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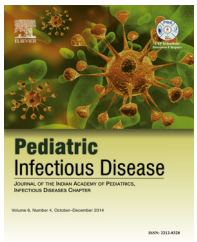

Notes from the Lab

Lab diagnosis of brucellosis

Bhaskar Shenoy [a,*], *Anupam Jaiswal* [b], *Anuradha Vinod* [c]

[a] *Consultant & Chief, Division of Infectious Diseases, Department of Pediatrics, Manipal Hospital, Bangalore 560017, India*

[b] *Specialist Pediatric ER, Department of Pediatrics, Manipal Hospital, Bangalore 560017, India*

[c] *Consultant Paediatrician, Department of Pediatrics, Manipal Hospital, Bangalore 560017, India*

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ABSTRACT

Brucellosis is a widespread zoonotic disease posing serious public health problem especially in developing countries. The diagnosis of brucellosis is a greater challenge, as it requires a high index of suspicion, clinical and laboratory evidence of diagnosis. Increased travel across the globe to endemic areas for work and pleasure over the last few years has led to increased diagnostic challenges in non-endemic countries.

Laboratory diagnosis of brucellosis is essential for diagnosis and effective treatment, as it involves two drugs or more for a prolonged period unlike many other infections. Among the various tests available, culture of the organism from the bone marrow, blood and other tissues is the gold standard for diagnosis in brucellosis. However, it is invasive, time consuming and at times less sensitive. These drawbacks have prompted the use of alternate methods to rapidly and accurately diagnose brucellosis and aid in early intervention or therapy.

The alternate methods predominantly include serological tests such as enzyme-linked immunosorbent assay, serum agglutination testing and molecular tests with varying advantages and drawbacks suited to various clinical situations. These tests help not only in diagnosis but also in follow-up of disease activity/response to therapy. This article reviews the various culture methods, serological tests and newer diagnostic methods available in making a laboratory diagnosis of brucellosis along with their advantages and drawbacks.

1. Introduction

Brucellosis is a re-emerging widespread zoonosis. It is transmitted by contact with fluids from infected animals (sheep, cattle, goats, pigs and other animals) or derived food products such as unpasteurized milk and cheese. Brucellosis is the most common zoonosis in the world accounting for more than five lakh cases occurring annually.[1] It is particularly a problem in developing countries with high morbidity and is an important cause of economic loss. The reported incidence of *Brucella* in endemic areas varies between <0.01 to >200 per 100,000 population. The low levels of incidence reported in known endemic areas may reflect low or absent levels of surveillance and reporting. However, the true incidence of brucellosis is unknown in most developing countries. Even

* *Corresponding author*. Tel.: +91 9845036174.
E-mail address: bshenoy@gmail.com (B. Shenoy).

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though brucellosis is less common in pediatric population than adults, places, where *Brucella melitensis* is endemic, pediatric cases are seen.

2. Clinical features

The incubation period varies from 1 to 4 weeks (occasionally several months). Brucellosis is a systemic disease with a broad clinical spectrum, ranging from asymptomatic disease to severe and/or fatal illness. Fever (mostly fever of undetermined origin) is the most common clinical presentation, arthralgia is also common. Physical signs include hepatosplenomegaly, generalized lymphadenopathy and arthritis (in some cases). Neurobrucellosis presents with manifestations like altered sensorium, seizures, increased intracranial pressure meningitis and encephalitis.

3. Diagnosis

The diagnosis of brucellosis is difficult on clinical grounds alone and hence invariably based on microbiological and serological laboratory tests. A high index of clinical suspicion that is based on epidemiological information along with travel and occupational history (and exposure to animals and exotic food) is critical in suspecting brucellosis clinically.

4. Lab diagnosis

The routine laboratory tests are nonspecific and include normal to low leukocyte counts with occasional pancytopenia. Minor abnormalities in liver enzymes are common.[2] In neurobrucellosis, the cerebrospinal fluid shows pleocytosis (10–200 WBC) with mononuclear cells predominantly. The protein levels are elevated and hypoglycorrhachia is also a common finding. When findings consistent with aseptic meningitis are found; an elevated level of ADA in CSF suggests *Brucella* meningitis.[3] The synovial fluid in *Brucella* arthritis presents similar to reactive arthritis (due to *Salmonella* and *Yersinia*) with a WBC count not exceeding 15,000 cells/μl with lymphocytic predominance.

5. *Brucella* cultures

The ideal method in making a diagnosis of brucellosis would be culturing the organism from blood, bone marrow, liver biopsy specimen and/or other body fluids or tissues.[4]

5.1. *Blood culture in brucellosis*

Isolation of *Brucellae* from blood cultures is restricted by the slow growth of this intra-cellular organism and by the previous use of antimicrobials. Successful diagnosis of brucellosis necessitates a careful selection of the best suitable culture method and validation of its performance. The sensitivity of culturing *Brucella* species from blood varies from 15% to 70%.[5]

There are three main types of culturing techniques:

- The traditional Ruiz-Castaneda method.
- The automated systems.
- The yield-optimizing methods such as the lysis centrifugation technique.

The traditional methods take longer time and extension of culture for days to weeks is usually needed to improve the yield rate. The use of various automated culture systems has improved the yield rate and has significantly decreased the period of time to obtain a positive culture. The vital automated system can detect *Brucellae* in 36–48% of blood cultures within 7–10 days.[6] The Bactec 9240 and 9120 automated systems can yield positive cultures in up to 95% of cases within 4–7 days. The Bact/Alert automated culture system yield is about 51% within 4–5 days. The lysis centrifugation method has high sensitivity in confirming chronic brucellosis and comparable to automated systems in acute cases.[7] Overall the BACTEC 9000 series is more sensitive and faster than Lysis centrifugation method.[8]

5.2. *Bone marrow culture*

The bone marrow culture is the ideal method and the gold standard in diagnosing brucellosis. It has the advantage that the sensitivity does not reduce with prior use of antibiotics.[9] While the time to detection is 7–21 days with blood culture, in comparison bone marrow cultures take lesser time and are much more sensitive especially in chronic cases.[9] In patients with brucellosis, bone marrow biopsies may show specific granulomatous changes and cultures of bone marrow aspirates may become positive thus helping in establishing the diagnosis.

Bone marrow examination and *Brucella* cultures of the marrow aspirate are indicated under the following circumstances:

1. In patients with fever of unknown origin, particularly in areas those are endemic for brucellosis,
2. In patients strongly suspected to have brucellosis on clinical grounds but have negative serology,
3. In patients having unexplained articular or hematological involvement, who live in endemic areas, and
4. In areas where advanced facilities such as automated culture systems or polymerase chain reaction (PCR) are not readily available.

6. Serological test

Despite having a plethora of serological tests for the diagnosis of brucellosis, none of these tests are 100% reliable or perfect. So, serological test results should always be considered or interpreted in conjunction with patient history, clinical manifestations and other laboratory findings. The serological tests detect antibodies to the antigens of *Brucella* in blood. The antigens include smooth-lipopolysaccharide (S-LPS) and cytosolic protein. The serological tests such as serum agglutination