Genus: *Hemophilus*

General characteristics

- Gram –ve coccobacilli.
- Require growth factors called X and V present in blood.
- Contain both pathogenic and non pathogenic species.
- Two pathogenic; H. influenzae type b causes meningitis in children and H. *ducreyi,* a sexually transmitted pathogen, causes chancroid; other *Haemophilus* species are among the normal microbiota of mucous membranes and only occasionally cause disease.

HAEMOPHILUS INFLUENZAE

Haemophilus influenzae is found on the mucous membranes of the upper respiratory tract in humans. It is an important cause of meningitis in children and causes upper and lower respiratory tract infections in children and adults.

Morphology and Identification

A. Typical Organisms

In specimens from acute infections, the organisms are short coccoid bacilli, sometimes occurring in pairs or short chains. In cultures, the morphology depends both on the length of incubation and on the medium. At 6–8 hours in rich medium, the small coccobacillary forms predominate (Figue 1). Later there are longer rods, lysed bacteria, and very pleomorphic forms (Figure 2). Organisms in young cultures (6–18 hours) on enriched medium have a definite capsule. The capsule is the antigen used for "typing" *H infl uenzae*.

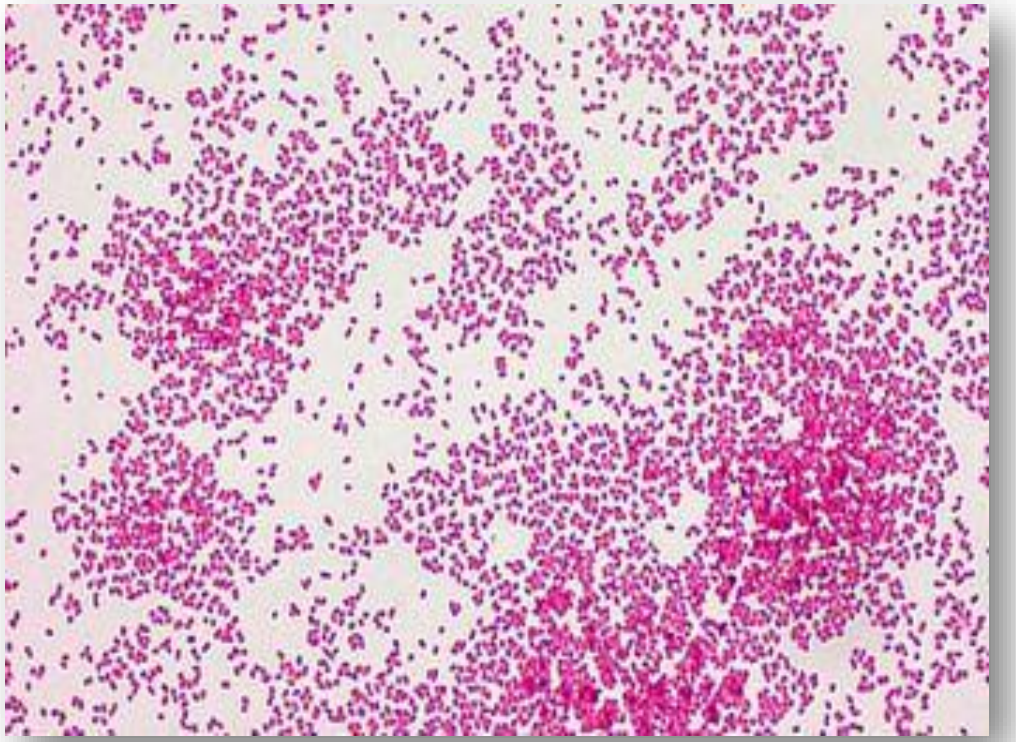


Figure 1. Gram negative coccobacilli (*H.influenzae*) in young culture

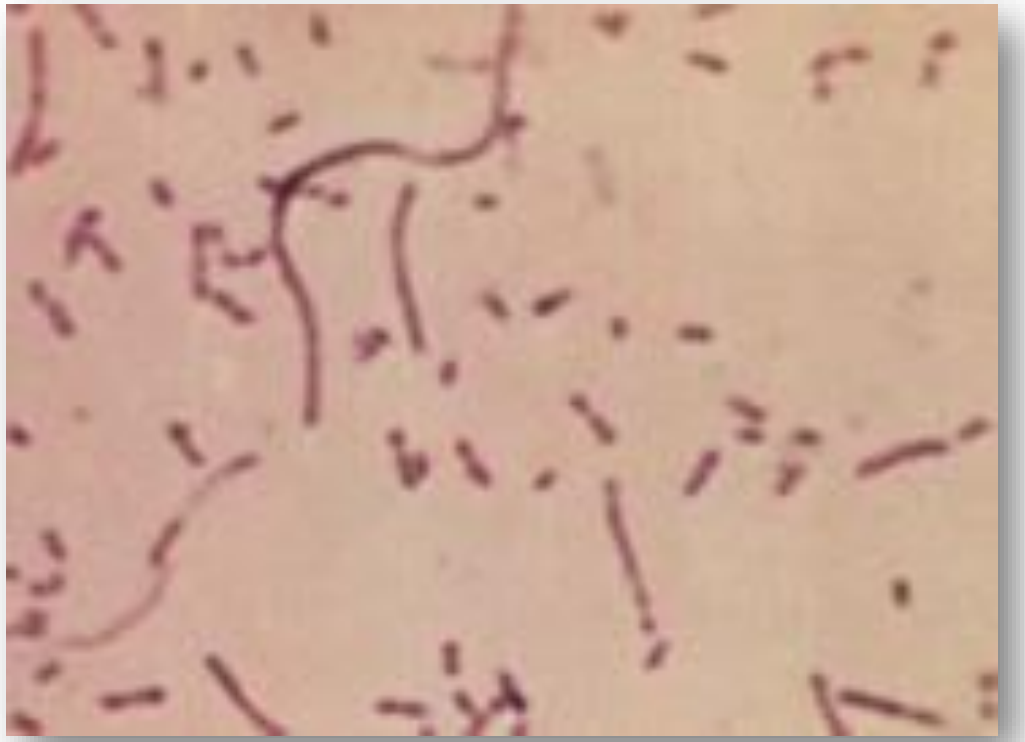


Figure 2. Pleomorphism in H.influenza (old culture)

Culture

On chocolate agar, flat, grayish brown colonies with diameters of 1–2 mm are present after 24 hours of incubation (Figure 3). IsoVitaleX in media enhances growth. *H influenzae* does not grow on sheep blood agar except around colonies of staphylococci ("satellite phenomenon"). *Haemophilus hemolyticus and H. parahemolyticus* are hemolytic variants of H. influenza and H. parainfluenzae respectively

