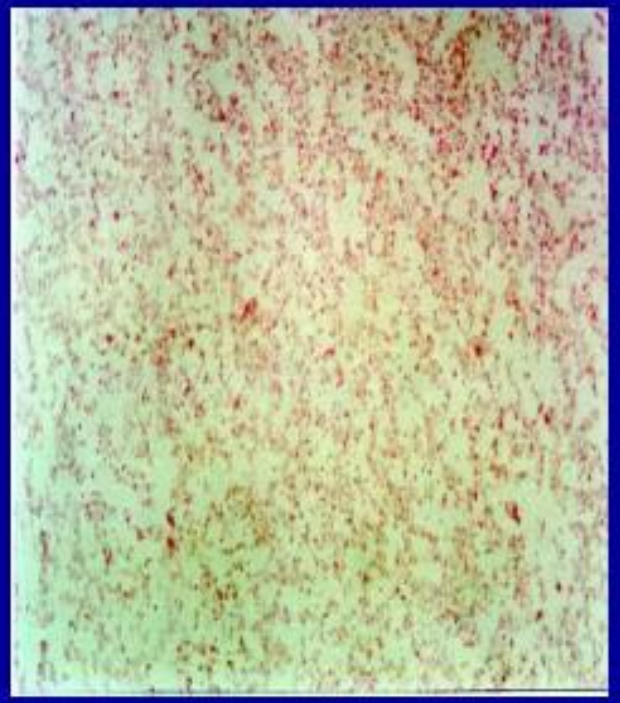
Lecture X

Causative agent of zoonotic infection (genus *Brucella*, *Bacillus*,*Listeria*, *Yersinia*, *Francisella*). Pathogenic members of *Corynebacterium*, *Bordetella*, *Haemophilus*, *Gardnerella*, *Legionella* genus

Francisella tularensis

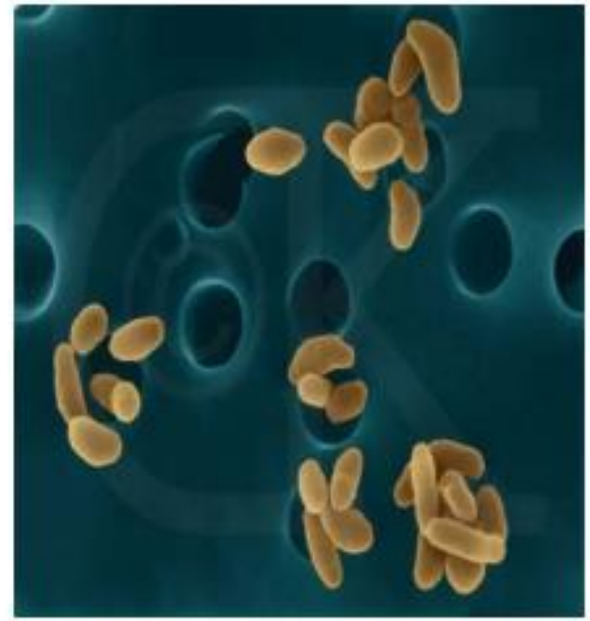
- Gram stain
  - Poorly staining, tiny Gram-negative coccobacilli

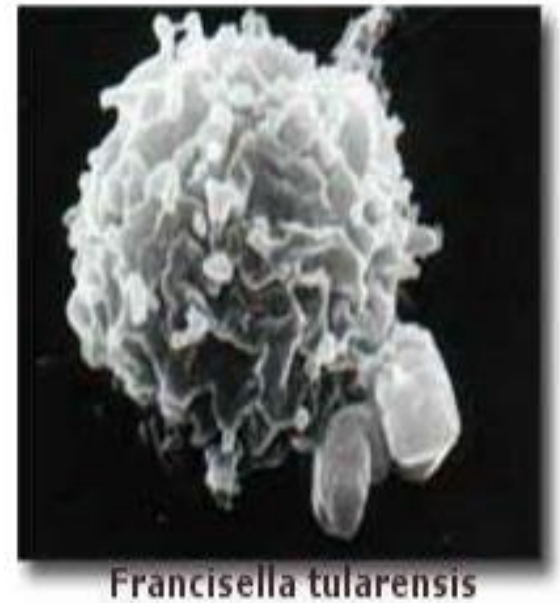


Morphology and Physiology

- Gram-negative rod, pleomorphic (coccoid to filamentous)
- Facultative anaerobe
- Requires a rich medium plus cystine to grow

Microbiology

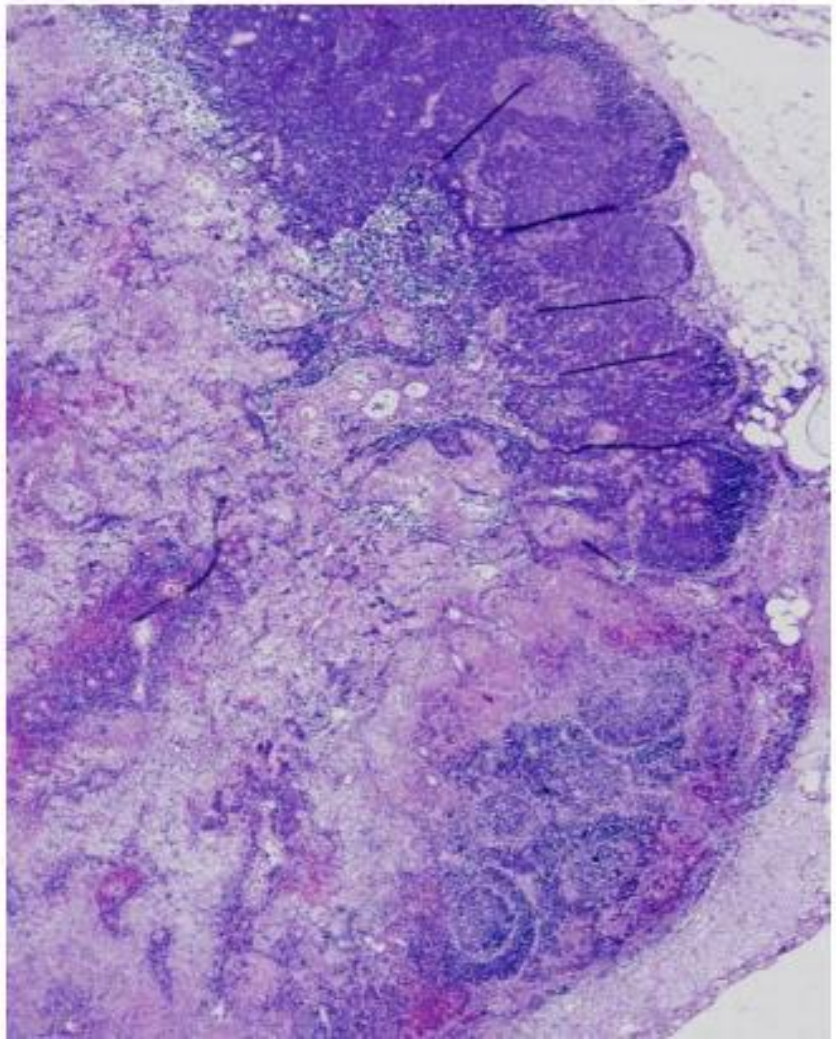




Tularemia Transmission

- Vectors
  - HARD TICKS
    - Transstadial/transovarian
    - Human transmission
    - MT: *Dermacenter variabilis*
  - BITING FLIES
    - *Chrysops*
    - *Tabanus*
    - Mechanical transmission
    - Infective for 14 days
- Direct contact
  - Skinning infected animals
- Ingestion
  - Infected tissues
  - Water (esp. *Microtus* spp)
- Inhalation

Tularemia Diagnosis



- History of exposure
- Culture
- Paired serology
  - ELISA, tube agglutination, microagglutination for IgM
- Histopathology
- Immunohistochemistry
- FA
- PCR

Transmission

- Organisms are harbored in the blood and tissues of wild and domestic animals, including rodents
- In US chief reservoir hosts are wild rabbits and ground squirrels

