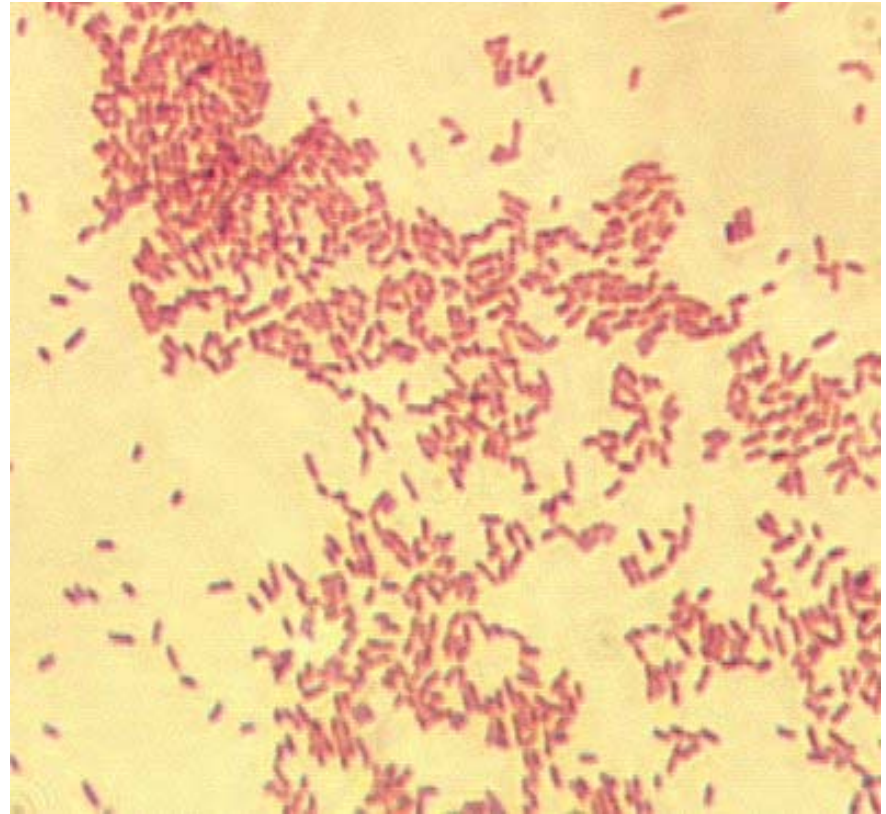


Brucella



Introduction

- Brucella species are strict parasites of animals and may infect humans.
- Brucellosis is a Zoonosis.
- It primarily affecting goats, sheep, cattle, buffaloes, pigs and other animals.
- It is transmitted to humans by contact with infected animals or through their products.

Contd.

- Brucellosis also known as Mediterranean fever, Malta fever, Undulant fever.
- David Bruce (1886) isolated a small microorganism from the spleen of fatal cases in Malta.
- This organism was named *Brucella melitensis*. (Brucella after Bruce, melitensis after Melita).
- Zammit (1905) showed that *Br. melitensis* was transmitted to humans by goat's milk.

The Many Names of Brucellosis

Human Disease

- Malta Fever
- Undulant Fever
- Mediterranean Fever
- Rock Fever of Gibraltar
- Gastric Fever

Animal Disease

- Bang's Disease
- Enzootic Abortion
- Epizootic Abortion
- Slinking of Calves
- Ram Epididymitis
- Contagious Abortion

