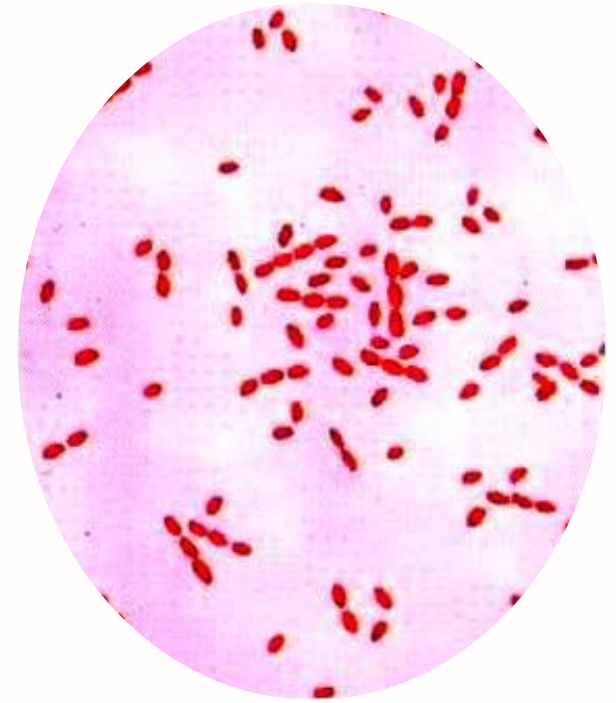


# BRUCELLA



# Many names of Brucellosis

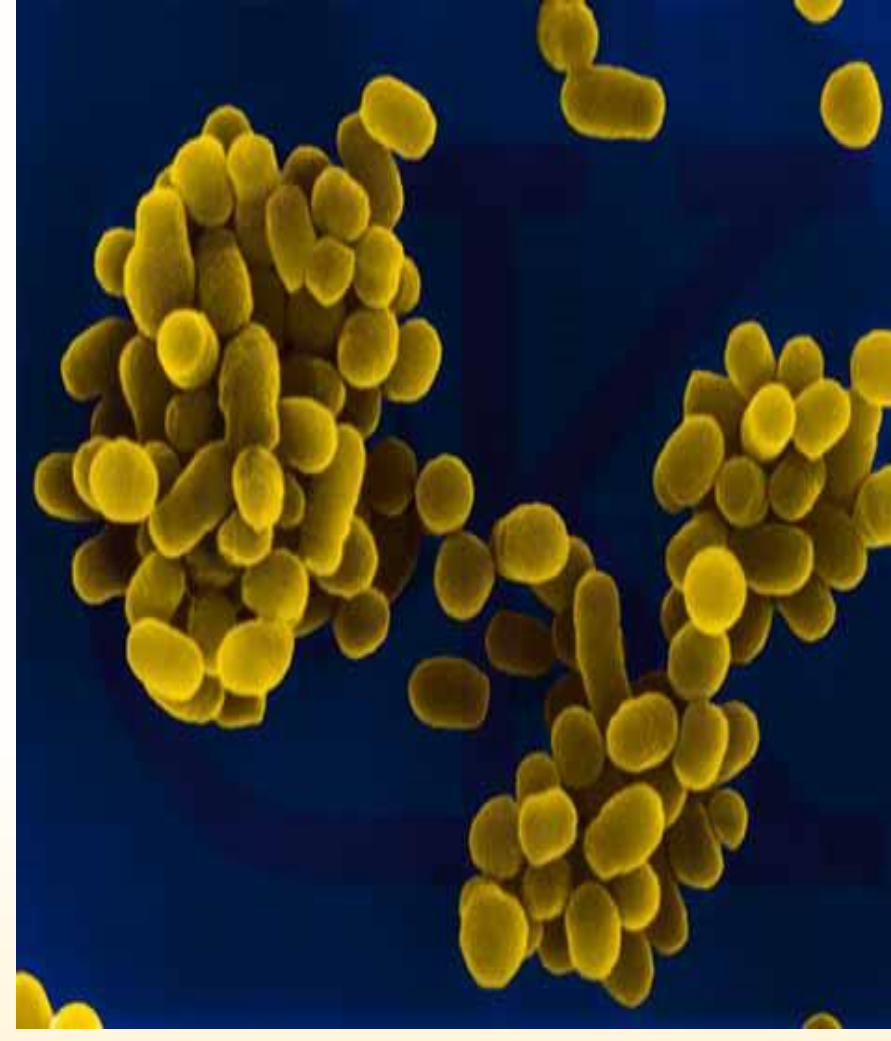
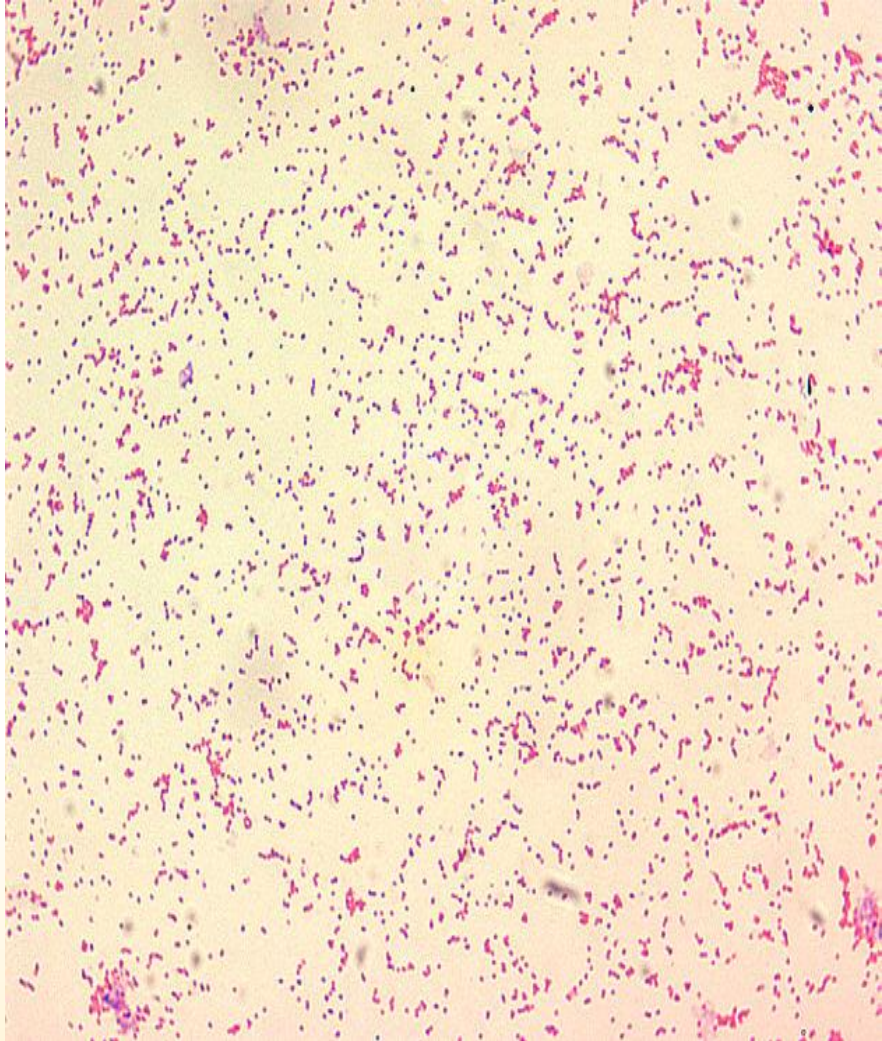
## **Human Disease**