Tularemia Lesion

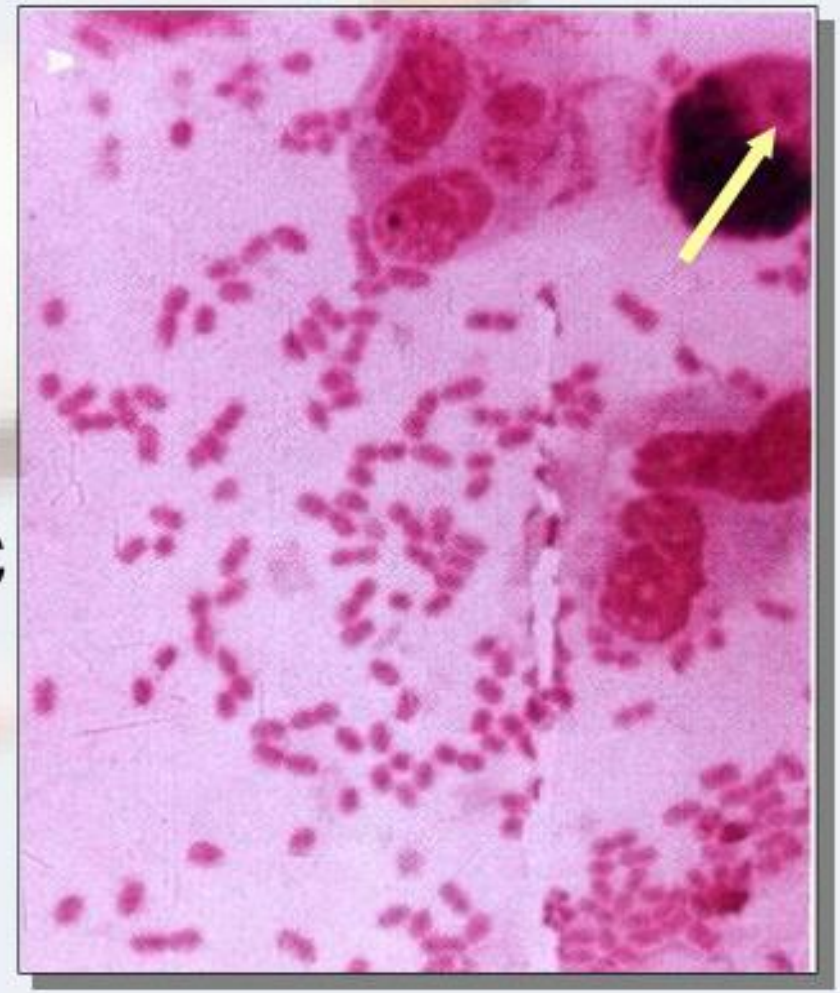
Skin Ulcer of Tularemia

Yersinia species

- *Yersinia pestis*
- *Yersinia enterocolytica*
- *Yersinia pseudotuberculosis*

Morphology/Physiology

- Gram-negative, pleomorphic bacillus
- Facultative anaerobe
- Optimal growth at 28° C
- Facultative intracellular parasites



Virulence Determinants

- Calcium dependence at 37° C (*lcr)*
- Proteins V & W
- *Yops*
- Envelope (F-1) antigen
- Coagulase and Fibrinogen Activator (fibrinolysin)

Yersinia Outer Proteins (*Yops*)

- Plasmid encoded lcr controlled proteins (11)
- Cytotoxic
- Inhibit phagocyte migration, engulfment and intracellular killing
- Inhibit platelet aggregation

F-1 Antigen

- Protein-polysaccharide complex
- Highly expressed in the mammalian host
- Not expressed in flea host
- Antiphagocytic
- Antibodies to F-1 are protective

Coagulase and Fibrinolysin

- Plasmid encoded proteins
- Coagulase causes clotting and microthrombi formation
- Fibrinolysin promotes dissemination

V and W antigens

- Plasmid encoded proteins
- Part of the low calcium (*lcr*) regulated response
- Rapid proliferation leading to overwhelming septicemia

The different forms of plague

- Sylvatic
  - In animals (rodents, rabbits, carnivores) only
- Bubonic
  - Flea (bite) to humans
- Pneumonic
  - Human (aerosol) to human

Bubonic vs pneumonic plague

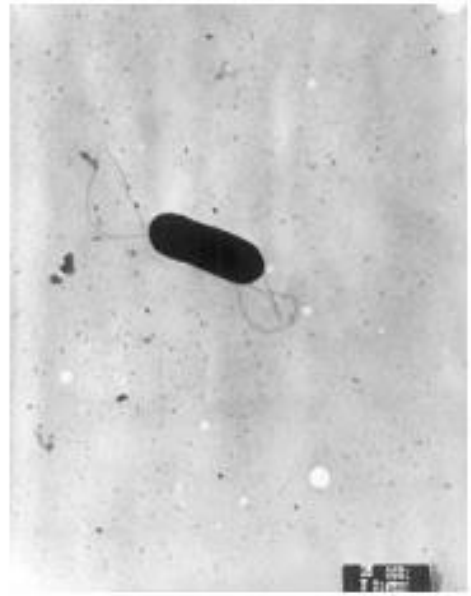
	Bubonic	Pneumonic
entry	flea-bite	inhalation
disease	buboes, hemorrhage, pneumonia	rapid pneumonia
exit	flea & exhalation	exhaled droplets

Diagnosis

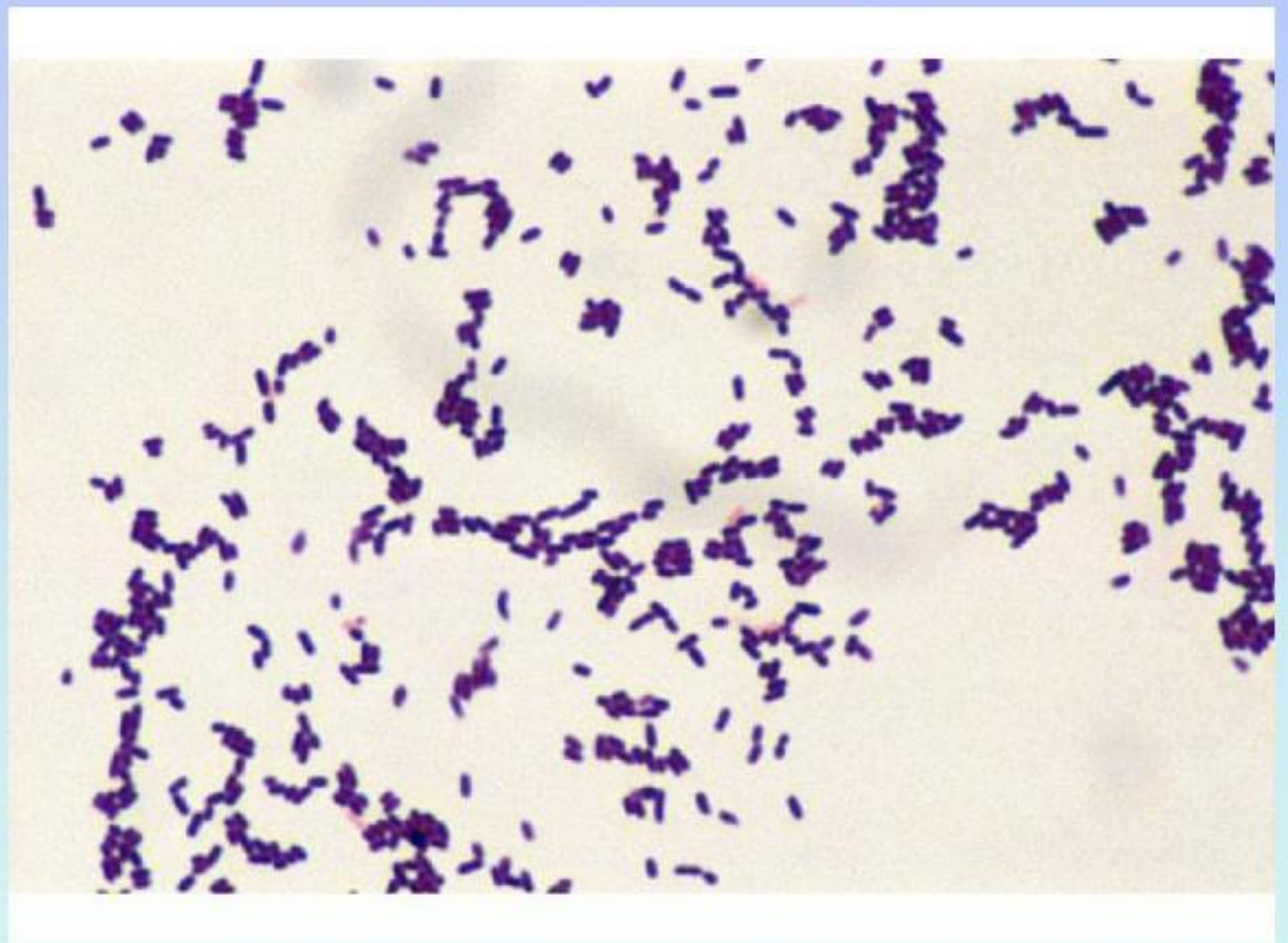
- Examination of Buboes aspirate stained smears for bipolar staining
- Fluorescent-antibody
- Culture/identify organisms (hazardous)