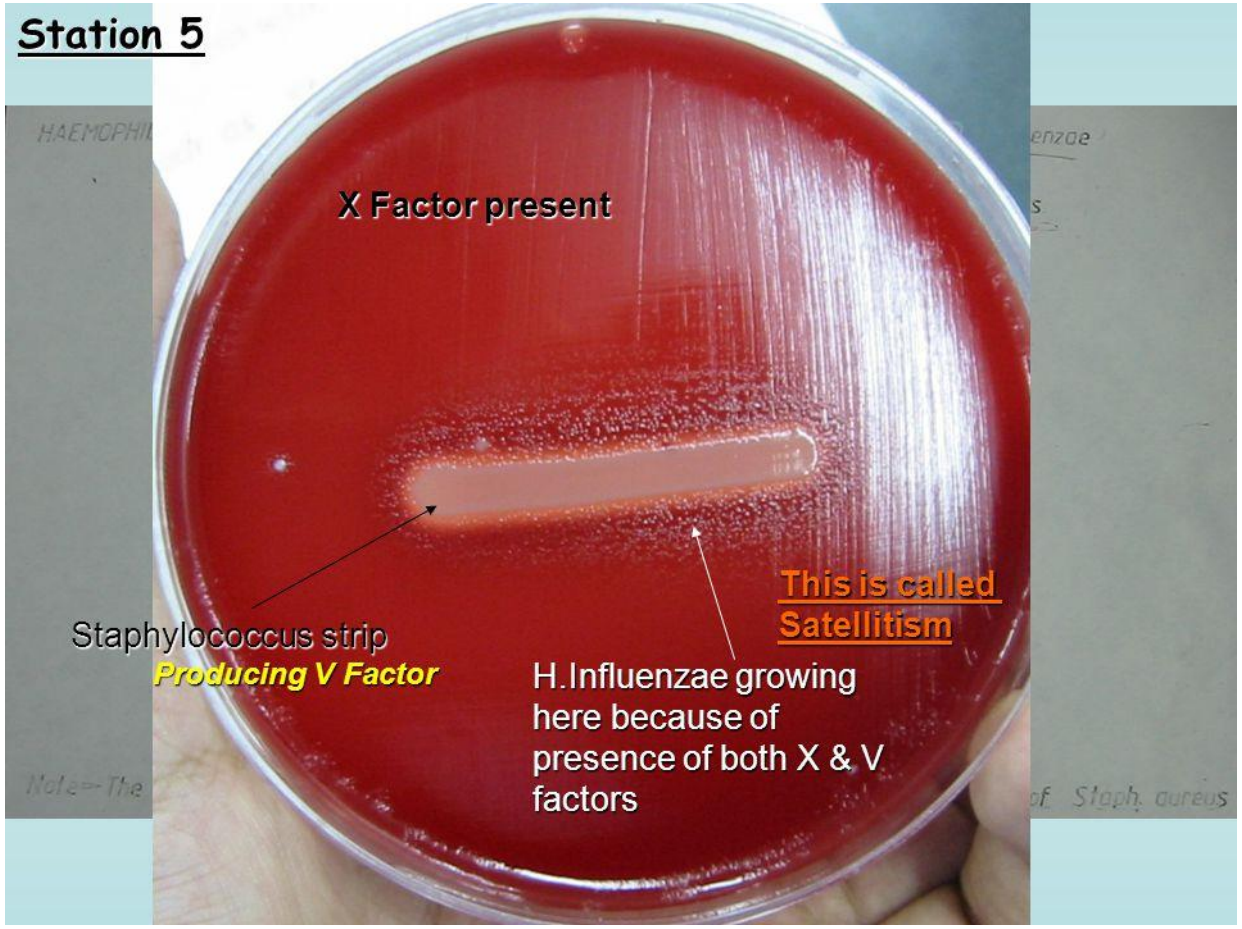


Figure 3. Satellite phenomenon

Growth Characteristics

Identification of organisms of the *Haemophilus* group depends partly on demonstrating the need for certain growth factors called X and V. Factor X acts as hemin; factor V can be replaced by nicotinamide adenine nucleotide (NAD) or other coenzymes. Colonies of staphylococci on sheep blood agar cause the release of NAD, yielding the satellite growth phenomenon. In addition to serotyping on the basis of capsular polysaccharides ,*H infl uenzae* and *H parainfl uenzae* can be biotyped on the basis of the production of indole, ornithine decarboxylase and urease.

Antigenic Structure

Encapsulated *H. influenzae* contains capsular polysaccharides of six types (a–f).The most important is **type b which causes invasive infection**. **Encapsulated *H. influenzae* can be typed by slide agglutination** (serological test)

Most *H. influenzae* organisms in the normal microbiota of the upper respiratory tract are not encapsulated and can not cause **invasive infections** but only **localized infections in the URT**.

The peak incidence *of H.influenzae* infections is children from **6 months to one year**.
Route of infection: Respiratory tract
Either asymptomatic infection **(carrier)** or infections like **sinusitis, otitis** (*strep.pneumoniae*) and pneumonia (non capsulated forms) or enter the blood to cause bacteremia, meningitis (95% by *H.influenzae* type b) and septic arthritis.
Main virulence factors: **capsule, endotoxin and IgA protease**

LABORATORY DIAGNOSIS

- **Clinical Specimens**
  - Blood, sputum, CSF, Joint fluid
- **Microscopy**
  - Gram Stain
  - Flourescent antibody stain
- **Culture**
  - Chocolate Agar (Factor V & X)
- **Serology**
  - Latex agglutination, Counter immunoelectrophoresis
- **PCR**

Table 1. Characteristics and growth requirements of hemophilus sp.

