

IN THE NAME OF GOD



BRUCELLAE



Introduction

- ⊙ **Obligate parasites** of animals and humans
- ⊙ **Intracellular**
- ⊙ **Metabolically inactive**
- ⊙ **7 Species**
- ⊙ *Brucella abortus* typically infects cattle
- ⊙ *Brucella melitensis* goats
- ⊙ *Brucella suis*, swine
- ⊙ *Brucella canis*, dogs
- ⊙ *Brucella ovis*, sheep
- ⊙ *Brucella neotome*, rat
- ⊙ *Brucella maris*

Introduction

Biovars

1. *Brucella abortus* 7 biovar
2. *Brucella melitensis* 3 biovar
3. *Brucella suis* 5 biovar
4. *Brucella canis, ovis, neotome* and *maris* 1 biovar

The Many Names of Brucellosis

Human Disease

Malta Fever

Undulant Fever

Mediterranean Fever

Animal Disease

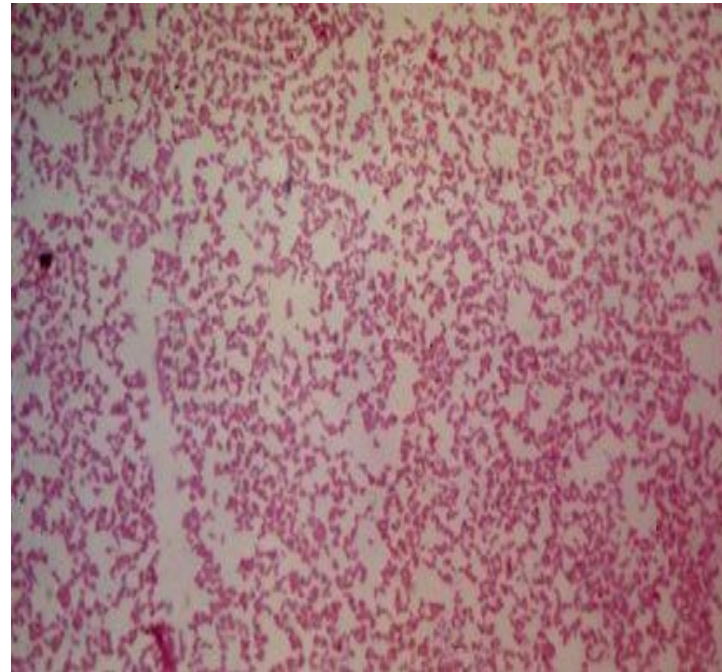
Bang's Disease

Contagious Abortion

Morphology and Identification

A. Typical Organisms

- ⊙ Small **gram-negative coccobacilli**
- ⊙ Gram negative but often **stain irregularly**
- ⊙ Strict aerobe
- ⊙ Nonfermenter
- ⊙ Nonmotile
- ⊙ Nonspore forming
- ⊙ **Intracellular** microbes



Morphology and Identification

B. Culture

- ⊙ Fastidious
 - Requires complex media containing amino acids, vitamins, salts, and glucose
 - **Prolonged incubation** for in vitro growth
- ⊙ Best medium is **Trypticase Soya Agar** with selective agents (Polymyxin, Cyclohexamide) or blood culture media
- ⊙ Translucent and glistening **colonies after 3 or more days** of incubation
 - Small
 - Convex
 - Smooth colonies



Morphology and Identification

C. Growth Characteristics

- ⦿ *B abortus* requires 5–10% CO₂ for growth
- ⦿ The other three species grow in air

Biochemical reactions

- ⊙ Catalase and Oxidase positive
- ⊙ Nitrates are reduced
- ⊙ Rapidly positive for urease
- ⊙ **Negative for IMViC reactions**
- ⊙ Brucellae use carbohydrates but produce neither acid nor gas
- ⊙ **Hydrogen sulfide** is produced by many strains

Susceptibility

- ⊙ Sensitive to heat and acidity
 - Killed at 60⁰ C in 10 minutes
- ⊙ Survival is long in refrigerated milk and cheese and ice creams
- ⊙ They are killed in milk by pasteurization

Morphology and Identification

D. Variation

- ⊙ **Virulent** organism forms a smooth, transparent colony
- ⊙ Rough form is **avirulent**
 - **D-alanine** and mutation

Transmission to Humans

- I. **Conjunctiva or broken skin** contacting infected tissues
 - Blood, urine, vaginal discharges, aborted fetuses, placenta
- II. **Mucous membranes (droplets)**
- III. **Ingestion**
 - Raw milk & unpasteurized dairy products
 - Cheese made from unpasteurized goats' milk is a **particularly common** vehicle
 - **Rarely** through **undercooked meat**
- Incubation varies
 - 5 days to three months

Who is at Risk?