Listeria Monocytogenes

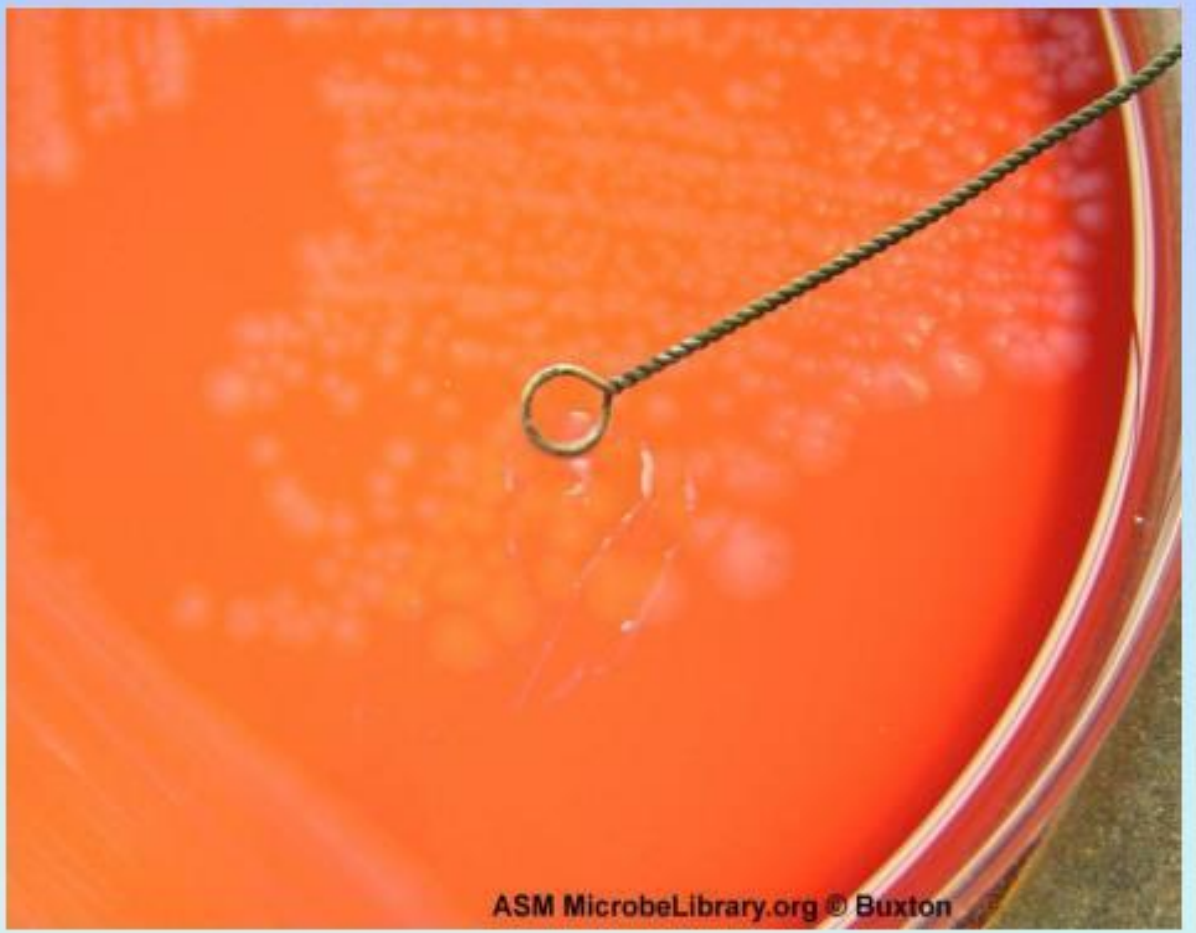
- Rod shaped Gram Positive bacteria (bacilli)
- 7 species of Listeria
- First documented in 1924