Species	Requires X	Requires V	Hemolysis
Haemophilus influenzae (H aegyptius)	+	+	–
Haemophilus parainfluenzae	–	+	–
Haemophilus ducreyi	+	–	–
Haemophilus haemolyticus	+	+	+

H influenzae is differentiated from related gram-negative bacilli by its requirements for X and V factors and by its lack of hemolysis on blood agar (Table 1).

Treament

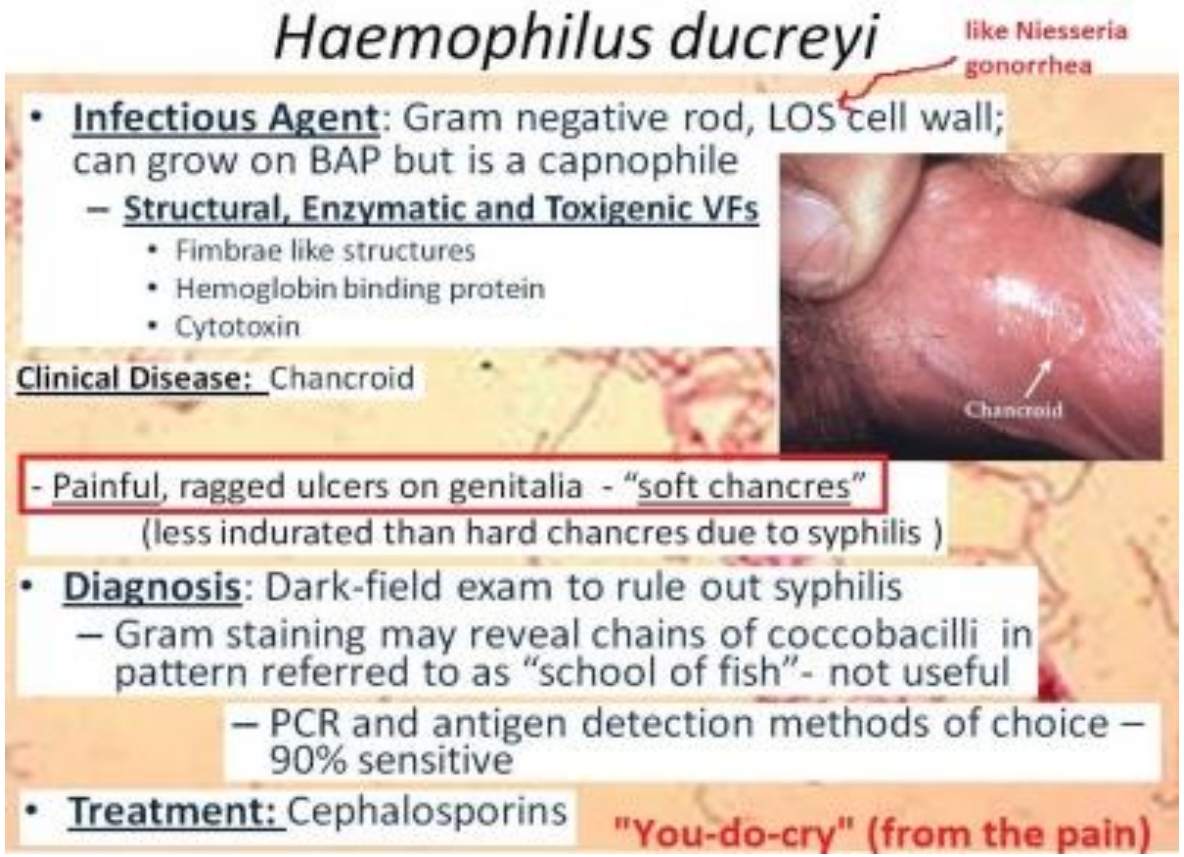
3rd generations cephalosporin like Claforan(Ceftriaxone) is the drug of choice for meningitis and Amoxiclav(amoxicillin+clavulanic acid) for sinusitis and otitis.