- Malta Fever
- Undulant Fever
- Mediterranean Fever
- Rock Fever
- Gastric Fever

# *Brucella* spp.

- Gram negative, small, cocobacilli
- Non-motile, non-capsulated, non-pigment producer
- Intracellular microorganisms causing chronic disease.
- Prolonged incubation period > 4 days
- Oxidase and catalase positive.
- Rapidly positive for urease.
- New isolate from organism growth slowly. Needs serum, glycerin, glucose, eggs, etc. in the medium.

## The appearance of light and electron microscopy



- The colonies are small, round, fluffy, slippery like the **dewdrop**, and are of the S type.
- Non acid and gas production from carbohydrate
- Opt temp: 37 °C, pH: 6.8-7
- Species are divided according to their biochemical properties, antigenic structures, the effects of dye substances on their growing.

# Appearance on blood agar



*Brucella* spp. are aerobic. However *B. ovis* and many strains of *B. abortus*, when first cultured, are unable to grow without the addition of 5-10 % carbon dioxide. All strains grow best at 37 °C in a medium enriched animal serum and glucose.



Brucella spp. have serologically cross reactions with

- *E.coli* O116 and O157,
- *Francisella tularensis*,
- *Vibrio cholerae*
- *Yersinia enterocolitica* O9,
- *Xanthomonas maltophilia*



