veterinary Research Institute (IVRI), Izatnagar, Uttar Pradesh, India. RBPT was strong positive for brucellosis, whereas SAT showed a positive titre of 1:160 IU for *Brucella*-specific antibodies. Isolation was attempted from blood sample and synovial fluid of the swollen 5th metatarsophalangeal joint on blood agar using standard protocol as described previously.^[3] Bacterial growth resembling *Brucella* species was observed after 5 days of incubation at 37°C under aerobic and microaerophilic conditions. This bacterial growth was characterized as *B. melitensis* using standard procedures of staining, biochemical and carbohydrate fermentation tests.^[4] This isolate was further confirmed by Bruce-Ladder polymerase chain reaction [Figure 2] as reported earlier that can detect and differentiate all the reported seven *Brucella* species.^[5] To further establish a zoonotic linkage, we also did *Brucella* sero-screening of five sheep and goats kept by the patient including the aborted goat, by serological tests, i. e., RBPT and SAT. All the tested animals were positive by RBPT and SAT (titres > 40 IU).

On the basis of these results, the patient was treated with doxycycline (100 mg, every 12 h) and rifampicin (450 mg, once a day) for 16 weeks.^[1] The patient started showing clinical improvement after 2 weeks of therapy. Gradually, there was relief from pain, marked decreased in the joint swellings and resolution of the wrist flexor tendon tenosynovitis. Later, patient recovered fully from the disease [Figure 1b].



Figure 1: Picture showing (a) *Brucella melitensis*-induced inflammation of ankle and 5th metatarsophalangeal joint before treatment (red arrow) and (b) recovered joints after 16 weeks of brucellosis specific treatment (blue arrows). The *B. melitensis* was isolated from the infected 5th metatarsophalangeal joint

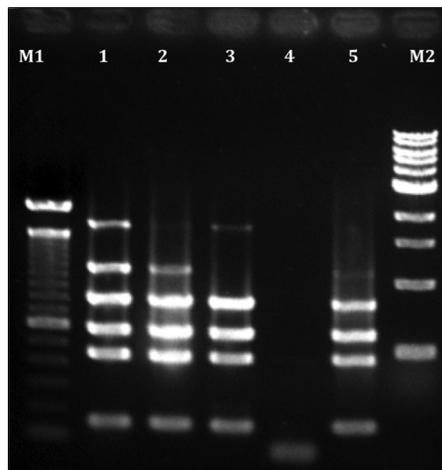


Figure 2: Confirmation of the isolated *B. melitensis* with Bruce-ladder polymerase chain reaction (PCR). Lane M1 is 100 bp and M2 1Kb ladders, 1 is isolated *B. melitensis*, 2 and 5 are *B. melitensis* controls, 3 is *B. abortus* and 4 is negative control

Discussion

Brucellosis remains to be a major debilitating disease worldwide. Globally, the incidence of the disease is <0.01 to >200 per 100,000 population, and is endemic in certain parts of the world.^[6] However, the true incidence of human brucellosis is not known in most of the countries including India. Human brucellosis has been reported occasionally from India both from general and occupationally exposed human populations.^[1,6] *B. melitensis* and *B. abortus* are the major species of concern in human beings.^[6] Peripheral arthritis is one of the important clinical musculoskeletal presentations of human brucellosis.^[7] This condition usually involves one to three joints at a time. Mostly unilateral involvement of hip, knee, ankle is observed. The first sacral joint and/or lumbar or dorsal spondylitic joints may also be involved.^[8] This case was unique due to the involvement of four joints along with painless tenosynovitis of forearm flexors. This degree of involvement can be attributed to the long duration (18 months) of the disease condition. It is reported that osteoblasts and fibroblasts like synoviocytes produce cytokines and chemokines in response to *Brucella* infection. These cytokines promote the secretion of metalloproteinases and induce osteoclastogenesis. This causes the bone loss and cartilage degradation as observed in brucellar arthritis.^[9]

There are reports showing presence of *Brucella* antibodies in patients suffering from arthritis but isolation of *Brucella* organism from the synovial fluid, which confirms the brucellar septic arthritis, is reported rarely worldwide including India.^[2,10-12] This can be attributed to the fastidious nature of this micro-organism.^[3,4] To our knowledge, in India, it is the second report of isolation of *B. melitensis* from the synovial fluid of a patient suffering from polyarthritis.^[10] The patient fully recovered from the

disease symptoms after complete course of *Brucella* specific treatment.^[13]

Brucella arthritis is more commonly observed in children or young adults.^[3,11] The age of the patient in this case was 39 years, which indicates that brucellar arthritis can occur in different age-groups with the involvement of multiple joints and is not necessarily a disease of young age-groups.

Conclusion

It can be concluded that patients with peripheral (pauci/poly) arthritis should be examined for brucellosis in routine, particularly cases not cured by standard arthritis treatment coupled with corroborative history of contact with brucellosis infected animals or animal products. Further, on the basis of symptoms, brucellosis should be differentially diagnosed from other disorders such as reactive arthritis, prolapsed inter-vertebral disc, tuberculosis, osteosclerosis, gout and psychosomatic backache. However, a higher suspicion for brucellosis should be maintained in an appropriate epidemiological setting. It is important to screen appropriate clinical specimens like serum and synovial fluid for *Brucella* diagnosis.

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