HAEMOPHILUS DUCREYI

Cause **chancroid** or **soft sore** which is venereal disease characterized by tender, non-indurated,irregular ulcer on genitalia. The disease must be differentiated from syphilis, herpes simplex infection, and lymphogranuloma venereum.

H ducreyi requires X factor but not V factor.
It is grown best from scrapings of the ulcer base that are inoculated onto chocolate agar containing 1% IsoVitaleX and vancomycin, 3 μg/mL; the agar is incubated in 10% CO_2 at 33°C.

Treatment
Intramuscular ceftriaxone, oral ciprofloxacin,
or oral erythromycin; healing results in 2 weeks.



THE BORDETELLAE

***Bordetella pertussis*, a highly communicable and important pathogen of humans, causes whooping cough (pertussis).**
***Bordetella parapertussis* can cause a similar disease**.
*Bordetella bronchiseptica c*auses diseases in animals, such as kennel cough in dogs and snuffles in rabbits, and only occasionally causes respiratory disease and bacteremia in humans, primarily in immunocompromised hosts.
Bordetella avium causes turkey coryza and is a rare cause of respiratory illness in humans.
Bordetella hinzii (bacteremia, respiratory illness, arthritis)
Bordetella holmesii (bacteremia among immunosuppressed patients)
Bordetella trematum (wound infections and otitis media).

BORDETELLA PERTUSSIS
Morphology and Identification
Typical Organisms
The organisms are minute gram-negative coccobacilli resembling *H influenzae.* With toluidine blue stain, bipolar metachromatic granules can be demonstrated. A capsule is present.