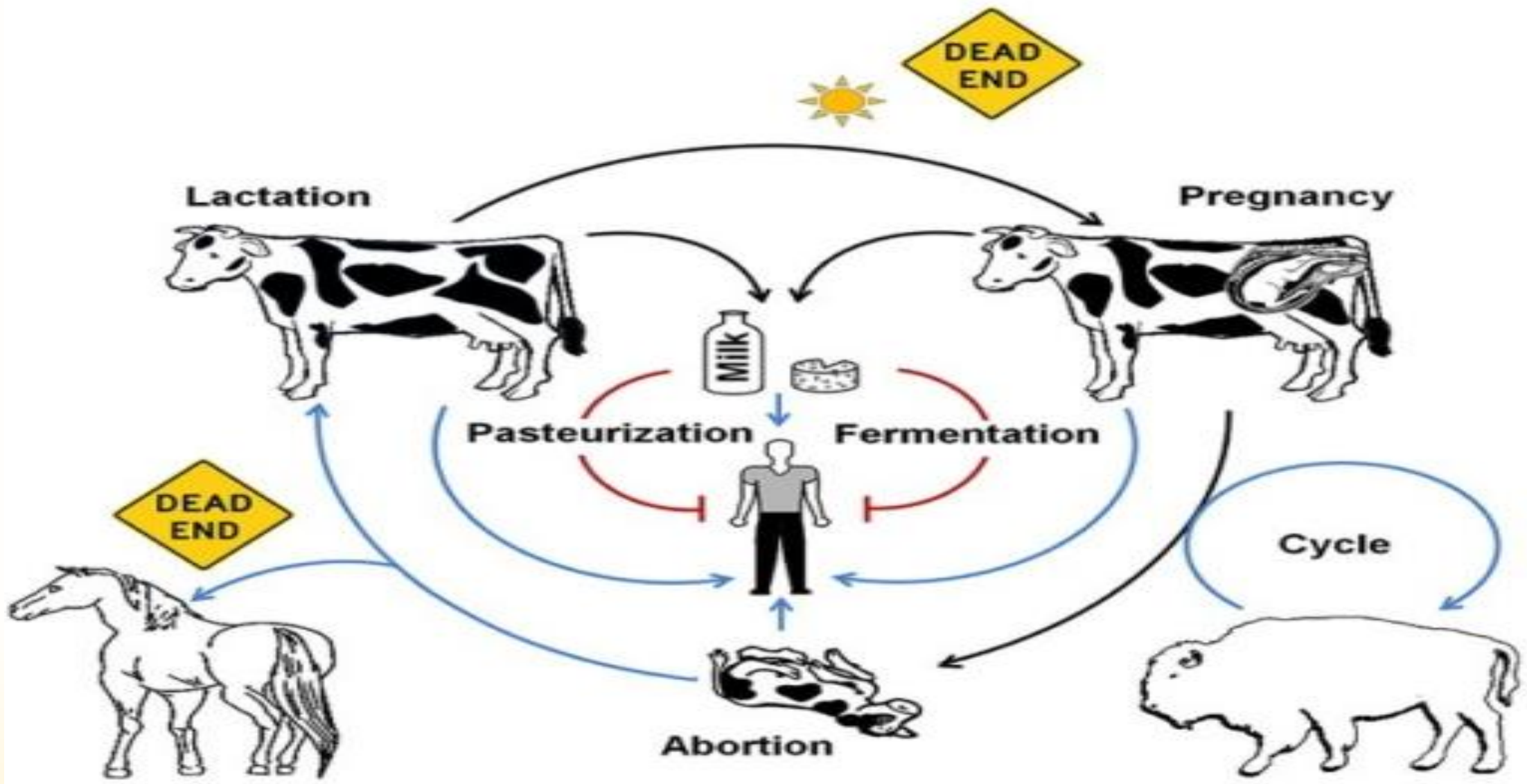
# Susceptibility

- Killed at 60 °C in 10 minutes.
- Pasteurization of milk.
- Survival is long in refrigerated milk, ice creams (30 days) and cheese consist 10% salt (45 days).

# Transmission to Humans

Human become infected by:

1. Inhaling the organisms.
2. Conjunctiva or broken skin on contact with infected materials including
  - Animal carcasses
  - Fetal membrane
  - Vaginal discharges
  - Fetuses
3. Ingestion of unpasteurized milk or milk products from infected animals.



- Four main causative species of Human Brucellosis

- *B. abortus*

- *B. melitensis*

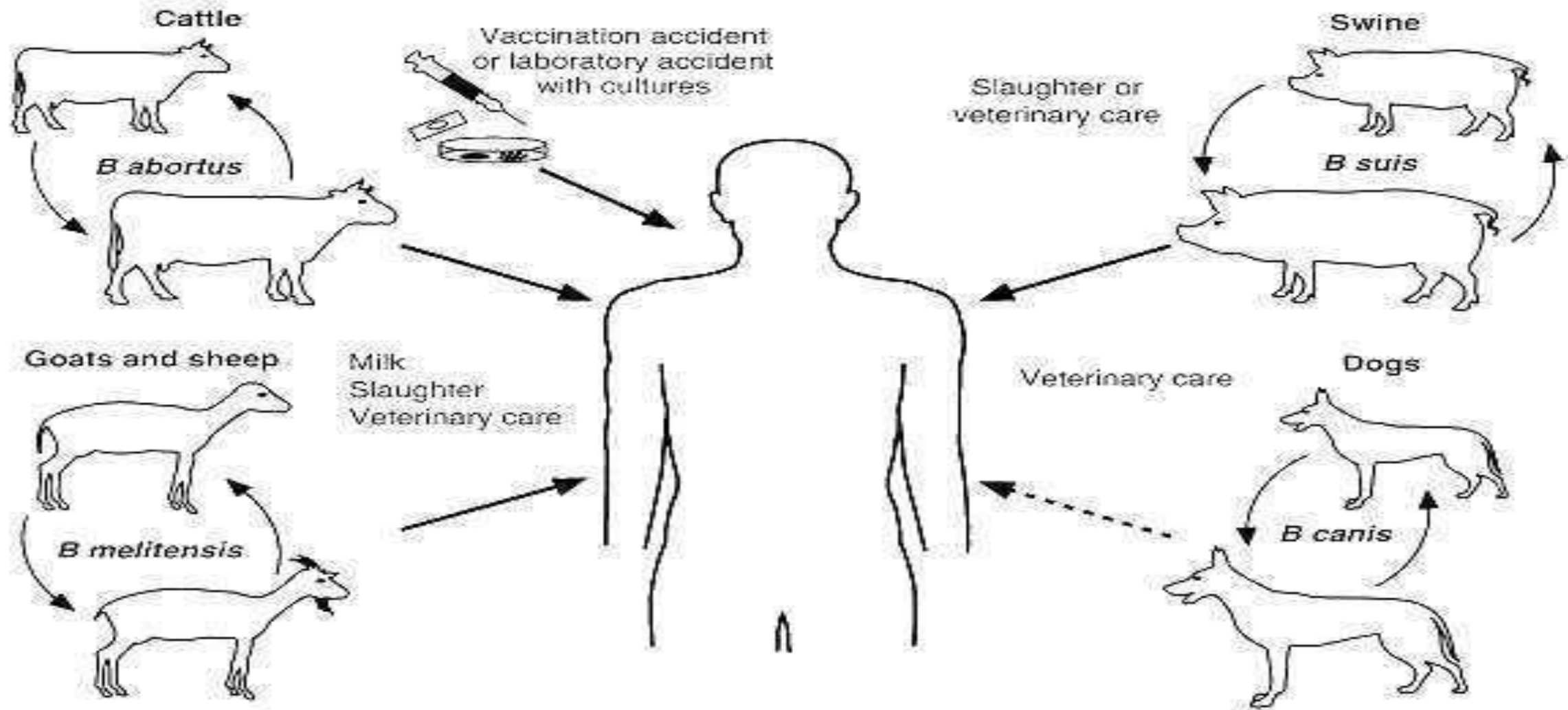
- *B. suis*

- *B. canis*

The other Brucella spp.

