

Case Study

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## Incidental Laboratory Identification of *Brucella melitensis* in a case with Pancytopenia

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### ABSTRACT

Brucellosis is a bacterial zoonosis usually associated with exposure to infected animals or their products. Although a significant public health problem in India, exact prevalence and distribution are unknown owing to the imprecision of diagnosis and inadequacy of reporting and surveillance. Although the febrile illness is common, its manifestations are highly variable. Bone marrow suppression and consequent pancytopenia have been rarely reported. We present a case of 50 years old female diagnosed with human brucellosis associated with pancytopenia and non-specific clinical presentation, that was diagnosed incidentally on blood and bone-marrow culture. This was confirmed by serological tests like the standard agglutination test. Culture isolation using automated blood culture (e.g. BacT/ALERT), followed by identification using automated identification systems (e.g. MALDI-TOF and VITEK-2) help to reach accurate and timely diagnosis aiding in the management of the patient.

#### Keywords

Brucellosis, occupation, dairy, bone marrow suppression, pancytopenia

#### Article Info

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### Introduction

#### Case Report

A 50 years old non-diabetic non-hypertensive female, milkmaid by occupation from southern India presented to the medicine outpatient department with complaints of vague non-localizing lower backache, easy fatigability, and generalized weakness for the

past one month along with intermittent non-projectile vomiting for seven days. She also complained of unexplained weight loss and anorexia for the last month.

On clinical examination, she had moderate pallor, with no signs of icterus, pedal edema, lymphadenopathy, clubbing, or cyanosis. She also had mild hepato-splenomegaly. During the initial course of stay in the hospital, the

patient developed spikes of mild grade fever not associated with chills, rigor, or any rashes. On routine hemogram, the patient was diagnosed to have normocytic anaemia (Hb 8.9gm/dL), thrombocytopenia (platelet count of 92,000/ $\mu$ L) as well as leucopenia (total leucocyte count of 3000/ $\mu$ L).

Because of the fever spikes, a blood culture specimen was sent to microbiology laboratory in BacT/ALERT automated adult aerobic blood culture bottle, which was immediately incubated in VirtuoBacT/ALERT system (bioMerieux) at 37°C. The bottle flagged positive after 63 hours of incubation. Gram staining was performed directly from flagged broth, which showed short gram-negative coccobacilli. Subculture from the bottle was performed on 5% sheep blood agar (SBA), chocolate agar (CA), and MacConkey agar (MAC), and incubated aerobically at 37°C. Faint colonies were visible after 20 - 22 hours of aerobic incubation. Gram staining from colony showed gram-negative coccobacilli (approx. 1.5 $\mu$ m  $\times$  1 $\mu$ m), which was catalase and oxidase test positive. Automated identification was performed using Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (VITEK-MS), which identified it as *Brucella* species. Identification was subsequently confirmed using conventional phenotypic tests. *Brucella* differential stain (Stamp's modification of Ziehl - Neelsen staining) was performed in which organism appeared reddish-pink coccobacilli. Urea was rapidly hydrolyzed within 20 minutes of aerobic incubation. Growth was enhanced on incubation in capnophilic (5 - 10% CO<sub>2</sub>) incubation. Species identification was made by using the VITEK-2 automated system using the gram-negative identification card, which was identified as *Brucella melitensis*. Subsequently, serology by standard agglutination test (SAT) was also performed on the patient's serum to detect the presence of

agglutinating antibodies against *Brucella*, which also turned out reactive with a titer of 1:320.

Paired blood culture was repeated after five days from initial blood culture which flagged positively with time to positivity of 66 hours and isolate was again identified as *Brucella melitensis*. Because of existing pancytopenia, diagnostic bone marrow aspiration was performed and the bone marrow sample was sent for aerobic culture directly in paired BacT/ALERT pediatric blood culture bottles. The bottles flagged positively at 55 hours and 60 hours of incubation respectively and yielded subsequently the same organism, *Brucella melitensis*. Bone marrow cytology revealed hypocellular patches, with suppressed leucopoiesis and erythroid hyperplasia.

Following laboratory confirmation, detailed history was evaluated for zoonotic exposure to the patient. The patient was a milkmaid by profession; frequently involved handling of cattle. She also gave an occasional history of intake of unpasteurized raw cow milk. In addition, she revealed that she had an episode of oral ulcers for the past 15 days, for which she has taken goat milk as a local remedy a few days back.

Two-dimensional echocardiography (ECHO) was performed to rule out infective endocarditis as vegetations could serve as a niche for this organism but ECHO does not reveal any vegetations. Because of persistent backache, an X-ray of the sacroiliac joint was performed to rule out sacroiliitis, which was found to be normal. No other features suggestive of focal brucellosis or complicated brucellosis could be elicited.

On reporting of *Brucella* from blood culture, the patient was started on oral doxycycline 200 mg twice daily and injectable streptomycin

1gram intramuscularly. She improved clinically after seven days of antimicrobial therapy and was subsequently discharged on injectable streptomycin and oral doxycycline for 3 months. Informed consent was obtained subsequently from the patient during the stay in the hospital.

## Results and Discussion

Brucellosis is a bacterial zoonosis and is usually associated with occupational or domestic exposure to infected animals or their products like ingestion of unpasteurized raw milk(1). Though it is a significant public health problem in India, the exact prevalence and distribution are unknown owing to the imprecision of diagnosis and the inadequacy of reporting and surveillance systems.