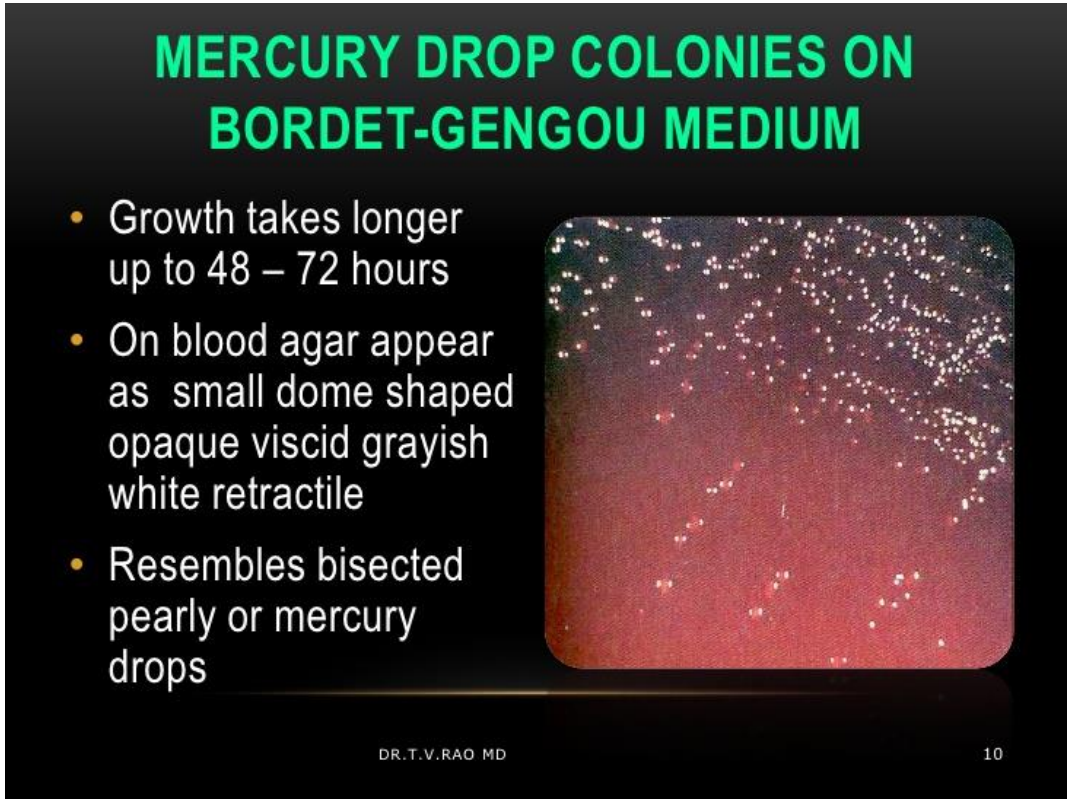
Culture

Primary isolation of *B pertussis* requires enriched media. Bordet-Gengou medium (potato-blood-glycerol agar) that contains penicillin G, 0.5 µg/mL, can be used; however, a charcoal-containing medium supplemented with horse blood (Regan Lowe) is preferable because of the longer shelf life.

The plates are incubated at 35–37°C for 3–7 days aerobically in a moist environment (eg, a sealed plastic bag). Growth is slow and after 48 to 72 hours' incubation on this media refractile, glistening (mercury drop-like appearance), small, smooth, dome-shaped, opaque and grayish white colonies appear (Figure 4). There is hazy area of hemolysis around colonies. Confluent growth gives aluminium paint appearance.

Growth Characteristics

- ❑ The organism is a strict aerobe
- ❑ Oxidase and catalase positive
- ❑ Nitrate, citrate, and urea negative, the results of which are useful for differentiating among the other species of bordetellae.
- ❑ It does not require X and V factors on subculture.
- ❑ Hemolysis of blood-containing medium is associated with virulent *B pertussis.*
- ❑ Non motile



Fi.4. Growth of B.pertussis on Bordet-Gengo agar.

Clinical Findings

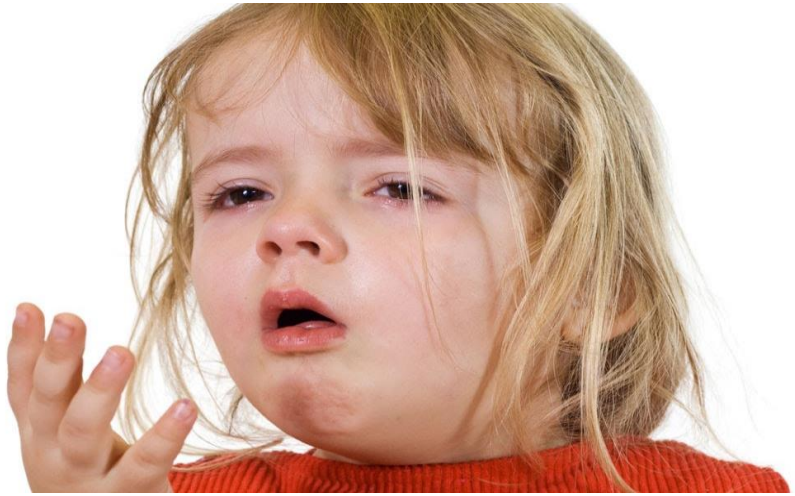
Incubation period of about 2 weeks. There 3 main phases

- **catarrhal phase**": mild coughing and sneezing. During this stage, the patient is highly infectious but not very ill.
- "**paroxysmal" phase** , the cough develops its explosive character and the characteristic "whoop" upon inhalation.

This leads to rapid exhaustion and may be associated with vomiting, cyanosis, and convulsions. The white blood count is **high (16,000–30,000/μL),** with an **absolute lymphocytosis**.

- Convalescence is slow. *B pertussis* is a common cause of prolonged (4–6 weeks) cough in adults.

Rarely, whooping cough is followed by the serious and potentially fatal complication of encephalitis. Several types of adenovirus and *Chlamydia pneumoniae* can produce a clinical picture resembling that caused by *B pertussis*



Diagnostic Laboratory Tests

A. Specimens

A saline nasal wash is the preferred specimen. **Nasopharyngeal swabs** or **cough droplets** expelled onto a "cough plate" held in front of the patient's mouth during a paroxysm are sometimes used but are not as good as the saline nasal wash.