Gram Stain Listeria



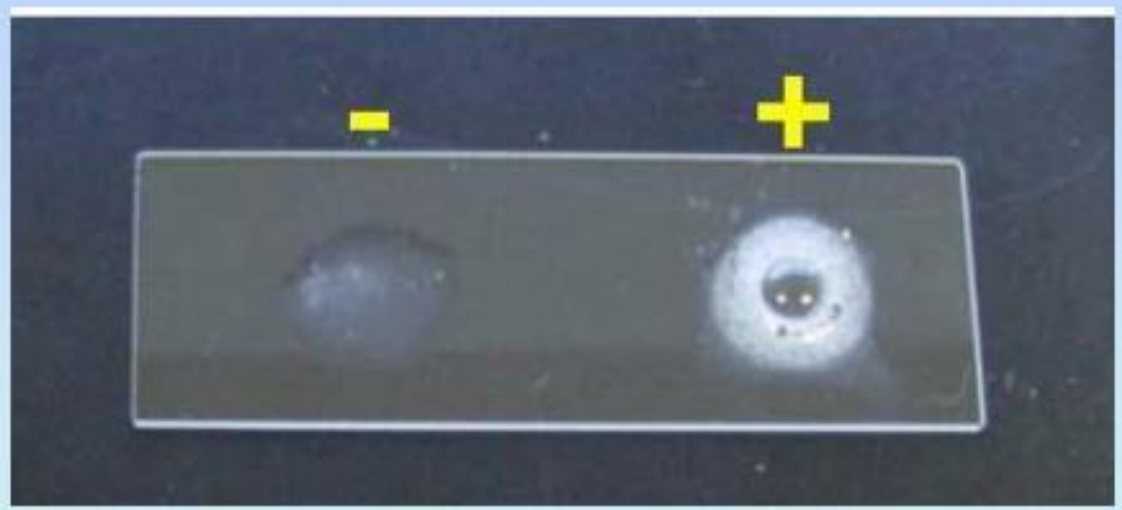
Listeria Culture




OXL
5011-10-51 BIOMERIEUX
05:23

CAMP test

Catalase +ve



Listeria identification

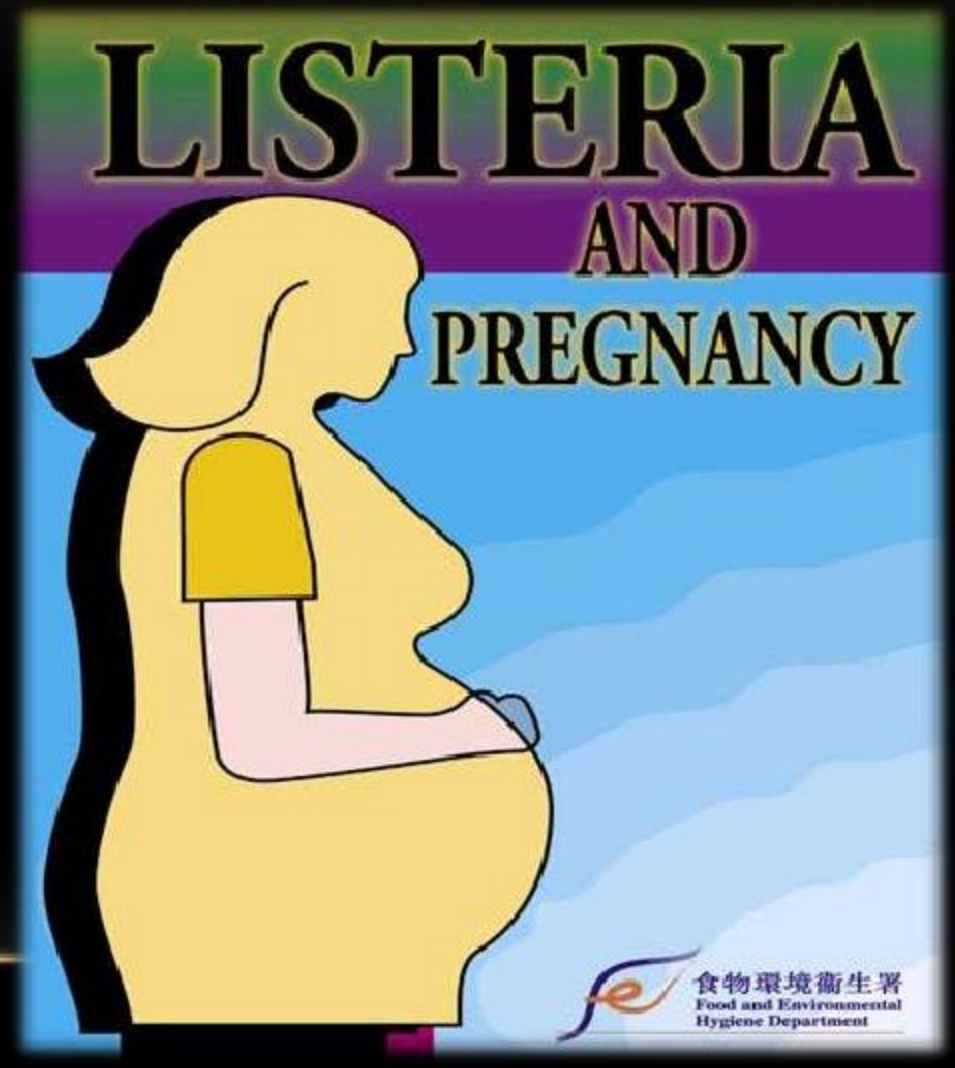
- Gram stain
- Beta-haemolysis
- CAMP test
- Tumbling motility
- Esculin hydrolysis
- Catalase positive, oxidase negative
- API coryne
- MALDITOF!

WHO ARE AT RISK WITH LISTERIOSIS

- Pregnant women
- New-borns
- People with weakened immune systems
- People who are taking immuno-suppressing medication.

LISTERIOSIS AND PREGNANCY

- **Pregnant women** - They are about 20 times more likely than other healthy adults to get Listeriosis. About one-third of listeriosis cases happen during pregnancy.



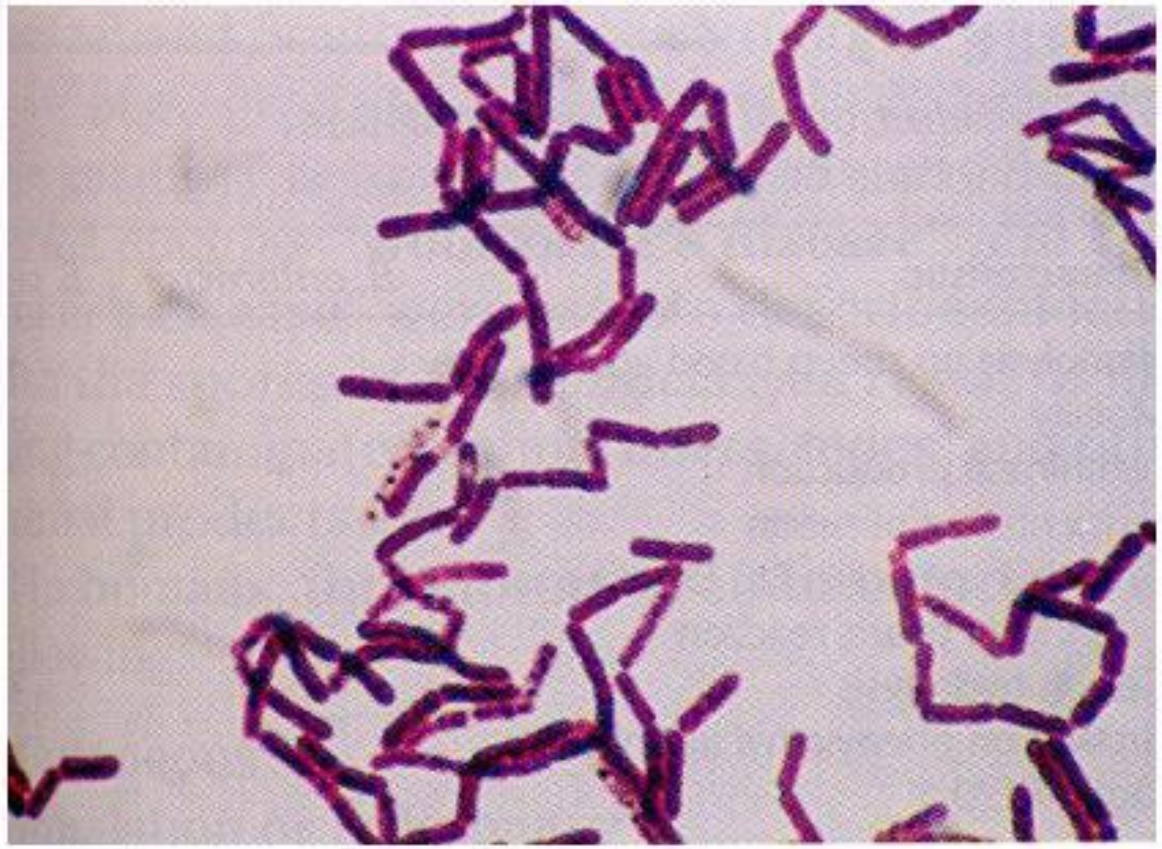
Bacillus

- Saprophytic
  - *B.cereus*
  - *B.subtilis*
  - *B.megaterium*
  - *B.Circulans*
- Pathogenic
  - *B.anthracis*

Bacillus

- Classification
  - All are large Gram-positive bacilli
  - Are aerobic
  - Form endospores
  - Most are found in dust and soil
  - *Bacillus anthracis* is the major pathogen in the group
- Morphology and Cultural Characteristics (*Bacillus anthracis*)

Gram stain of *Bacillus sp.*



Endospores of *Bacillus sp.*

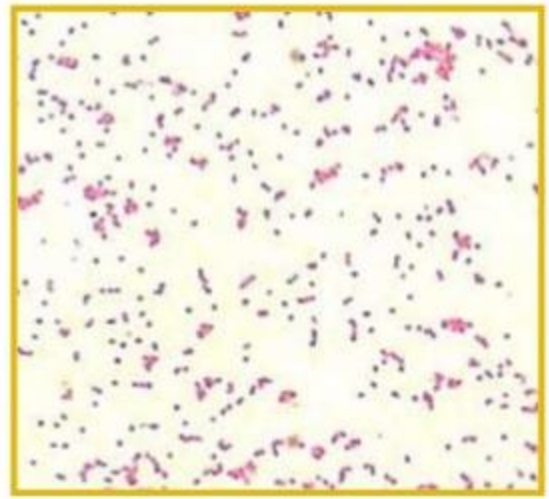
Bacillus anthracis

- Grow well on ordinary lab media producing large granular colonies with a coarse texture.