*Brucella* is an obligately aerobic, catalase-positive, oxidase-positive, fastidious, small intracellular gram-negative coccobacillus. *Brucella melitensis* accounts for the major proportion of human infections(2,3). Brucellosis is a multisystem infection that may present with a broad spectrum of clinical presentations with the most common being acute febrile illness and musculoskeletal symptoms. It tends to involve reticuloendothelial cells. Its clinical manifestations are highly variable, and definitive symptoms indicative of the diagnosis may be lacking. Bone marrow involvement can lead to hematological complications such as anemia, leukopenia, or pancytopenia, and are rarely reported (4,5,6). Thus even on the smallest amount of clinical suspicion, the diagnosis must be attempted using bacteriologic and/or serologic tests. *Brucella* species have been reported in patients presenting with autoimmune hemolytic anemia (2), febrile neutropenia (7),

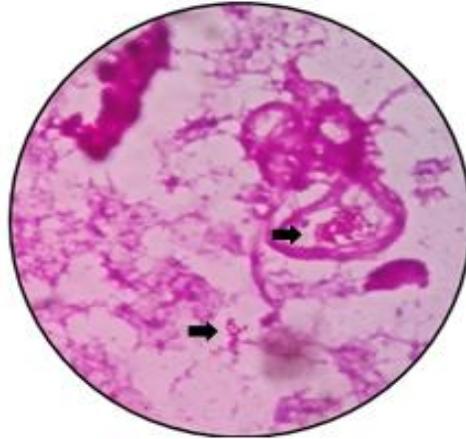
immune thrombocytopenic purpura (8), disseminated intravascular coagulation (9), myelodysplastic syndrome (4). *Brucella* infection is known to induce several hematological abnormalities already well described in the above-mentioned literature. Leukopenia is more common, occurring in 16 to 25% of patients (5) whereas the frequency of pancytopenia ranges from 3 to 20% (6). Our patient presents with pancytopenia similar to several other studies (3-7). Pancytopenia associated with brucellosis is attributed to hypersplenism, hemophagocytosis, and granulomatous lesions of the bone marrow, which is usually hypercellular. Bone marrow hypoplasia is rarely reported. (3) This is probably because the organisms exert a direct inhibitory effect on marrow cells or they induce lymphocytes to release mediators that inhibit hematopoiesis(3).

*Brucella* is a facultative intracellular bacterium, and disseminate through the hematogenous route in the body (10). There is a strong predilection for organs of the reticuloendothelial system consequently, brucellosis often presents with hematologic abnormalities (7, 10).

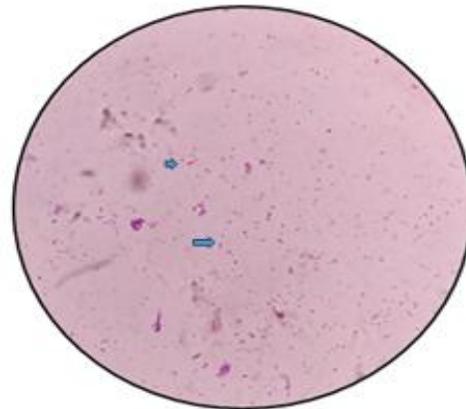
In this study performed a literature review on PubMed search database using the search term "*Brucella*" and "pancytopenia". Around 40 literatures were found of brucellosis presenting as pancytopenia. On literature review, most common age group reported with brucellosis and cytopenia were children and adolescents (11-12). Adults have been reported sporadically (4-7), as in our case.

We could not determine initially whether the patient had pancytopenia secondary to brucellosis, or the brucellosis occurred in preexisting pancytopenia.

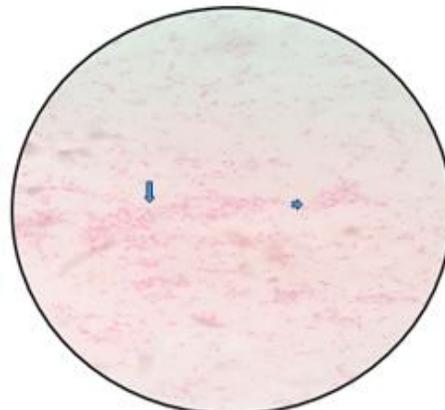
**Fig.1** Gram negative coccobacilli resembling *Brucella* species at 100X magnification in Gram stain from flagged blood culture bottle



**Fig.2** Gram negative coccobacilli resembling *Brucella* species at 100X magnification in Gram stain from colony



**Fig.3** Reddish pink acid-fast coccobacilli resembling *Brucella* species at 100X magnification in Stamp's modified Ziehl-Neelsen staining from colony



**Fig.4** Small non-pigmented grey colonies of *Brucella* species on Chocolate agar after 48 hours of capnophilic incubation



However, isolation of *Brucella* from bone-marrow culture and the remarkable clinical response after starting targeted therapy for the infection support the hypothesis that *Brucella* was the cause of the pancytopenia. Simultaneous isolation of *Brucella* from blood and bone-marrow culture has also been reported from other studies (13).

The possible source of the infection was traced back to one of three zoonotic exposure - i) milkmaid by profession; frequently involved handling of cattle; ii) history of intake of unpasteurized raw cow milk; iii) history of goat milk as a local remedy a few days back, as found in other studies (12). These findings suggest the potential source of exposure to this zoonotic pathogen in this patient with bone marrow suppression and pancytopenia.

Bone marrow hypoplasia is rarely reported and should be kept in mind in the etiology of aplastic anemia in a country where brucellosis is frequently encountered. Pancytopenia with hypocellular bone marrow although rare, may complicate the course of *Brucella* infection. We conclude that it is always important to obtain a comprehensive patient history and consider brucellosis as one of the broad

differential diagnoses for any patient with a vague constellation of symptoms of back pain, easy fatiguability, and generalized weakness and subsequently warrant automated blood cultures.

#### **Conflicts of Interest**

The authors declare that there are no conflicts of interest regarding the publication of this article.

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