

# BRUCELLOSIS - UPDATE ON LABORATORY DIAGNOSIS

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## Introduction

Human brucellosis is a zoonotic disease with a major impact on public health.

The number of undetected cases still remains high and this can be attributed to the non-specific clinical picture of human brucellosis, low awareness of the disease in non-endemic countries and shortcomings in laboratory diagnosis.

The number of human cases is directly correlated with the number of infected animals within a defined region. Once the disease has been transmitted from its animal reservoir to humans, only early diagnosis and adequate antibiotic therapy can prevent serious sequelae in patients.

## Pathogenesis & Pathology

The common routes of infection in humans are the intestinal tract (ingestion of infected milk), mucous membranes (droplets), and skin (contact with infected tissues of animals). Cheese made from unpasteurised sheep or goats' milk is a particularly common vehicle.

The *Brucella* spp. that infect humans have apparent differences in pathogenicity:

- *B. abortus* usually causes mild disease without suppurative complications; non-caseating granulomas of the reticulo-endothelial system are found.
- *B. canis* also causes mild disease.
- *B. suis* infection tends to be chronic with suppurative lesions; caseating granulomas may be present.
- *B. melitensis* infection is more acute and severe.

## Clinical Findings

The incubation period is 1 – 6 weeks. The onset is insidious, with malaise, fever, weakness, arthralgia, and sweats. The fever usually rises in the afternoon, and falls during the night accompanied by drenching sweat. There may be gastrointestinal and nervous symptoms. Lymph nodes enlarge, and the spleen becomes palpable. Hepatitis may be accompanied by jaundice. Deep pain and disturbances of motion, particularly in vertebral bodies, suggest osteomyelitis. These symptoms of generalised *Brucella* infection generally subside in weeks or months, although localised lesions and symptoms may continue.

Following the initial infection, a chronic stage may develop, characterized by weakness, aches and pains, low-grade fever, nervousness, and other non-specific symptoms compatible with neuropsychiatric abnormalities. *Brucella* spp. cannot be isolated from the patient at this stage, but the agglutination titre may be high.

The diagnosis of "chronic brucellosis" is difficult to establish with certainty unless local lesions are present.

## Diagnostic Laboratory Tests

### Culture

The number of bacteria in clinical samples may vary widely, with the isolation of *Brucella* being highly dependent on the stage of disease (acute versus chronic), antibiotic pre-treatment, the existence of an appropriate clinical specimen and the culturing methods used. Isolation rates are much higher during the first two weeks of symptomatic disease and in blood or bone marrow cultures taken during the pyrexial phase.

### Serology

In contrast to bacterial culture, serological testing is fast, non-hazardous and more sensitive, and is therefore preferred in routine clinical practice. However, serological tests can only indirectly detect *Brucella* infections by high or rising titres of specific antibodies. Serological tests can also not be used for speciation due to considerable cross-reactivity between *Brucella* species. Cross-reaction between *Brucella* and other gram-negative bacteria (e.g. *Yersinia enterocolitica*, *Francisella tularensis* and *Vibrio cholera*) may lead to false-positive serology results.

Agglutination titres  $\geq$  1:160 or a fourfold rise in titre between acute and convalescent sera taken at least 14 days apart are considered to be indicative of active infection. However, diagnostic high titres can be detected months or even years after acute infection despite therapeutic success and negative blood cultures. Patients in endemic regions may have persistent antibody titres due to ongoing exposure to *Brucella*.

Effective antibiotic therapy is usually accompanied by a decline in antibody titres, whereas persisting high IgG titres can be an indication of treatment failure. Relapse is often characterised by a second peak of anti-*Brucella* IgG (but usually not IgM) immunoglobulins.

IgM antibody levels rise during the first week of acute illness, peak at 3 months, and may persist during chronic disease. Even with appropriate antibiotic therapy, high IgM levels may persist for up to 2 years in a small percentage of patients. IgG antibody levels rise about 3 weeks after onset of acute disease, peak at 6 – 8 weeks, and can remain detectable lifelong despite successful treatment.

### Molecular testing

Polymerase chain reaction (PCR) assays can be used to detect *Brucella* DNA in pure cultures and in clinical specimens. Direct detection of *Brucella* DNA in patient samples is a challenge because of the small number of bacteria present in clinical samples and inhibitory effects arising from matrix components.

## Challenges in laboratory testing

In endemic countries with limited financial means, diagnosis of brucellosis should rely on two criteria: clinical presentation and laboratory diagnosis. This diagnosis should be based either on a positive culture from blood or organ samples or on positive serological results, preferably demonstrating rising titres. In rare cases, clinical diagnosis without laboratory confirmation can justify therapy.

## Treatment

*Brucella* spp. may be susceptible to tetracyclines or ampicillin. Symptomatic relief usually occur within a few days after treatment with these drugs is begun. However, because of their intracellular location, the organisms are not readily eradicated completely from the host. For best results, treatment must be prolonged. Combined treatment with a tetracycline (such as doxycycline) for 6 weeks PLUS either streptomycin for 2 – 3 weeks or rifampicin for 6 weeks is recommended.

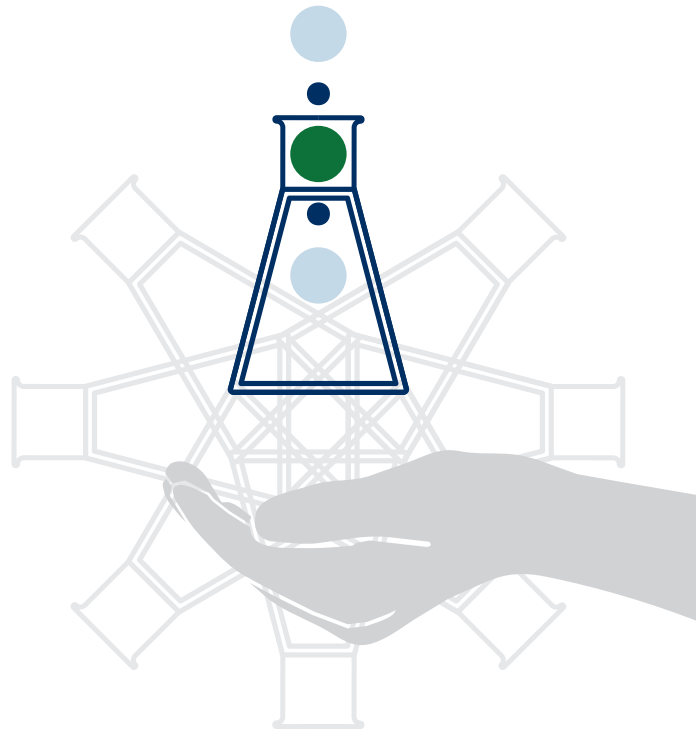
## Epidemiology, Prevention, & Control

Because of occupational exposure (e.g. farmer and veterinarian), *Brucella* infection is much more frequent in men. The majority of infections remain asymptomatic (latent).

Active immunisation of humans against *Brucella* infection is experimental only. Control rests on limitation of spread and possible eradication of animal infection, pasteurisation of milk and milk products, and reduction of occupational hazards wherever possible.

## References

1. Jawetz, Melnick, & Adelberg's Medical Microbiology; 24th edition, 2007;
2. Al Dahouk S, et al. New developments in the diagnostic procedures for zoonotic brucellosis in humans; Rev Sci Tech 2013; 32: 177 – 188.



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