- *B. neotomae*

- *B. ovis*



[https://www.researchgate.net/publication/262377206\\_Public\\_Health\\_Importance\\_of\\_Goat\\_Borne\\_Diseases\\_and\\_Zoonoses/figures?lo=1&utm\\_source=google&utm\\_medium=organic](https://www.researchgate.net/publication/262377206_Public_Health_Importance_of_Goat_Borne_Diseases_and_Zoonoses/figures?lo=1&utm_source=google&utm_medium=organic)

## Main Causative Species of Human Brucellosis

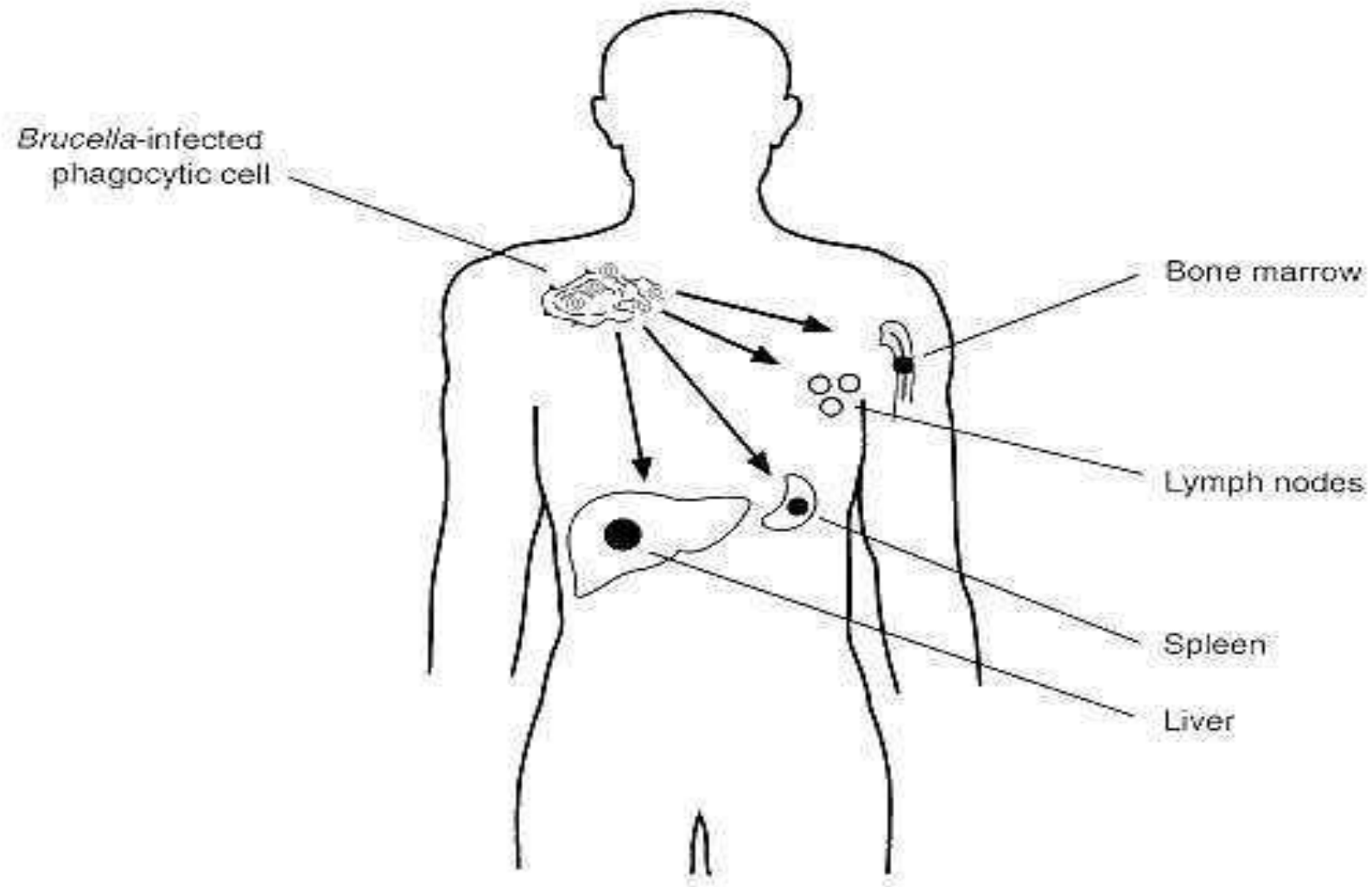
<b>Species</b>	<b>Main animal host</b>	<b>Human Pathogen</b>	<b>Pathogenicity</b>
<i>B. abortus</i>	cattle	yes	Moderate
<i>B. melitensis</i>	goats, sheep	yes	Highest
<i>B. suis</i>	pigs	yes	High
<i>B. canis</i>	dogs	yes	Moderate

# Who is at risk?

- Occupational disease: Dairy farmers, veterinarians, lab workers etc..
- Travelers
- Consumers of unpasteurized dairy products.



# The Spread of Brucella in the Body





# Antigenic structure

- **A** and **M** antigens are common to 3 main *Brucella* spp. (*B. canis* has no A and M antigen) *B. melitensis* has the highest concentration of M antigen and causes the most serious infections. *B. abortus* has the highest concentration of A antigen. The difference between species is related to the amount of the two main antigens
- *B. abortus*: A: M = 20:1
- *B. melitensis*: A:M = 1:20
- *B. suis*: A:M = 2:1
- Additionally they have superficial L envelope antigen.

# *Brucella abortus*

- Principal host- cattle
- Worldwide but some countries have eradicated it.
- Notifiable disease in many countries.
- Cause abortions, arthritic joints



# *Brucella suis*

- Principal host – swine
- Eradicated: Holland, Denmark
- Low incidence: Middle East, North Africa

# *Brucella canis*

- Principal host- dog
- Individuals who are in close contact with dogs, or breeders/ veterinary staff are risk of becoming infected.
- Rarely causes disease in humans.

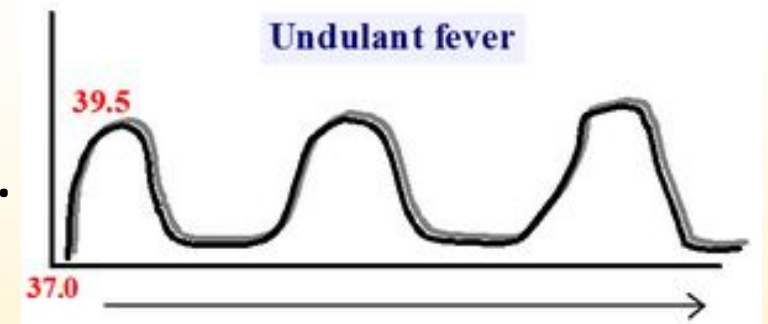


# *Brucella melitensis*

- Principal hosts-goats and sheep
- Most pathogenic in human
- Because of unpasteurized dairy products

# Signs and symptoms

- The incubation period is usually about 10-30 days although infection may persist for several months before causing any symptoms. *B.melitensis* and *B. suis* tend to cause more severe disease. Signs and symptoms are often variable and non-specific, with chills, fever, sweats and anorexia.
- The fever is characteristically (dirulant) **undulant**.