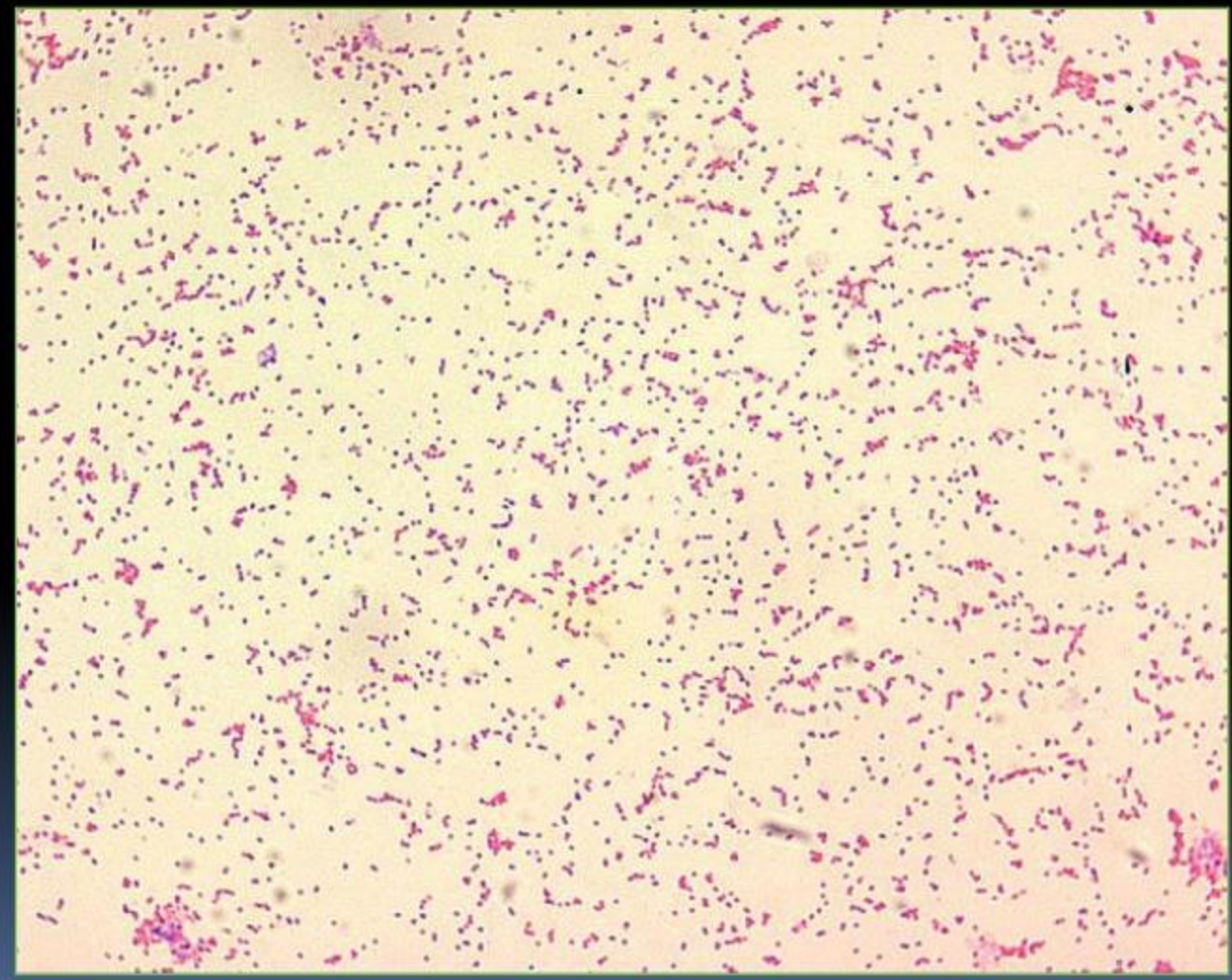
Cutaneous Anthrax

Brucella spp.

- Gram negative coccobacillus
  - Facultative, intracellular organism
- Multiple species
  - Associated with certain hosts
- Environmental persistence
  - Withstands drying
  - Temperature, pH, humidity
  - Frozen and aborted materials, dust, soil