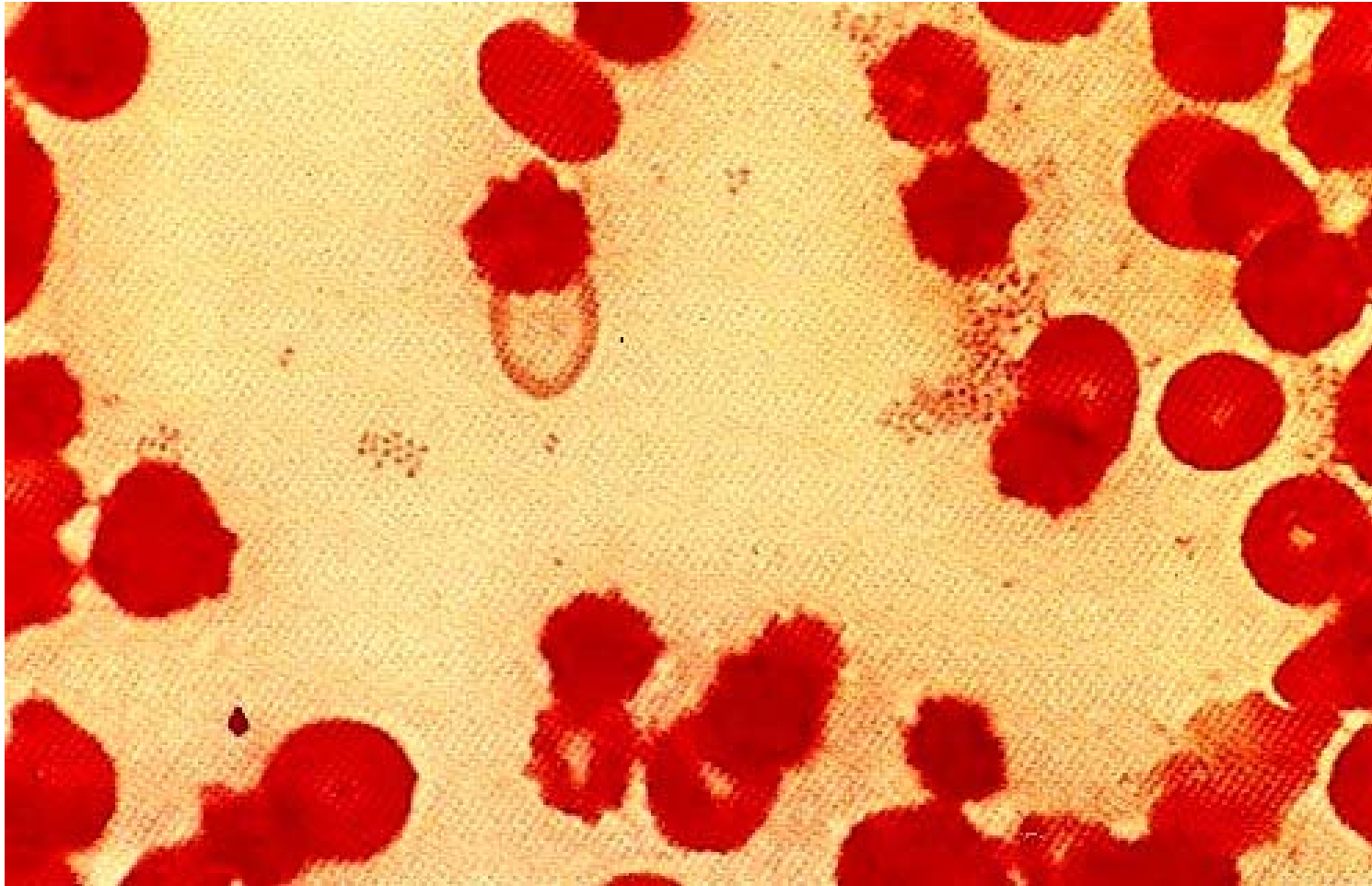
Six species

- *Brucella melitensis* - sheep & goat, man susceptible
- *B. abortus* - cattle, man
- *B. suis* - pigs, man
- *B. canis* - dogs, man
- *B. ovis* - rams
- *B. neotomae* - wood rats

Morphology

- Brucellae are coccobacilli or short rods.
- Size is $0.5\text{-}0.7\mu\text{m} \times 0.6\text{-}1.5\mu\text{m}$.
- Arranged singly or in short chains.
- They are nonmotile, noncapsulated, nonacid fast.
- They are Gram negative and nonacid fast.
- Bipolar staining is not uncommon.