# Symptoms

- **Fever** is the most common symptom which is associated with **chills**.
- Constitutional symptoms of brucellosis including **anorexia, asthenia, fatigue, weakness, and malaise** (>90% of cases).
- **Bone and joint symptoms** include arthralgia, low back pain, spine and joint pain, and, rarely, joint swelling.

# Symptoms

- **Neuropsychiatric symptoms** of brucellosis are common including Headache, depression, and fatigue.
- **Gastrointestinal symptoms** include abdominal pain, constipation, diarrhea, and vomiting.
- **Neurologic symptoms** of brucellosis can include weakness, dizziness, unsteadiness of gait and urinary retention.

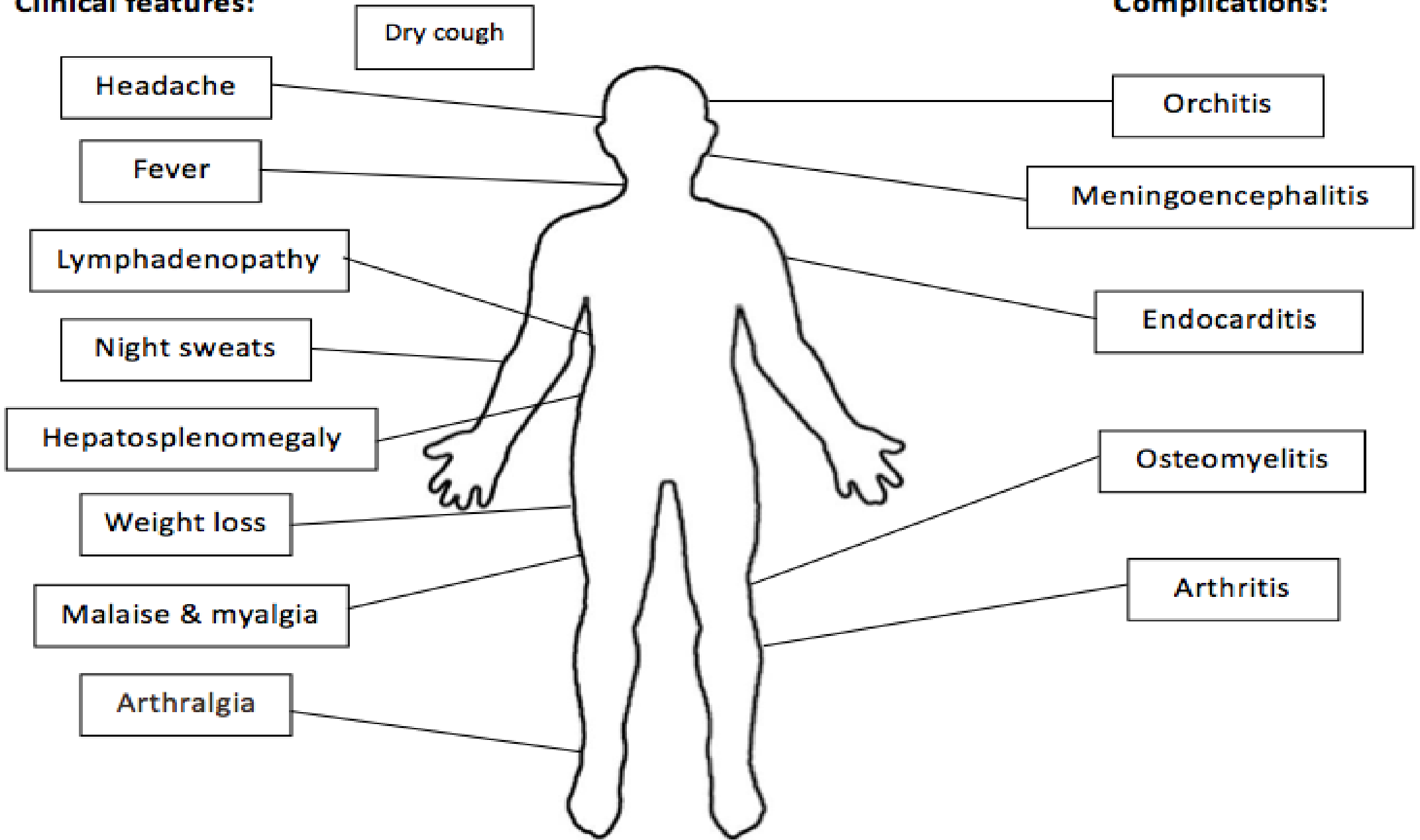


# Symptoms

- **Cough and dyspnea** develop in up to 19% of persons with brucellosis; however, these symptoms are rarely associated with active pulmonary involvement.

**Clinical features:**

**Complications:**



# Diagnosis in Humans

- Brucella are easily transmitted by aerosols, ingestion and percutaneous inoculation. Samples suspected to contain Brucella must be treated as high risk. Cultures must be handled under containment conditions appropriate to class 3 pathogens.

- Most often recovered from blood and bone marrow and less often from material obtained from spleen and liver abscesses.
- They grow on standard laboratory media.
  - brucella
  - blood
  - chocolate
  - trypticase soy agar

- Because of long incubation period (4-6 weeks). The diagnosis can be done serologically (1: 160) with Wright test (tube agglutination test)
  - Recent brucellosis when a four fold or greater rise in titer occurs during the first month or two of illness.