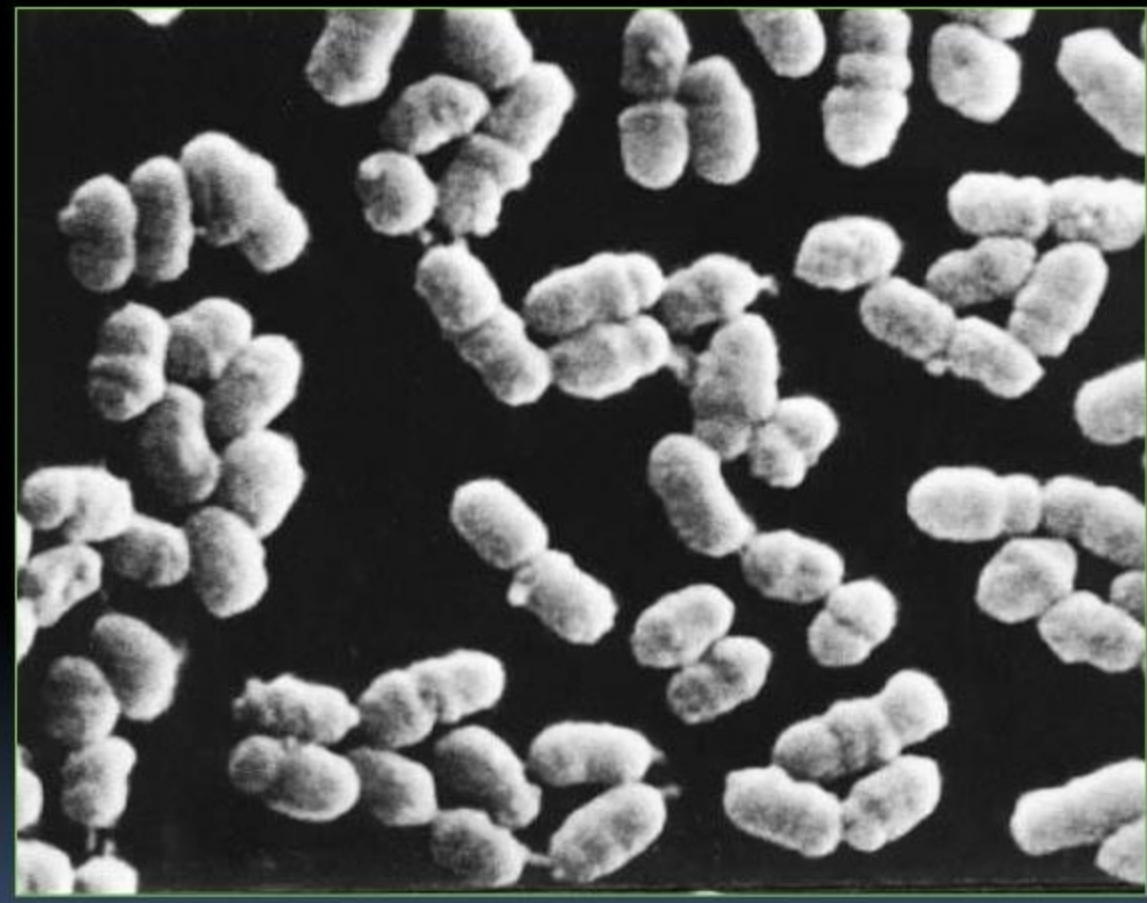


Brucella species
gram-negative bacilli



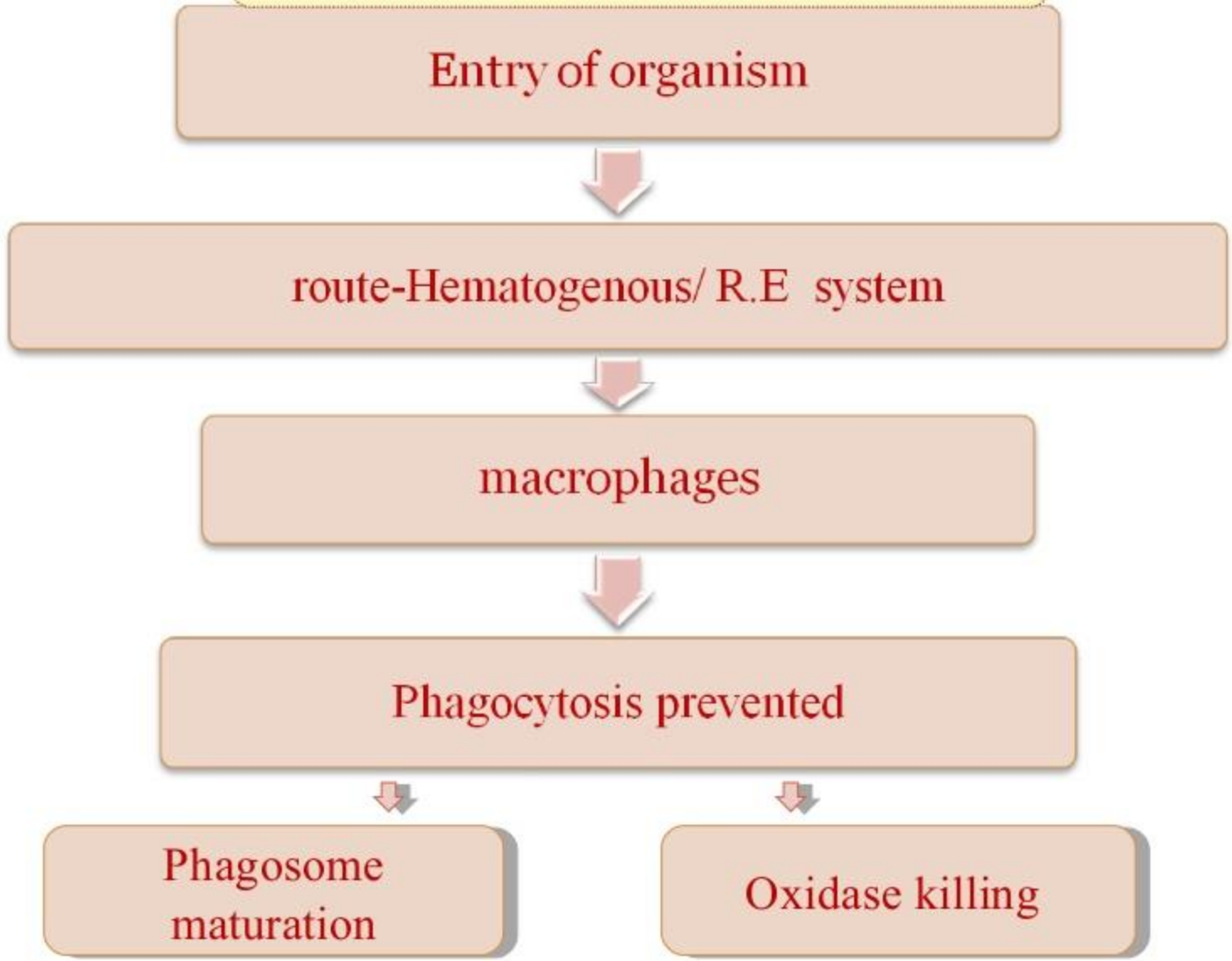
Brucella melitensis colonies

Brucella abortus



Species	Biovar/ Serovar	Natural Host	Human Pathogen
B. abortus	1-6, 9	cattle, bison, buffalo	yes
B. melitensis	1-3	goats, sheep	yes
B. suis	1, 2, 3	swine	yes
	2	European hares	yes
	4*	reindeer, caribou	yes
	5	rodents	yes
B. canis	none	dogs, other canids	yes
B. ovis	none	sheep	no
B. neotomae	none	rodents	no
B. maris *B. pinnipediae,* *B. cetaceae(?)*		marine mammals	yes?

PATHOGENESIS



- Preferentially localization of Gravid uterus (unknown factors – Allontoic factors, Erythritol)

Populations at Risk

- Occupational disease
  - Cattle ranchers/dairy farmers
  - Veterinarians
  - Abattoir workers
  - Meat inspectors
  - Lab workers
- Hunters
- Travelers
- Consumers
  - Unpasteurized dairy products

Clinical Signs: Cattle and Bison

- Third trimester abortions with *B. abortus*
- Retained placenta
  - Once expelled will have a leathery appearance
- Endometritis
- Birth of dead or weak calves
  - Respiratory distress and lung infections
- Low milk yield

Clinical Signs: Sheep and Goats

- *B. melitensis*
  - Late term abortions
    - Retained placenta
    - Birth of dead or weak lambs/kids
- Goats
  - Articular, periarticular hygroma localizations
- *B. ovis*
  - Abortions, fertility problems in sheep
    - Orchitis, epididymitis
    - Abnormal breeding soundness exam

Clinical Signs: Swine

- *B. suis*
  - Prolonged bacteremia
  - Abortion, early or late gestation
  - Fertility problems
  - Lameness, posterior paralysis, spondylitis metritis, abscesses

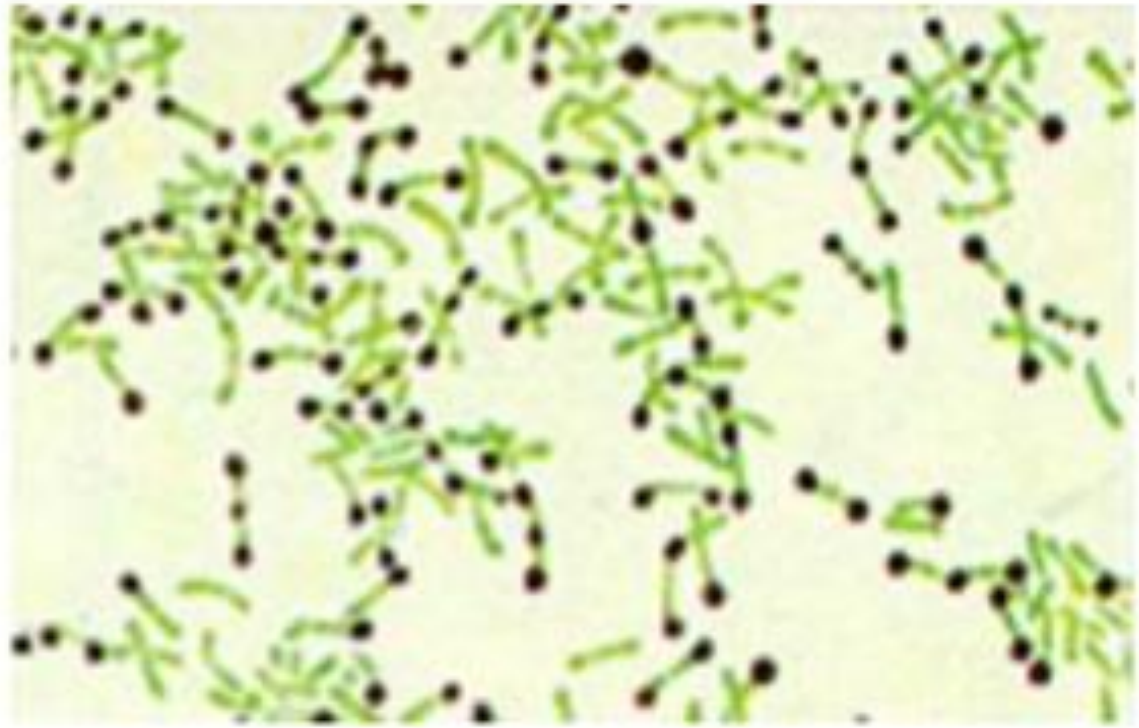
Clinical Signs: Horses

- *B. abortus* most common
  - Susceptible to *B. suis*
- Fistulous Withers or Poll Evil
  - Inflammation of the supraspinous bursa
  - Exudative process
    - Bursal sac fills with clear viscous liquid
    - Can eventually rupture

Clinical Signs: Dogs

- *B. canis*
  - Abortions
    - Last trimester
    - Prolonged vaginal discharge
  - Bacteremia
  - Failure to conceive, stillbirths, Prostatitis, epididymitis
- Also susceptible to
  - *B. melitensis*, *B. abortus*, and *B. suis*

CORYNEBACTERIUM DIPHTHERIAE



MORPHOLOGY

- Slender Gram-positive rods, pleomorphic; easily decolousised;
- 0.6-0.8µ diameter and 3-6 µ length;
- Irregular swelling at one or both ends ('club shaped');
- Non-capsulate, Non-sporing and nonmotile
- Granules containing polymetaphosphate are seen in the cells;
- Take up bluish purple color against lightly stained cytoplasm, when stained with Loeffler's Methylene Blue, and hence called 'Metachromatic granules';
- Also called, 'volutin granules' or 'Babes Ernst granules';
- They are often situated at poles- 'polar bodies'

MORPHOLOGY

- Special stains for demonstrating the granules :
  - Albert's stain
  - Neisser's stain
  - Ponder's stain
- The bacilli are arranged in pairs, palisades or small groups; the bacilli lie at various angles to each other, resembling the letters, V or L;
- This is called, "Chinese letter pattern" or "cuneiform pattern";