Gram Staining

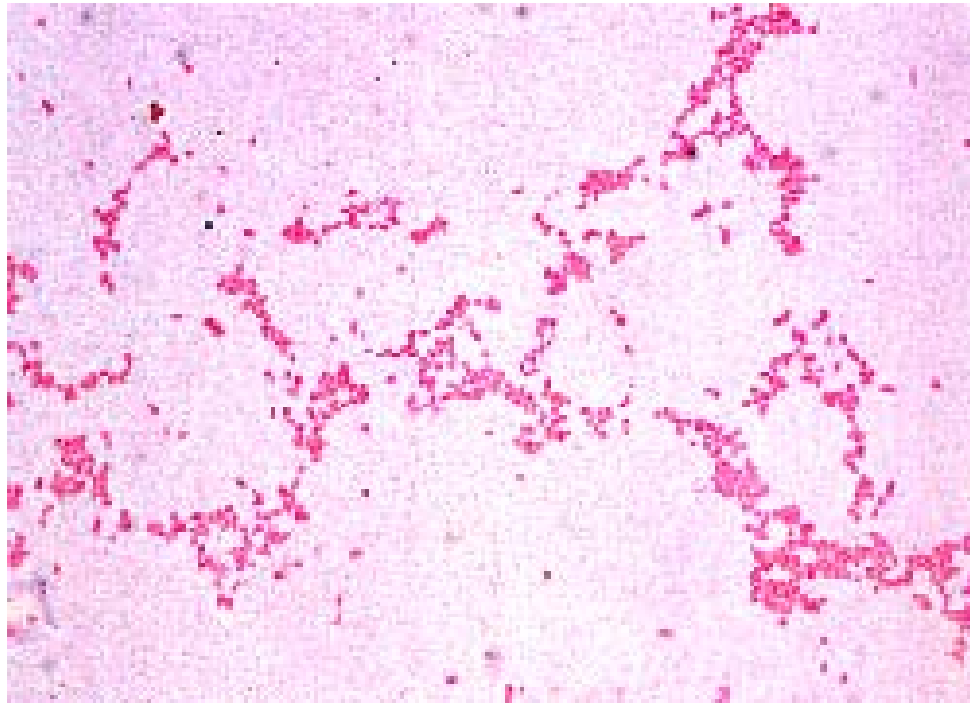


Culture characteristics

- Brucellae are strict aerobes.
- Br. abortus is capnophilic.
- The optimum temperature is 37°C and pH 6.6-7.4.
- Liver infusion media were widely used for the cultivation of Brucella.
- Media employed are Serum dextrose agar, serum potato infusion agar, trypticase soy agar, tryptose agar.
- The addition of bacitracin, polymyxin and cycloheximide makes the media more selective

Contd.

- Growth is uniform in liquid media.
- In solid media, colonies are small, moist, translucent and glistening.
- Mucoid, smooth and rough types of colonies appear, associated with change in antigenic structure and virulence.
- Erythritol has a specially stimulating effect on the growth of brucellae.



Gram stain from an isolated colony - tiny, averaging $0.5 - 0.7 \times 0.6 - 1.5 \mu\text{m}$ in diameter, appearing as Gram-negative coccobacilli

The appearance of the bacterial cells as observed in direct gram stains of clinical materials has been described as "**grains of sand**"



Colonies - Relatively small, measuring 0.5 - 1.0mm in diameter, entire, convex, and glistening, with no evidence of hemolysis

Biochemical reactions

- Ordinarily carbohydrates are not fermented.
- Brucellae are catalase positive, oxidase positive.
- They are urease test and nitrate reduction test positive.
- Citrate is not utilised.
- Indole is not produced and MR and VP tests are not negative.

Resistance

- Brucellae are destroyed by heat at 60°C in 10 minutes.
- They are killed by pasteurisation.
- They remain viable for 10 days in refrigerated milk. one month in ice creams, four month in butter.
- It remains viable for varying time in cheese depending on its pH.
- They may survive for many weeks in meat.

Brucella bacteriophage

- Several bacteriophages that lyse the Brucella strains have been isolated.
- All these phages are serologically similar.
- The Tblisi (Tb) has been designated as the reference phage.
- At RTD It lyses only Br. abortus, Br. Suis at 10,000 RTD, While Br. melitensis is not lysed at all.

Classification

Classification depends upon-

- CO_2 requirements
- H_2S production
- Sensitivity to dyes (basic fuchsin and thionin)
- Agglutination by monospecific sera
- Phage lyses
- Oxidative metabolic tests by amino acids and carbohydrates

Contd.

Three main species are there-

- 1.Br.melitensis**-Infecting primarily goats or sheep.
Three biotypes are there
- 2.Br.abortus**-Infecting cattle and swine. Nine biotypes
- 3.Br.suis**-Strains that produce H_2S are known as 'American' strains. Those that don't produce known as 'Danish' strains. There four biotypes are present.

Pathogenicity

- All three major species of brucellae are pathogenic to human beings.
- *Br. melitensis* is the most pathogenic.
- The incubation period is 10-30 days.
- Human infection may be of three types-
 1. Latent infection with only serological but no clinical evidence.
 2. Acute or subacute brucellosis
 3. Chronic brucellosis

Contd.

- Acute brucellosis is mostly due to *Br.melitensis*.
- It is usually known as undulant fever which is periodic nocturnal fever that may occur weeks, months or years particularly in untreated patients.
- It is associated with prolonged bacteremia and irregular fever.
- The symptoms are muscular and articular pains, asthmatic attacks, nocturnal drenching sweats, exhaustion, anorexia, constipation, nervous irritability and chills.
- The usual complications are articular, osseous, visceral or neurological.