- Immunofluorescence method
- PCR
- **Castaneda strip test:** Strip with color Brucella antigen. On addition of patients serum, If antibodies are present, prevents the flow of serum.
- Brucella skin test: delayed hypersensitivity test

## Diagnosis in Animals

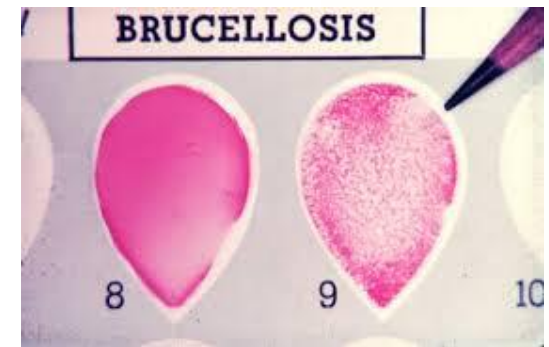
- Culture of urine and milk
- Rapid latex agglutination test
- Rose Bengal test
- Milk ring test

# Rose Bengal test

- It is one of the easiest methods to implement and the most widely used for identifying Brucellosis antibodies in sera.

## Principle of the test:

- The RB is a rapid slide agglutination test.
- It is now often used widely for diagnosing human disease.
- The test uses a suspension of *B. abortus* smooth cells stained with Rose Bengal dye (pink color) to detect Brucella agglutinins.
- The stained bacterial suspension agglutinates when mixed with samples containing specific IgG or IgM antibodies present in the patient sample.





**Milk Ring Test:** Frequently used test.

A drop of colored brucella antigen (B.abortus/B.melitensis with hematoxylin) is added to milk in a test tube, incubated in a water bath at 70°C for 40-50 minutes.

**Positive:** Blue ring at the top leaving the milk unstained.

**Negative:** No ring. Milk remains uniformly blue.



# Treatment

First option combination of doxycycline-rifampin

- Tetracycline
- Aminoglycosidase
- Trimethoprim sulfamethoxazole (for prolonged period of time: at least 6 weeks).

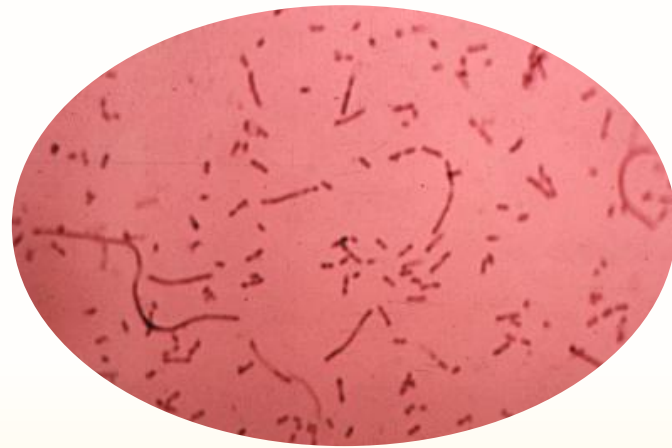
# Prevention

- Control of disease in domestic animals.
- Routine pasteurization of milk
- In Labs, biosafety precautions

# KEY POINTS FOR BRUCELLA

- Brucella are highly infectious coccobacilli that cause a septicemic illness, undulant fever,. Most human disease is caused by *B. melitensis*, *B. abortus* or *B. suis*.
- The disease is atypical zoonosis most commonly acquired from infected animals or from infected meat or dairy products.
- Brucellosis is diagnosed by isolation of the organism from blood; serology or PCR tests.

# *Haemophilus*



- Small, Gram negative rods
- Non-motile
- Transmitted via respiratory droplets, or direct contact with contaminated secretions
- Normal flora of the human respiratory tract and oral cavity.

# *Haemophilus* species

1. *H. influenzae*: type b is an important human pathogen
2. *H. ducreyi*: chancroid ( painful penile ulcers)
3. Other *Haemophilus* are normal flora
  - *H. parainfluenzae* : pneumonia, endocarditis
  - *H. aphrophilus*: endocarditis, sinusitis, abscess
  - *H. aegyptius*: pink eye

# Differentiation of Species

Species	Hemolysis	Growth factor X	Growth factor V
<i>H. influenzae</i>	-	+	+
<i>H. aegyptius</i>	-	+	+
<i>H. ducreyi</i>	-	+	-
<i>H. parainfluenzae</i>	+	-	+
<i>H. aphrophilus</i>	-	-	-

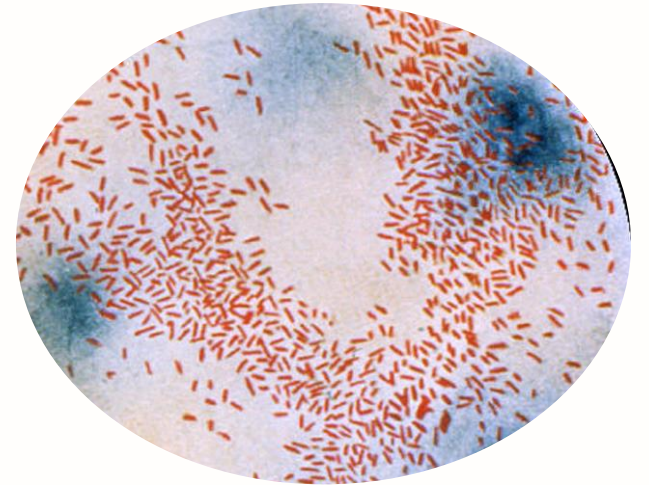


# *H. influenzae*

- Small, pleomorphic rods or cocobacilli
- Some strains produce **polysaccharide capsule**
- There are six capsular types (a-f)
- Catalase and oxidase positive
- They reduce nitrate to nitrite, ferment glucose.

## Resistance:

- Heating at 55 °C for 30 minutes destroys.
- Drying and disinfectants destroy.