CULTURAL CHARACTERISTICS

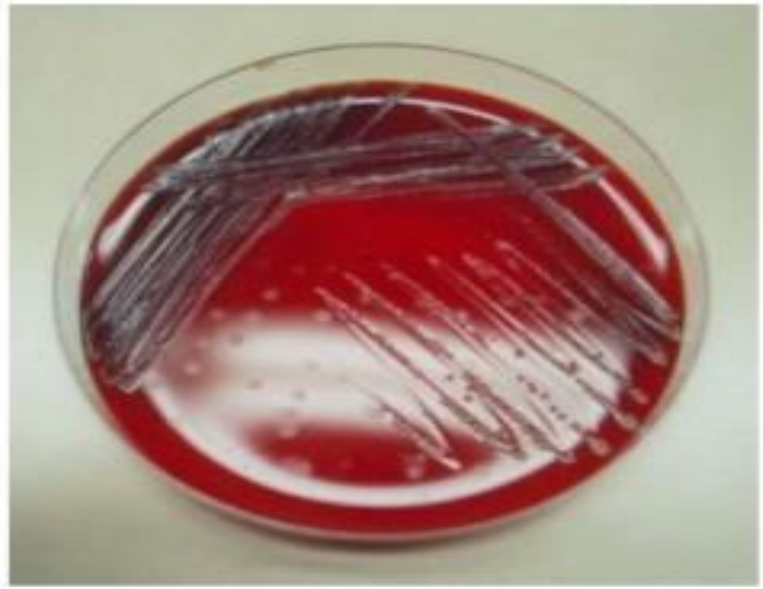
- Aerobe and facultative anaerobe;
- Optimum temperature is 37^0C
- Growth scanty on ordinary media;
- **Enrichment with:** blood, serum or egg is necessary for good growth;
- Potassium tellurite(0.04%) acts as a *'selective agent',* as it inhibits growth of most oral commensals and retards the growth of *Candida albicans* and *S.aureus;*

MEDIA FOR CULTIVATION

- Blood agar
- Loeffler's serum slope
- Tellurite blood agar

COLONY CHARACTERISTICS

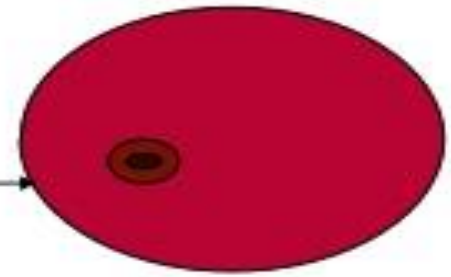
- Blood agar : small, granular and gray with irregular edges; Hemolysis may or may not present;
- Loeffler's serum slope:
  - Very rapid growth;
  - Colonies in 6-8 hrs
  - Initially circular white opaque colonies and acquire yellowish tint on incubation



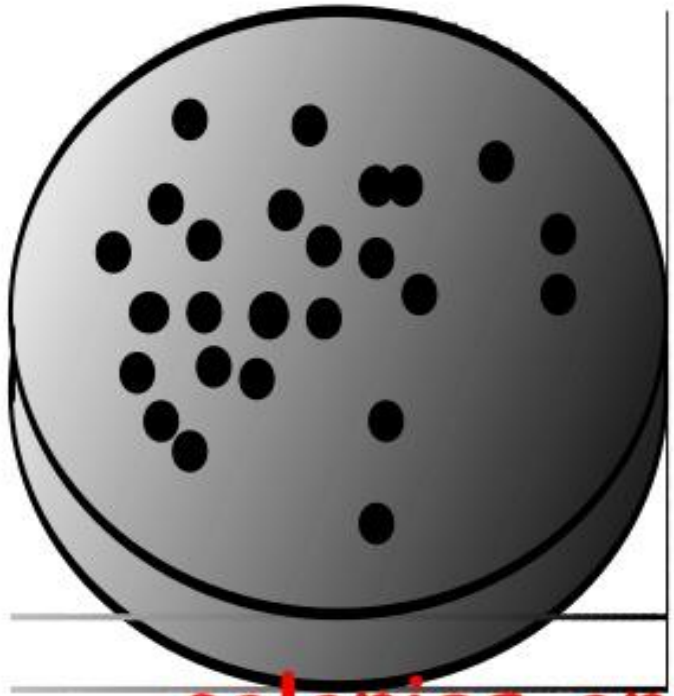
COLONY CHARACTERISTICS

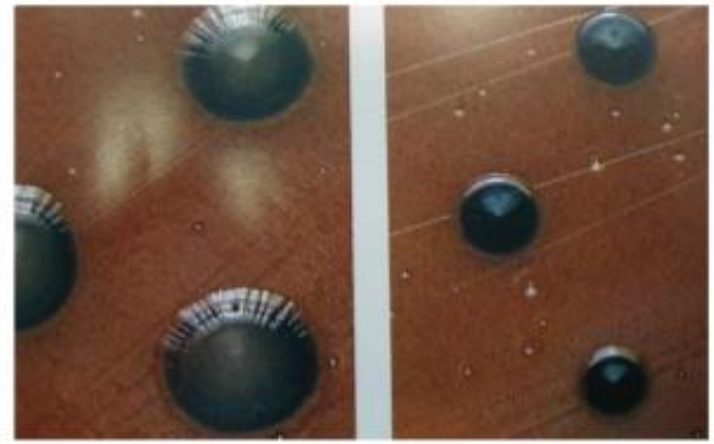
- Tellurite blood agar:
  - Growth slow; colonies seen after 48 hrs;
  - The colonies are brown to black with a brown-black halo because the tellurite is reduced to metallic tellurium;
  - Staphylococcus also produce such colonies

A diagrammatic representation →



Colonial morphology





colonies on tellurite agar

BIOTYPES

- **McLeod and Anderson** classified diphtheria bacilli, based on the colony characteristics on Tellurite medium and other properties like biochemical reactions and severity of disease;
- 3 biotypes :
  - *gravis*
  - *intermedius*
  - *mitis*
- 4th biotype : *belfanti* has also been described

Feature	gravis	intermedius	mitis
Morphology	shot rods, few granules some degree of pleomorphism	long barred forms poor granulation Pleomorphism	long curved prominent granules Pleomorphism
Colony on tellurite blood agar (48 hrs)	**Daisy head colony** (flat colony with raised dark centre and crenated edge; radial striations)	**Frog's egg colony** (dull granular centre with glistening periphery and lighter ring near edge)	**Poached egg colony** (shiny , flat with central elevation)
Consistency of the colonies	Brittle not easily emulsifiable	intermediate	soft, buttery easily emulsifiable
Hemolysis	**Variable**	**nonhemolytic**	**hemolytic**
Glycogen/ starch fermentation	Positive	Negative	Negative

BIOCHEMICAL REACTIONS

- Hiss serum sugars – for testing fermentation reactions;
- Ferment- *glucose, galactose, maltose and dextrose*; but not *lactose, sucrose, mannitol;*
- Proteolytic activity is absent;
- Do not hydrolyse urea;
- Do not form phosphatase;
- Produce cystinase (halo on Tinsdale's medium)

RESISTANCE

- Cultures remain viable for 2-3 wks at 25-30^{0}C
- Destroyed by heat
- Resistant to light, desiccation or freezing;
- Easily destroyed by antiseptics
- Susceptible to – Penicillin, erythromycin and broad spectrum antibiotics;

ANTIGENIC STRUCTURE AND TYPING

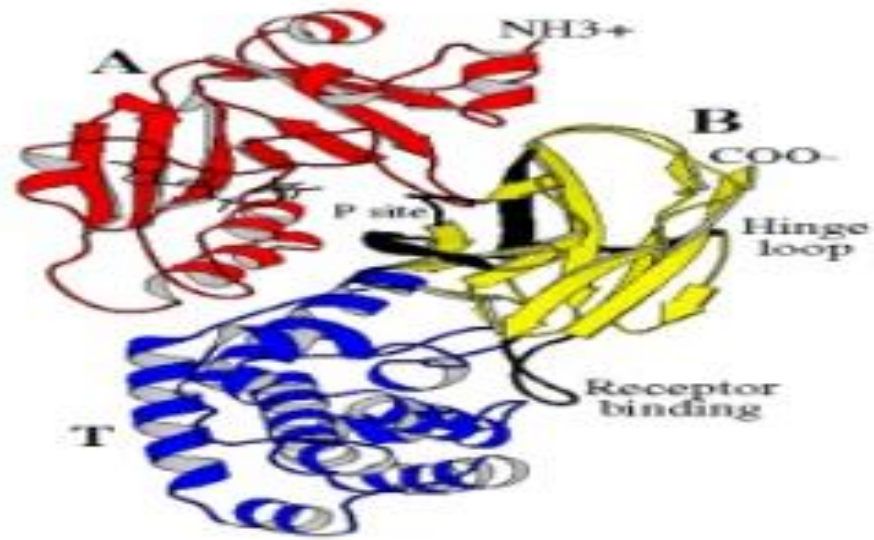
- Serotyping : Antigenically heterogenous
  - gravis: 13 types
  - intermedius : 4 types
  - mitis : 40 types
- Bacteriophage typing : 15 types
- Bacteriocin typing : diphtheriocin typing