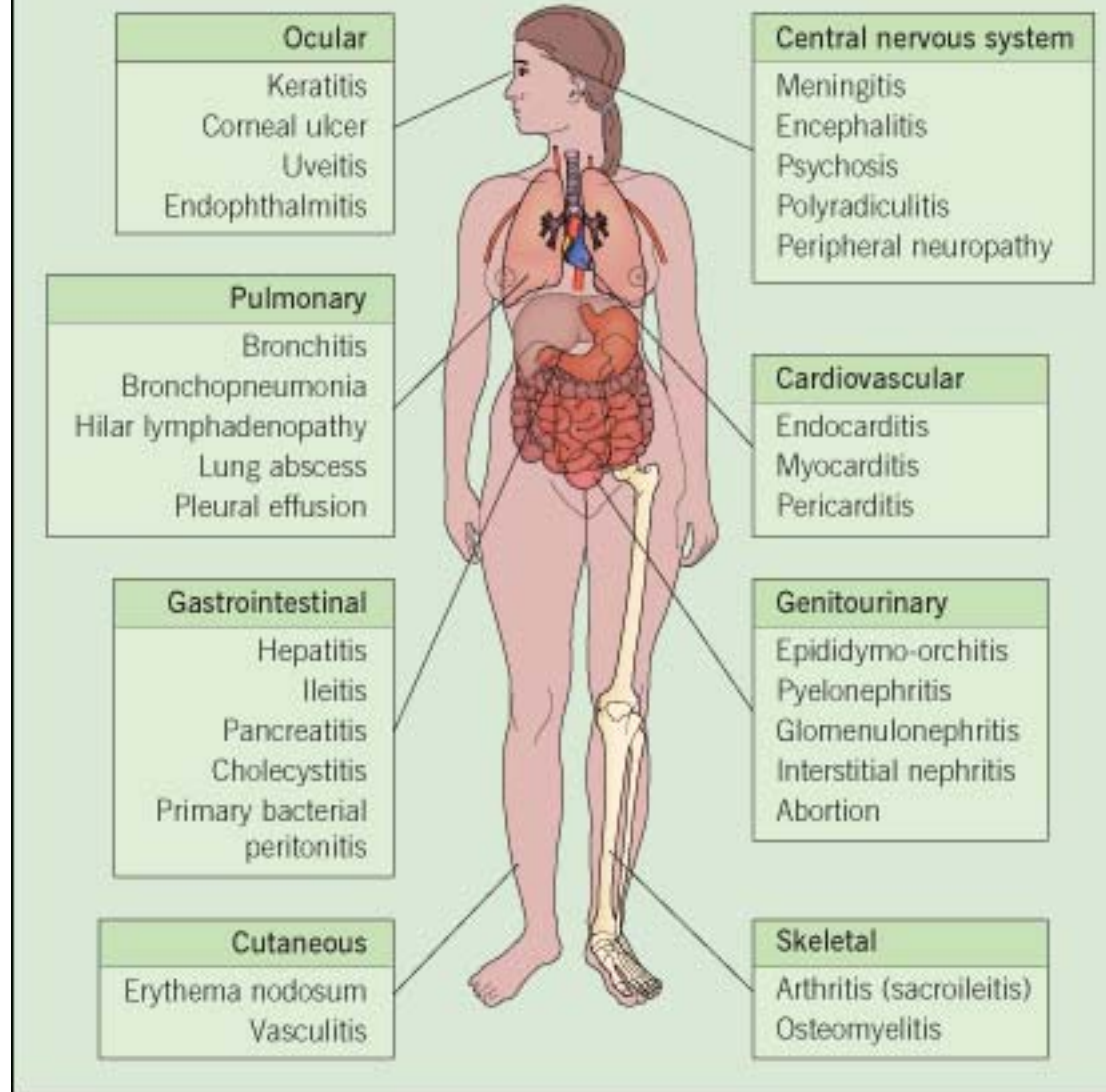
Contd.