# Growth requirements

- *H. influenza* growth depends on a requirement for two factors, termed **X** (haemin-hematin) and **V** (NAD-NADP).
- **X factor:** (Heat stable) required for the synthesis of cytochrome c and other iron-containing respiratory enzymes
- **V factor:** (Heat labile) is nicotinamide adenine dinucleotide (NAD), NAD phosphate. It is essential for oxidation- reduction process in cell metabolism.

- Heating blood agar for a few minutes at 70-80 °C until it turns brown (chocolate agar) much improves the growth of *H. influenzae*. This process removes serum NADase, which limits the amount of V factor, and also liberates extra X and V factors from red cells into the medium.

# Satellitism



Growth is stimulated in the vicinity of a streak of *S. aureus*, which synthesizes V factor

<http://www.medical-labs.net/haemophilus-influenzae-diagnosis-using-satellite-test-1659/haemophilus-influenzae-satellitism-test/>

# Antigenic Structure

1. Capsular polysaccharide: prevent phagocytosis
2. Outer membrane proteins (OMP): similar with *E.coli* but less toxic, it is related with adherence and spreading of bacillus
3. Lipopolysaccharides (LPS)

- *H. influenzae* can be divided into six serotypes on the basis of capsular polysaccharide. Type b is the most common and most invasive (type b).
- 95 % of invasive disease caused by type b (Hib).

- *H. influenzae* is associated with two types of infection which are quite distinct in their epidemiological profiles; invasive and non-invasive infections.

### **The most common Invasive infections;**

- **Meningitis-** (~50% of cases of invasive disease) infection of membranes covering the brain. Fever, decreased mental status, and stiff neck. Mortality 2-5%, brain damage 15-30 %.
- **Epiglottitis-** (17%) infection and swelling of epiglottis, the tissue in the throat that covers the larynx during swallowing. (2-5 age children)
- **Septic arthritis-** (8%)
- **Cellulitis** (6%)
- **Pneumonia** (15%)

## **Less common infections;**

- Osteomyelitis (2%)
- Pericarditis (2%)

## **Non-invasive infections;**

- Otitis media – middle ear infection
- Conjunctivitis – pink eye
- Sinusitis
- Acute bronchitis



# Diseases of *Haemophilus* spp

## *H influenzae*

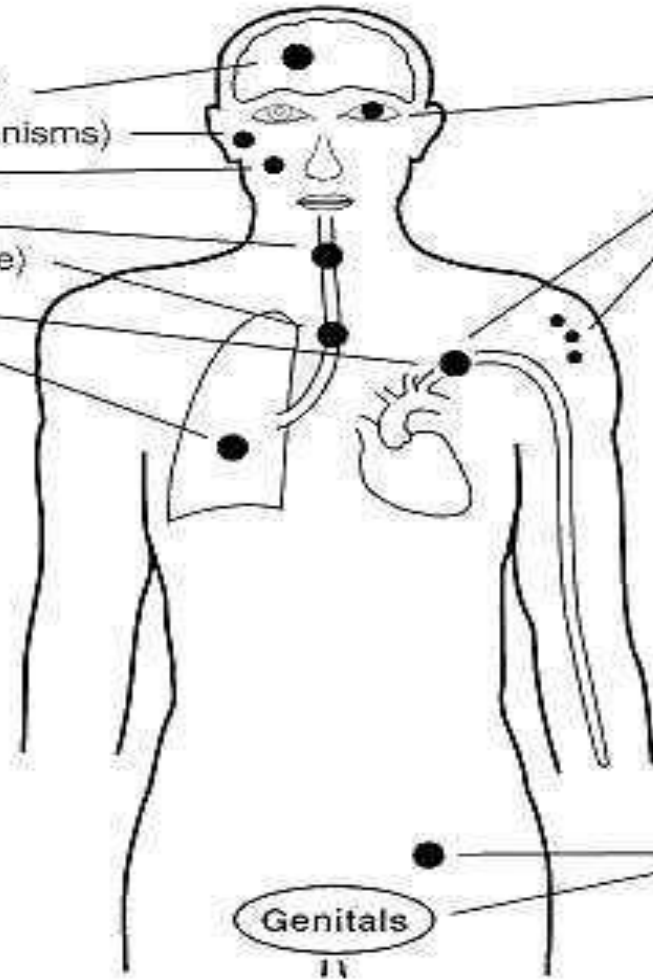
- Meningitis (type b organisms)
- Otitis media (nontypable organisms)
- Sinusitis (nontypable)
- Epiglottitis (type b)
- Tracheobronchitis (nontypable)
- Bacteremia (type b)
- Pneumonia (nontypable)

## *H aegyptius*

- Conjunctivitis
- Purpuric fever (Brazilian)

## *H ducreyi*

- Chancroid (papules & ulcers of genitals, lymph nodes)



Genitals  
11

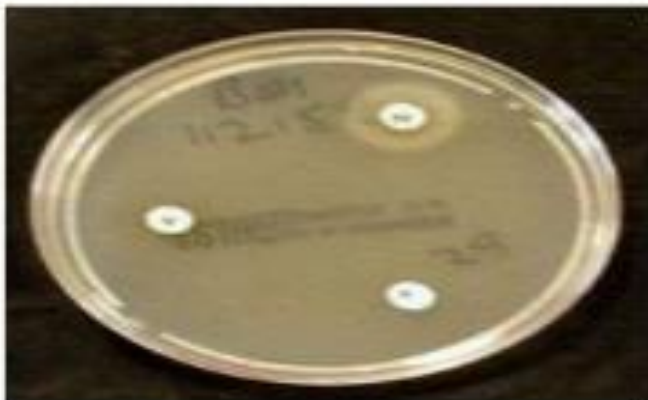
- **Transmission:** Droplet infection and discharge from the upper respiratory tract during the infectious period.
- Incubation period unknown, probably short, 2-4 days.

### **Infectious Period**

- As long as the organism is present, even in the absence of nasal discharge.
- Noninfectious within 24 to 48 hours after the start of effective antibiotics

# Laboratory Diagnosis

- Microscopy: Gram negative pleomorphic organism
- On chocolate agar the colonies are smooth, grey or colorless. Confirmation of the identity depends on demonstrating a requirement for one or both of the growth factors, X and V.