B. Direct Fluorescent Antibody Test

The fluorescent antibody (FA) reagent can be used to examine nasopharyngeal swab specimens. However, false-positive and false-negative results may occur; the sensitivity is about 50%. The FA test is most useful in identifying *B pertussis* after culture on solid media.

C. Culture

The saline nasal wash fluid is cultured on solid medium agar. The antibiotics in the media tend to inhibit other respiratory flora but permit growth of *B pertussis*.

D- Polymerase Chain Reaction

Polymerase chain reaction (PCR) is the most sensitive method to diagnosis pertussis.

Treatment

Administration of erythromycin during the catarrhal stage of disease promotes elimination of the organisms and may have prophylactic value. Treatment after onset of the paroxysmal phase rarely alters the clinical course. Oxygen inhalation and sedation may prevent anoxic damage to the brain.

Prevention

Every infant should receive three injections of pertussis vaccine during the first year of life followed by a booster series for a total of five doses. Pertussis vaccine is usually administered in combination with toxoids of diphtheria and tetanus (DTaP). Five doses of pertussis vaccine are recommended before school entry. The usual schedule is administration of doses at 2, 4, 6, and 15–18 months of age and a booster dose at 4–6 years of age.

THE BRUCELLAE

- The brucellae are obligate parasites of animals then transmitted tohumans
- Intracellularl pathogen.
- *4 species infect human*

Brucella melitensis typically infects goats
Brucella suis, swine
Brucella abortus, cattle;
Brucella canis, dogs.
The disease in humans, brucellosis (undulant fever, Malta fever), is characterized by an acute bacteremic phase followed by a chronic stage that may extend over many years and may involve many tissues.

Morphology and Identification

A. Typical Organisms

They are gram negative coccobacilli aerobic, nonmotile, and nonspore forming.

B. Culture

Small, convex, smooth colonies appear on enriched media in 2–5 days.

C. Growth Characteristics

Brucellae are adapted to an intracellular habitat, and their nutritional requirements are complex. Some strains have been cultivated on defined media containing amino acids, vitamins, salts, and glucose. Fresh specimens from animal or human sources are usually inoculated on trypticase-soy agar or blood culture media. All aerobic except *B abortus* requires 5–10% CO2 for growth.

Brucellae use carbohydrates but produce neither acid nor gas in amounts sufficient for classification.

Catalase and oxidase are produced by the four species that infect humans.

Hydrogen sulfide is produced by many strains, and **nitrates are reduced to nitri**tes.

Brucellae are moderately sensitive to heat and acidity.

They are killed in milk by pasteurization.

Antigenic Structure

Differentiation among *Brucella* species or biovars is made possible by their characteristic sensitivity to dyes and their production of H2S. Because brucellae are hazardous in the laboratory, tests to classify them should be performed only in reference public health laboratories using appropriate biosafety precautions.

Pathogenesis and Pathology

The common routes of infection in humans are
1- the intestinal tract (ingestion of infected milk) and home made cheese.
2- mucous membranes (droplets)
3-skin (contact with infected tissues of animals).

The organisms progress from the portal of entry via lymphatic channels and regional lymph nodes to the thoracic duct and the bloodstream, which distributes them to the parenchymatous organs. Granulomatous nodules that may develop into abscesses form in lymphatic tissue, liver, spleen, bone marrow, and other parts of the reticuloendothelial system. In such lesions, the brucellae are principally intracellular. Osteomyelitis, meningitis, or cholecystitis also occasionally occurs.

B melitensis infection is more acute and severe, while other species cause mild form.

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 in these species. 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. The onset is insidious, with malaise, fever, weakness, aches, and sweats. The fever usually rises in the afternoon; its fall during the night is accompanied by drenching sweat. There may be gastrointestinal and nervous symptoms.

Chronic stage characterized by weakness, aches and pains, low-grade fever, nervousness, and other nonspecific manifestations compatible with psychoneurotic symptoms. Brucellae cannot be isolated from the patient at this stage, but the agglutinin titer may be high.