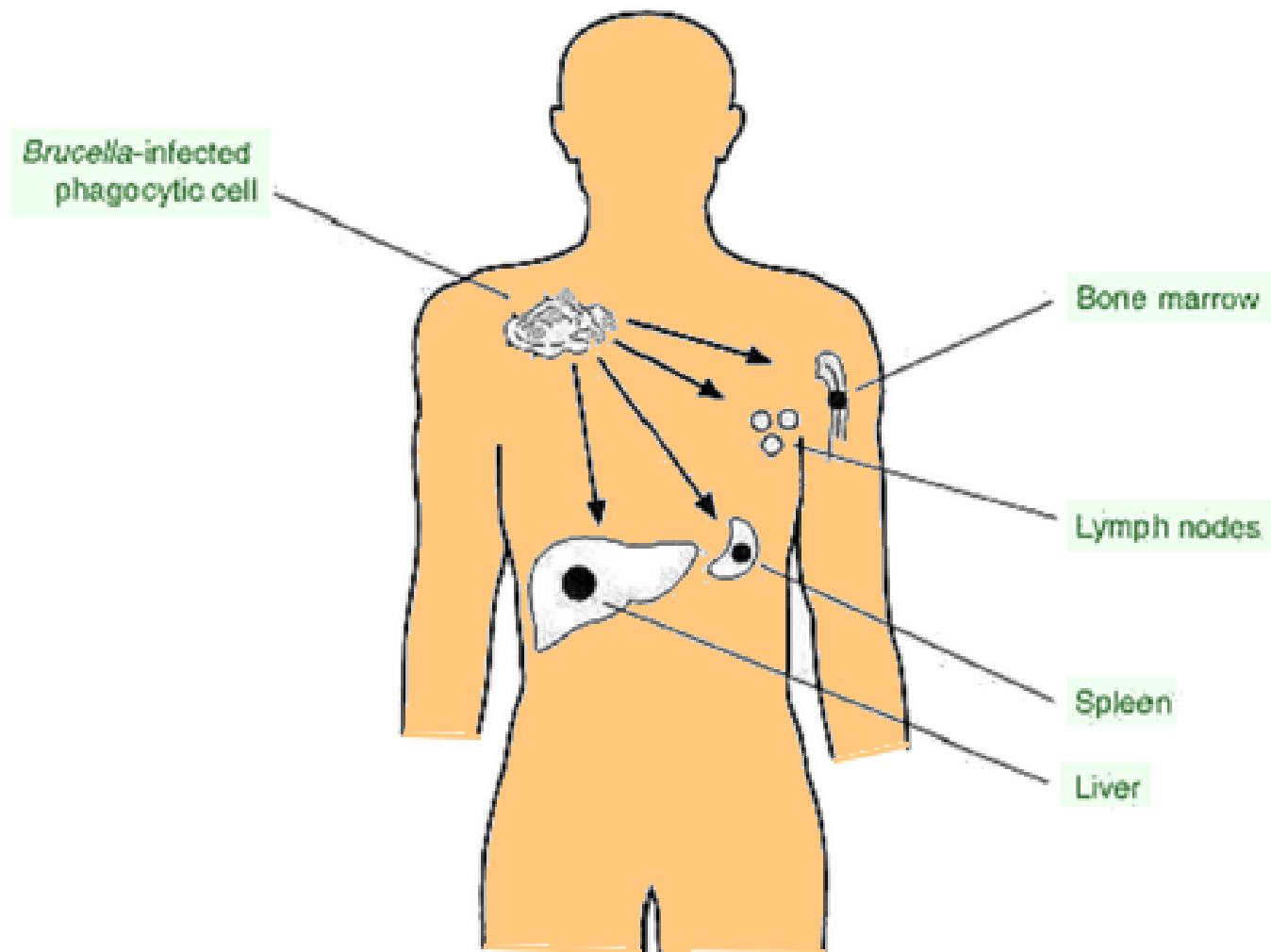
- Chronic brucellosis may be nonbacteremic. It is a low grade infection with periodic exacerbations.
- The symptoms are generally related to a state of hypersensitivity in the patient.
- The common clinical manifestations are sweating, lassitude and joint pains, with minimal or on pyrexia.

Contd.

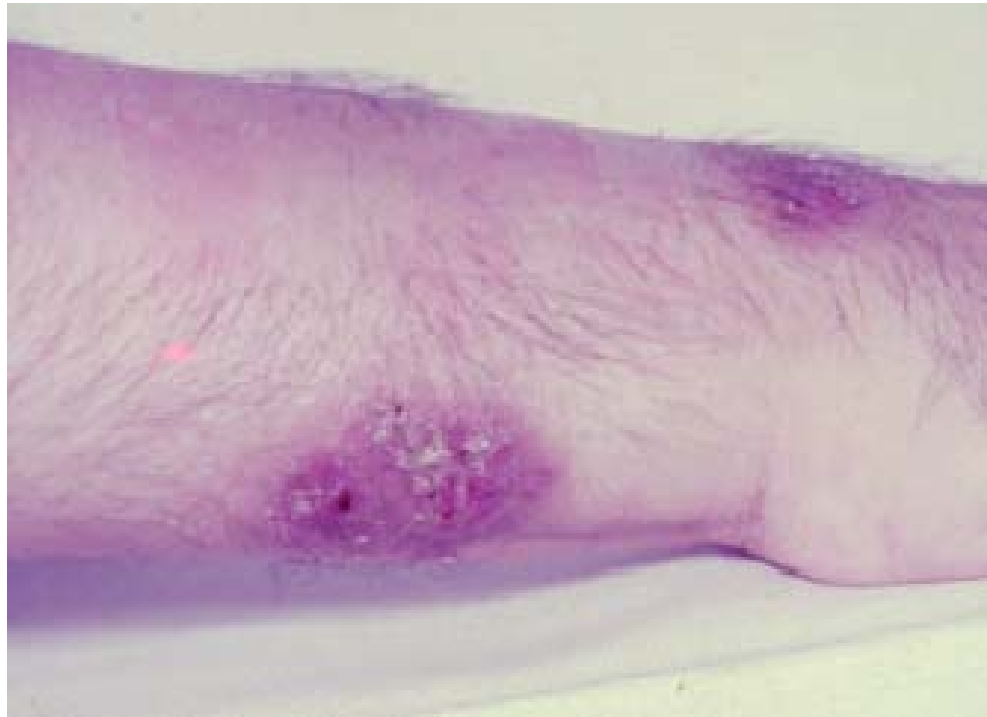
- Brucellosis is primarily an intracellular pathogen affecting the reticuloendothelial system.
- It is refractory to chemotherapy and there is coexistence of viable bacilli with high levels of circulating antibodies.
- Immunity in brucellosis is mainly cell mediated.
- Activated macrophages can kill the bacteria.
- Tissue reaction to brucella consists of granuloma formation.
- The brucellae spread from the initial site of infection through lymphatic channel to the local lymph glands.
- Then they disseminate throughout the body through blood stream.
- They have a predilection for the placenta.