⊙ Occupational Disease

- Veterinarians
- Cattle ranchers
- Meat inspectors
- Lab workers



⊙ Hunters

⊙ Travelers

⊙ Consumers of unpasteurized dairy products

Antigenic Structure

- ⊙ Antigen A and M
- ⊙ OMP
- ⊙ GMP and adenine

Pathogenesis and Pathology

1) Entry

- Skin, mucous membranes and ingestion

2) lymph nodes

3) Taxicab

4) Bloodstream

- ❖ liver, spleen, bone marrow and reticuloendothelial system

5) Granulomatous nodules

- ❖ liver, spleen and bone marrow

Pathogenesis and Pathology

- ⊙ Granuloma consist of epithelioid and giant cells, with central necrosis and peripheral fibrosis
- ⊙ The brucellae that infect humans have apparent differences in pathogenicity:
 - *B abortus* usually causes mild disease without suppurative complications; noncaseating granulomas of the reticuloendothelial system
 - *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

Pathogenesis and Pathology

- ⊙ Placentas and fetal membranes of cattle, swine, sheep, and goats contain erythritol, a growth factor for brucellae.
- ⊙ The proliferation of organisms in pregnant animals leads to placentitis and abortion
- ⊙ There is no erythritol in human placentas, and abortion is not part of *Brucella* infection of humans

Clinical Findings

- ⊙ The incubation period ranges from 1–4 weeks
- 1. **Acute stage** (primary clinical manifestation)
 - Malaise, fever, weakness, aches and sweats
- 2. The **fever** usually **rises** in the **afternoon**; its **fall** during the **night** is accompanied by drenching sweat
- 3. There may be **gastrointestinal** and **nervous symptoms**
- 4. **Lymph nodes enlarge**, and the spleen becomes palpable
- 5. **Hepatitis** may be accompanied by jaundice
- 6. **Osteomyelitis**

Clinical Findings

❑ **chronic stage**

- ❑ Weakness
- ❑ Aches
- ❑ Pains
- ❑ Low-grade fever
- ❑ Nervousness
- ❑ Nonspecific manifestations compatible with psychoneurotic symptoms.
- ❑ **Brucellae cannot be isolated from the patient at this stage, but the agglutinin titer may be high**
- ❑ The diagnosis of “chronic brucellosis” is difficult to establish with certainty unless local lesions are present

Diagnostic Laboratory Tests

Because brucellae are hazardous in the laboratory, tests to classify them should be performed only in **reference public health laboratories** using appropriate biosafety precautions

A. Specimens

- ⊙ Blood should be taken for culture
- ⊙ Biopsy material for culture (lymph nodes, bone)
- ⊙ Serum for serologic tests
- ⊙ **Bone marrow** and **blood** are the specimens from which brucellae are most often isolated

Diagnostic Laboratory Tests

B. Culture

- ⊙ Brucella species bacteria grow on commonly used media, including
 - A. Trypticase-soy medium with or without 5% sheep blood
 - B. Brain–heart infusion medium
 - C. Chocolate agar

- ⊙ All cultures should be incubated in 8–10% CO₂ at 35–37°C and should be observed for 3 weeks before being discarded as being negative results

Diagnostic Laboratory Tests

D. Blood culture

- The method of choice for bone marrow is to use **pediatric Isolator tubes**, which do not require centrifugation
- **Negative culture** results for *Brucella* do **not exclude** the disease because brucellae can be cultivated from patients only during the acute phase of the illness or during recurrence of activity

Diagnostic Laboratory Tests

- ⊙ After a few days of incubation on agar media
- ⊙ They are nonhemolytic
- ⊙ The observation of tiny gram-negative coccobacilli
- ⊙ Catalase positive
- ⊙ Oxidase positive
- ⊙ All further work on such a culture should be done in a biologic safety cabinet.

Diagnostic Laboratory Tests

- ⊙ A positive urease test
 - **B suis** and some strains of **B canis** can yield a positive test result less than 5 minutes
 - Other strains take a few hours to 24 hours
- ⊙ Molecular methods have been developed to rapidly differentiate among the various biovars.

Diagnostic Laboratory Tests

C. Serology

- ⊙ IgM antibody levels rise during the **first week** of acute illness, **peak at 3 months**
- ⊙ **IgG** antibody levels rise about **3 weeks** after onset of acute disease, **peak at 6–8 weeks**, and remain high during chronic disease
- ⊙ **IgA** levels parallel the IgG levels
- ⊙ The usual serologic tests may fail to detect infection with *B canis*

Diagnostic Laboratory Tests

C. Serology

1. Agglutination test

- ⊙ IgG agglutinin titers above 1:80 indicate active infection.
 - Cholera vaccine
 - Tularemia agglutinins
- ⊙ Blocking antibodies (IgA antibodies)
 - Coombs antiglobulin method
 - antihuman globulin

Diagnostic Laboratory

C. Serology

3. ELISA assays

- ⊙ IgG, IgA, and IgM antibodies may be detected using enzyme-linked immunosorbent assay (**ELISA**)
- ⊙ Use cytoplasmic proteins as antigens
- ⊙ These assays tend to be **more sensitive and specific** than the agglutination test especially in the setting of chronic disease

Treatment

- ⊙ Brucellae may be susceptible to:
 - Tetracyclines
 - Rifampin
 - Trimethoprim– sulfamethoxazole
 - Aminoglycosides
 - Quinolones
- ⊙ Because of their **intracellular** location, the organisms are **not readily eradicated** completely from the host
 - Treatment must be **prolonged**
 - **Combined treatment** with a tetracycline (eg, doxycycline) and either streptomycin for 2–3 weeks or rifampin for 6 weeks is recommended

Epidemiology, Prevention, and Control

- ⊙ The common sources of infection for humans are unpasteurized milk
- ⊙ Milk products
- ⊙ Cheese
- ⊙ Occupational contact

The airborne route may be important

Epidemiology, Prevention, and Control

- ⊙ Because of occupational contact, **Brucella infection is much more frequent** in men
- ⊙ The majority of infections remain asymptomatic (latent).

Epidemiology, Prevention, and Control

- ⊙ Active immunization of humans against *Brucella* infection is experimental
- ⊙ Vaccination with Rb51 in cattle