Diagnostic Laboratory Tests

A. Specimens

Blood should be taken for culture, biopsy material for culture (lymph nodes, bone, and so on), and serum for serologic tests.

B. Culture

Brucella species bacteria grow on commonly used media, including trypticase-soy medium with or without 5% sheep blood, brain–heart infusion medium, and chocolate agar. Blood culture media (see below) readily grow *Brucella* species bacteria. All cultures should be incubated in 8–10% CO_2 at 35–37°C and should be observed for 3 weeks before being discarded as being negative results; liquid media cultures should be blindly subcultured during this time. one marrow and blood are the specimens from which brucellae are most often isolated.

Clinical Manifestation:

- High fever ,
- Malaise (discomfort),
- Anorexia (malnutrition),
- Arthralgia (Pain in a joint),
- Fatigue,
- Headache,
- Sweating,
- Weight loss &
- Depression.

Media used in semiautomated and automated blood culture systems readily grow brucellae, usually within 1 week; however, holding the cultures for 3 weeks is recommended. 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. After a few days of incubation on agar media, the brucellae form colonies in the primary streak that are smaller than 1 mm in diameter. They are nonhemolytic. The observation of tiny gram-negative coccobacilli that are catalase positive and oxidase positive suggests *Brucella* species. Christensen's urea slant should be inoculated and observed frequently. Positive urease test result is characteristic of *Brucella* species. Bacteria that meet these criteria should be quickly submitted to a reference public health laboratory for presumptive identification. *Brucella* species are category B select agents. Molecular methods have been developed to rapidly differentiate among the various biovars.

C. Serology

Immunoglobulin M (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 remain high during chronic disease. IgA levels parallel the IgG levels. The usual serologic tests may fail to detect infection with *B canis.*

Rose Bengal test
It is latex agglutination test uses standardized heat-killed, phenolized, smooth *Brucella abortus* antigens stained with rose Bengal dye (pink color) to detect Brucella agglutinins.

Procedure:

- Allow the reagents and sample to reach room temperature.
- Place 50µL of the sample and one drop of each positive and negative control into separate circles on the slide test.
- Shake the Rose Bengal reagent gently before using and add a drop of this reagent next to the sample to be tested.
- Mix both drops with a stick, spreading them over the entire surface of the circle. Use different stirrers for each sample.
- Rotate the slide with a mechanical rotator at 80-100 rpm for 2 minutes, and read the results (this is the optimum time limited).

Reading the result & Interpretation:

- No agglutination= absence of specific antibodies

- Agglutination (even slight) = presence of specific antibodies

  If agglutination appear after 15 seconds = (1:640)
  If agglutination appear after 30 seconds = (1:320)
  If agglutination appear after 1 min. = (1:160)
  If agglutination appear after 1.30 min. = (1:80)

- Patient history should be taken into account before giving the result.

- This test is a screening test only for the detection of Brucella agglutinins. If result is positive it must be confirmed by other serological tests for Brucellosis.

Advantage:

- Rapid, inexpensive, sensitivity and specificity.

Limitation:

- Low sensitivity and specificity

False positive results
Cross react with
1- Vibrio cholera vaccine
2- tularemia agglutinins

False negative results
1- presence of blocking antibodies These are IgA antibodies that interfere with agglutination by IgG and IgM and cause a serologic test result to be negative in low serum dilutions (prozone), although positive in higher dilutions. These antibodies appear during the subacute stage of infection, tend to persist for many years independently of activity of infection, and aredetected by the Coombs antiglobulin method

2- Mercaptoethanol test (2-ME)
2-ME destry IgM and leaving IgG used to differentiate recent infection from past infection

Note: The physician should not depends only on the results of serological test because this disease is self limited

3. ELISA assays—IgG, IgA, and IgM antibodies may be detected using enzyme-linked immunosorbent assay (ELISA), which 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

Combined treatment with a tetracycline (eg, doxycycline) and either streptomycin for 2–3 weeks or rifampin for 6 weeks is recommended.