CLINICAL MANIFESTATIONS OF BRUCELOSIS





Brucellosis: Pictorial Retrospective



The skin lesions evolve as an erythematous macular, papular, or pustular lesion on the hands and arms at the sites of direct exposure to infected animal material. The lesions illustrated in the photograph are from an archival slide collection depicting cutaneous brucellosis on the arm of a farmer who hand-milked cows.

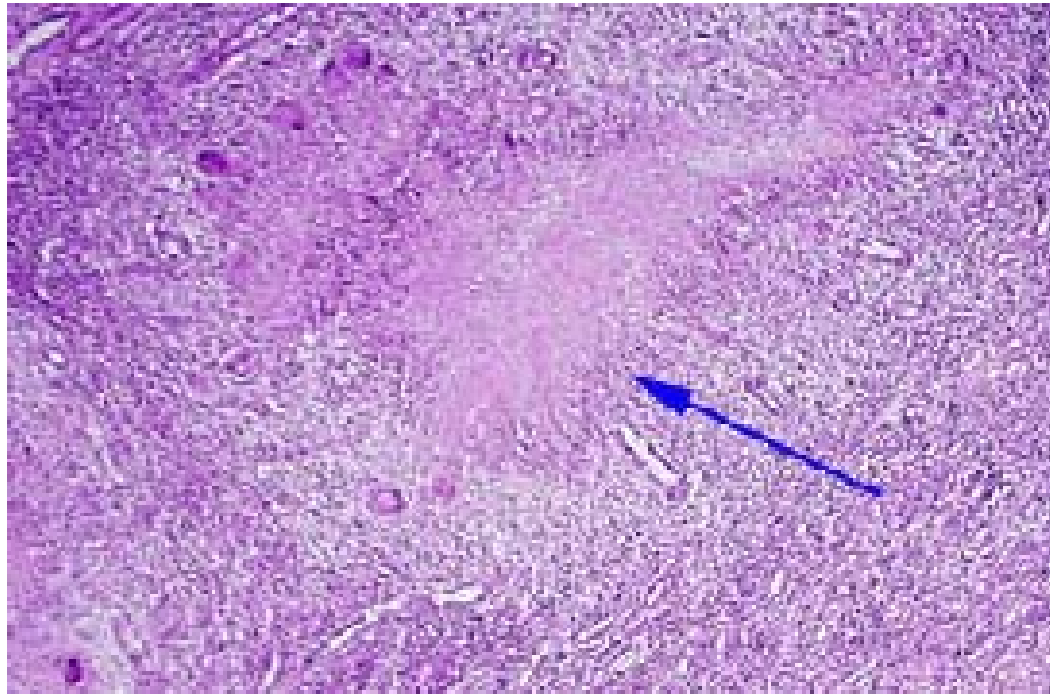
Brucellosis: Pictorial Retrospective



Localized disease may involve almost any organ system, including the spleen, in which multiple granulomas may be seen. The granulomas in the photograph range from 1 - 3 cm in diameter.

Initially, multiple splenic abscesses may be observed, that later develop into circumscribed granulomas that characteristically undergo secondary fibrosis and calcification.

Brucellosis: HPE



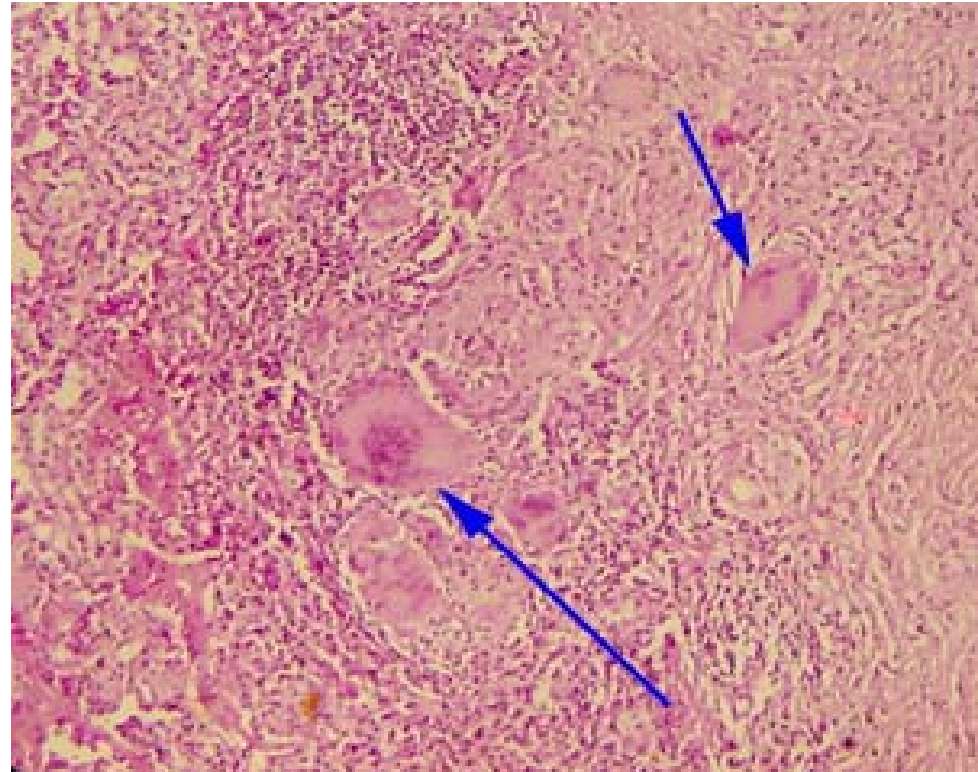
The reaction in lymph nodes, spleen, liver, and other organs is granulomatous. Although caseous necrosis *per se* is rarely observed, focal areas of stellate necrosis may be observed, with festooning of macrophages at the periphery, the so-called Splendore-Hoepfer effect (blue arrow).