*Haemophilus influenzae* requires X and V factors for growth. In this culture *Haemophilus* has only grown around the paper disc that has been impregnated with X and V factors. There is no bacterial growth around the discs that only contain either X or V factor.

- PCR
- The capsular type of *H. influenzae* isolates is determined by slide agglutination with type specific antisera.
- Antigen detected: The detection of type b polysaccharide antigen in body fluids or pus is useful. A rapid latex agglutination test with rabbit antibody to type b antigen is used most commonly.

# Treatment

- Cefotaxime
- Ceftazidime
- Amoxicillin clavulanate
- Clarithromycin
- For Hib hospitalization is required.  
3<sup>rd</sup> generation cephalosporin or  
chloramphenicol plus ampicillin

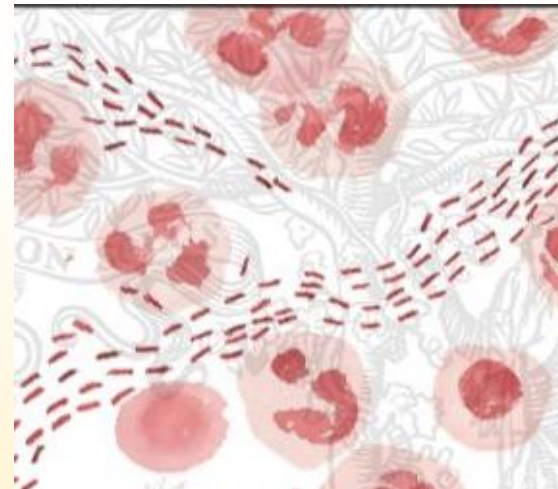
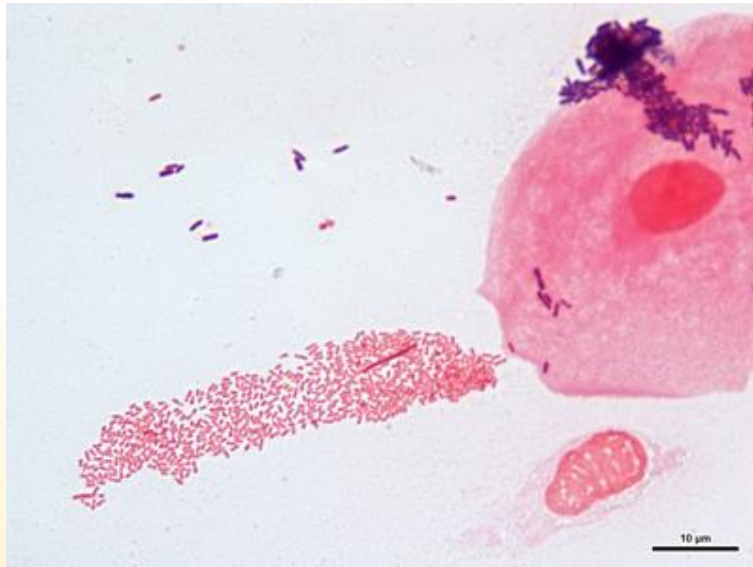
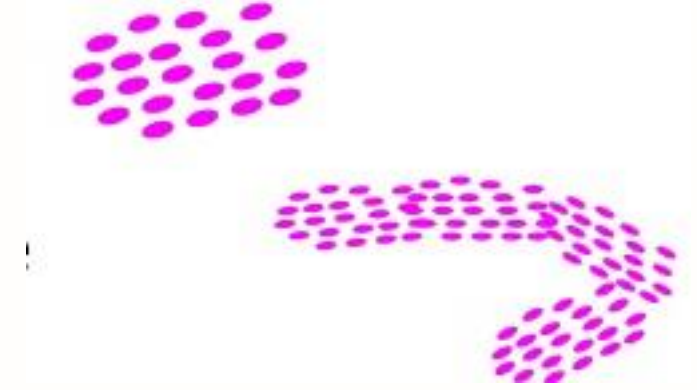
# Vaccines

- Haemophilus B conjugate vaccine. (Hib)
- Vaccine has reduced Hib meningitis in children by 95 %.

# *Haemophilus ducreyi*

- Responsible for a **sexually transmitted infection** **chancroid**, which is most prevalent in tropical regions, particularly Africa and south-East Asia. Patients present with painful penile ulcers (soft chancre) and inguinal lymphadenitis.
- Infection is localized spreading to only to regional lymph nodes.
- Lymph nodes enlarged and painful.

- Short cocobacillus, non-spore, non-motile, non-capsulated
- Needs X factor for growing
- Bipolar staining
- Bacilli in small groups appear as parallel chains or fish flock



# Treatment of *H. ducreyi*

- Sulphonamides
- Erythromycin
- Ciprofloxacin
- Ceftriaxone



# *Haemophilus aegypticus*

- Also called Koch-Weeks bacillus
- Identified as bio type of *H. influenzae*
- pink eye
- Sulphonamides and Gentamycin effective



# The other *Haemophilus* species

- *H. parainfluenzae*
- *H. haemolyticus*
- *H. parahaemolyticus*
- *H. paraprophaeolyticus*
- *H. pittmaniae*
- These species are the normal flora members of respiratory tract and rarely associated with infections.

# KEY POINTS FOR HAEMOPHILUS

- Most strain of *H. influenzae* are non-capsulate but some strains possess a polysaccharide capsule (a-f)
- *H. influenzae* type b (Hib) is a major human pathogen that causes invasive infections including meningitis, and epiglottitis
- Non-capsulate strains cause approximately 10% of invasive infections and 90% of non-invasive respiratory infections including otitis media

- 20% of *H. influenza* strains are ampicillin resistant , ceftriaxone is the treatment of choice for invasive disease.
- Conjugate Hib vaccines
- *H. ducreyi* causes chancroid, sexually transmitted genital ulcers that are common in Africa and South-East Asia.

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