Brucellosis: Pictorial Retrospective

The granulomatous reaction often also is characterized by the presence of numerous multi-nucleated giant cells (blue arrows). This reaction occurs because of the ability of organisms to remain viable intra-cellularly

Organisms are difficult to demonstrate with tissue gram stains. Culture is necessary to establish a diagnosis in most instances.

Pathologists should include chronic brucellosis in the differential diagnosis of all granulomatous lesions with histology as seen in the photograph.



Epidemiology

- Human brucellosis is acquired from animals, directly or indirectly.
- The modes of infection are by ingestion, contact, inhalation or accidental inoculation.
- The most important vehicle of infection is raw milk.

Laboratory diagnosis

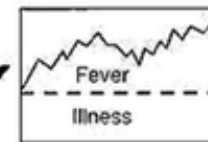
- The clinical manifestations of human brucellosis are variable.
- Clinical diagnosis is almost impossible.
- Laboratory help is essential.
- Laboratory methods for diagnosis include culture, serology and hypersensitivity tests.
- Blood culture is the most definitive method for the diagnosis of brucellosis.

History and Physical Exam

Clinical

Occupation
Raw milk
Fresh cheese
Travel
Symptoms
Signs

Course of disease

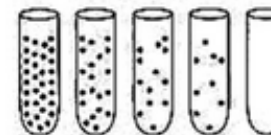


Laboratory

Blood cultures



Quantitative serology



Contd.

- Blood is inoculated into a bottle of trypticase soy broth.
- It is incubated at 37°C under 5-10% CO₂.
- Subcultures are made on solid media every 3-5 days, beginning on the fourth day.
- Cultures should not be declared negative in less than 6-8 weeks.
- BACTEC cultures may become positive in 5 to 6 days.

Laboratory Diagnosis Culture of Brucellae



- **Castaneda** method - 2 phase media
- Both liquid serum dextrose broth & solid serum dextrose agar are within the same bottle
- Blood inoculated into the broth & incubated in upright position
- For subculture, bottle is tilted so that broth flows over the surface of agar slant
- Again incubated in upright position
- Colonies appear on slant
- Reduces chances of contamination & risk of infection to laboratory personnel

Contd.

- Blood cultures are positive only in about 30-50% of cases.
- *Br. melitensis* and *Br. suis* are isolated more readily than *Br. abortus*.
- Bone marrow cultures yield a higher rate of isolation
- Bone culture remain positive long after the blood culture has become negative.
- Cultures may also be obtained from lymph nodes, CSF, urine, and abscesses.

Serological tests

- Paramount importance
- Antibodies appear within 7 - 10 days of onset of disease
- Reach max titre in 3 - 4 wks then slowly decline
- Ig M appear first. Rapidly followed by Ig G & to a lesser extent Ig A
- **Agglutination** identifies mainly Ig M
- Both Ig M & Ig G fix **complement**
- **Blocking or non- agglutinating antibodies** - Ig G & Ig A antibodies bind the antigen & prevent agg by Ig M

Serological tests

1. Standard Tube Agglutination test (SAT)
2. 2 - Mercaptoethanol (2ME) agglutination test
3. Complement fixation test
4. Anti - human globulin (Coombs') test
5. Enzyme linked immunosorbent assay (ELISA)
6. Radioimmunoassay (RIA)

Serological tests

- **SAT** - most widely used
- Tube agglutination test
- Serial 2 fold dilutions of patient serum (uninactivated) from 1 in 20 to 1 in 640
- Standardized antigen (heat killed smooth Brucella cells in saline containing phenol 0.5%) is added
- Incubated at 37°C for 24 hrs

SAT



Serological tests

- Most patients develop titres of 640 or more by the end of 3 - 4 wks
- Single titre greater than 1 in 160 is presumptive evidence of recent Brucella infection
- Prozone phenomenon due to antibody excess or blocking antibodies

Laboratory Diagnosis

Serological tests

- **Anti human globulin (Coombs') test** detects blocking antibodies
- Organisms + serum & incubated for half hour
- Centrifuged, washed & resuspended in normal saline
- Treated with anti human globulin
- Agglutination if blocking antibodies are present

Serological tests

- **Complement fixation** - imp in chronic cases
Detects both Ig G & Ig M
- **2ME agglutination test** demonstrates Ig G (2ME resistant)
2ME reduces the disulphide bonds that link Ig M
- **ELISA & RIA** are sensitive tests
Distinguish bet Ig M, Ig G & Ig A

Brucellin test (Skin test)

- Detect delayed (type IV) hypersensitivity
- Not useful for diagnosis of acute infection
- Remains positive for yrs after infection
- Only indicates past history of exposure
- **Brucellin** - protein extract of organisms is injected intradermally
- Positive reaction - slightly raised, sometimes tender, erythematous plaque, 2 - 6 cm in dia within 6 - 24hrs

Detection in animals

- Brucellae may be demonstrated microscopically in pathological specimens by suitable staining or by immunofluorescence.
- Rapid plate agglutination test and the Rose Bengal card test are the rapid methods for the detection of brucellosis in herds of cattle.
- For the detection of uninfected animals in dairies, pooled milk sample may be tested for bacilli by culture and for antibodies by several techniques.
- **By milk ring test-**
- A sample of whole milk is mixed well with a drop of the stained brucella antigen.

Contd.

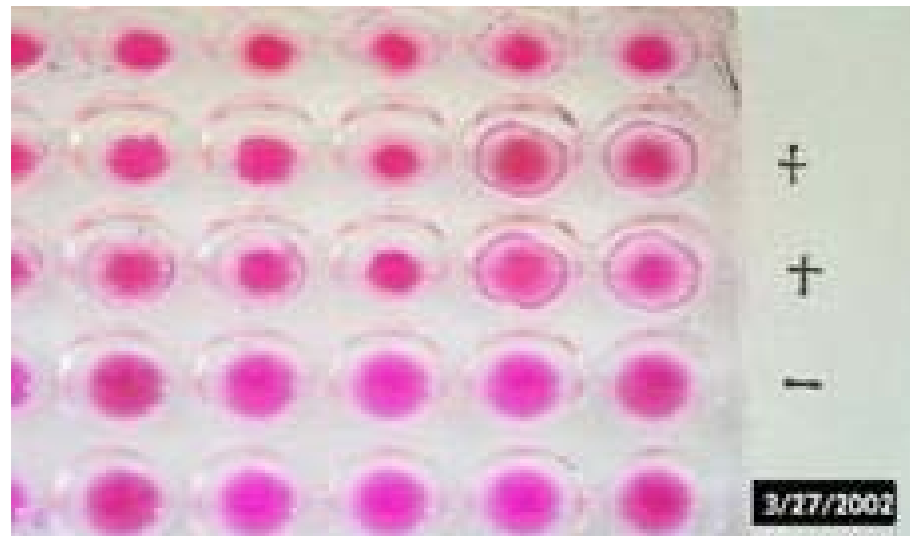
- Brucella antigen (a concentrated suspension of killed Br. abortus stained with hematoxylin)
- The mixture of whole milk and stained brucella antigen are incubated in a water bath at 70°C for 40-50 minutes.
- If antibodies are present in the milk, the bacilli are agglutinated and rise with the cream to form a blue ring at the top, leaving the milk unstained.
- If antibodies are absent, no coloured ring is formed and the milk remains uniformly blue.
- The whey agglutination test is another useful method for detecting the antibodies in milk.

Milk ring test



Rose Bengal Test

This is a simple but sensitive screening test which is carried out on all sera. Equal volumes (25 μ l) of serum and antigen (killed *Brucella abortus* organisms) are mixed in a plate for 4 minutes. In positive cases an agglutination reaction will take place between the antibodies in the serum and the antigen which can be seen as a ring in the well of the plate



Treatment

- Difficult - intracellular nature
- Prolonged use of antibiotics
- Until recently -
 - Tetracycline for 6 wks with
 - Streptomycin for initial 2-3 wks
- WHO recommended -
 - Rifampicin &
 - Doxycycline for 6 wks

Prevention and Control

- Education about risk of transmission
 - Farmer, veterinarian, abattoir worker, butcher, consumer, hunter, public
- Wear proper attire if dealing with infected animals/ tissues
 - Gloves, masks, goggles
- Avoid consumption of raw dairy products

Prevention and Control

- Immunize in areas of high prevalence
 - Calves with live attenuated *B. abortus* strain 19
 - Young goats and sheep with *B. melitensis* Rev-1
 - No human vaccine
- Eradicate reservoir
 - Identify, segregate, and/or cull infected animals