VIRULENCE FACTORS

- Virulent strains of diphtheria bacilli produce a very powerful exotoxin.
- The 'virulence' of diphtheria bacilli is due to their capacity to-
  - Establish infection and growing rapidly
  - Quickly elaborate an exotoxin
- Avirulent strains are common among convalescents , contacts and carriers, particularly those with extra-faucial infection

DIPHTHERIA TOXIN

- The pathognomonic effects are due to the toxin;
- Almost all the gravis and intermedius strains and 80-85% of mitis strains are toxigenic
- Toxin is a protein;
- Mol. Wt.: 62,000
- Two fragments, A and B;
- Extremely potent :
  - 0.1 µg lethal to guinea pig
- Inactive when released



Toxin – mechanism of action

- Fragment B : binds to a cell surface receptor and helps in transport of toxin into the cell;
- After entering the cell, A subunit is released ;
- A subunit catalyses the transfer of 'adenosine diphosphate ribose (ADPR)' from NAD+
- ADPR binds with the elongation factor EF 2
- "ADPR-EF2" complex is inactive → protein synthesis stops abruptly → necrotising and neurotoxic effects of the toxin;

PATHOGENICITY

- Commonest site of infection: Upper respiratory tract (fauces, larynx,nose)
- Ocassionally, other cutaneous or mucocutaneous areas (otitic/conjunctival/ genitovulval/vaginal/ prepucial/skin)
- Faucial diphtheria is the commonest type;
- Sore throat is frequently the presenting symptom;

PATHOLOGY

- After infection, the bacilli multiply on the mucous membrane or skin abrasion;
- The toxigenic strains start producing toxin;
- Diphtheria is a 'toxemia';
- The bacteria confine to the site of entry but the exotoxin is absorbed into the mucus membrane and causes destruction of epithelium and a superficial inflammatory response;

PATHOLOGY

- The toxin causes local necrotic changes;
- The resulting fibrinous exudate, together with the epithelial cells, leucocytes, erythrocytes and bacteria constitute : "pseudomembrane"
- Any effort to remove it will tear off capillaries beneath it and cause bleeding;
- Mechanical complications are due to pseudomembrane and systemic effects are due to the toxin;

Toxin-systemic absorption

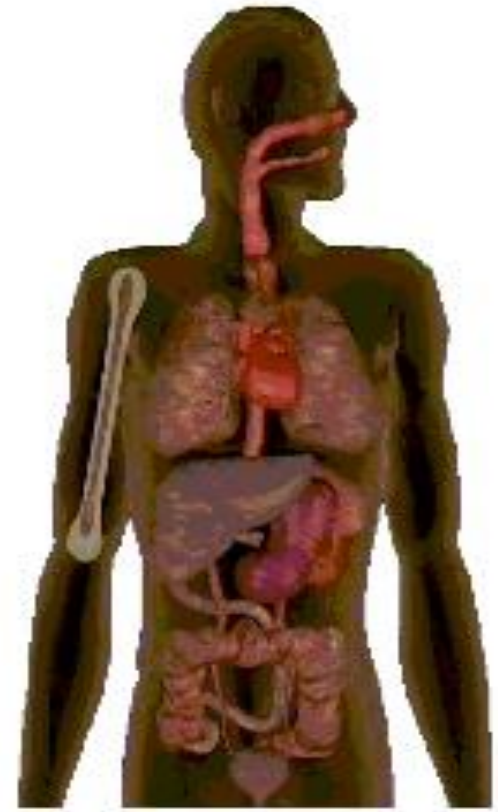
- The bacilli continue to produce the toxin;
- The toxin is absorbed systemically and damages heart muscle, liver, adrenals etc.;
- The toxin also cause nerve damage, especially of soft palate(palatine) and eye muscles (ciliary);
- Toxin absorption is negligible in case of skin infection with toxigenic strains;
- Nontoxigenic strains can also produce local disease but systemic effects are absent;

CLINICAL DISEASES

- Incubation period : usually 3-4 days;
- Acute infection : in the form of –
  - **Membranous tonsillitis**
  - **Nasal infection**
  - **Laryngeal infection**
  - **Skin infection –uncommon;**

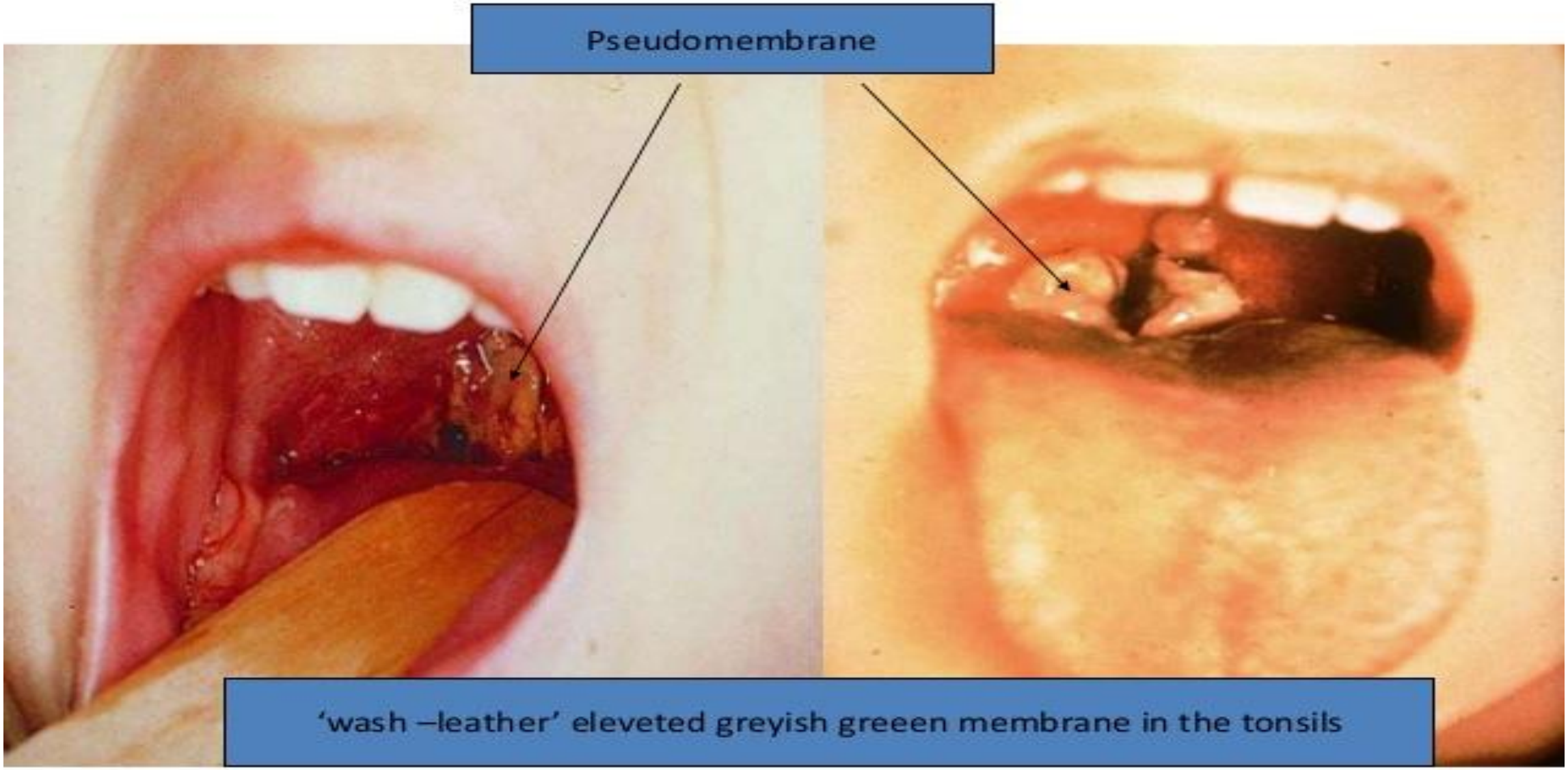
Diphtheria

- Nasopharyngeal diphtheria
  - Pharyngeal
  - Laryngeal
- Cutaneous diphtheria
- Systemic complications



CLINICAL DISEASES

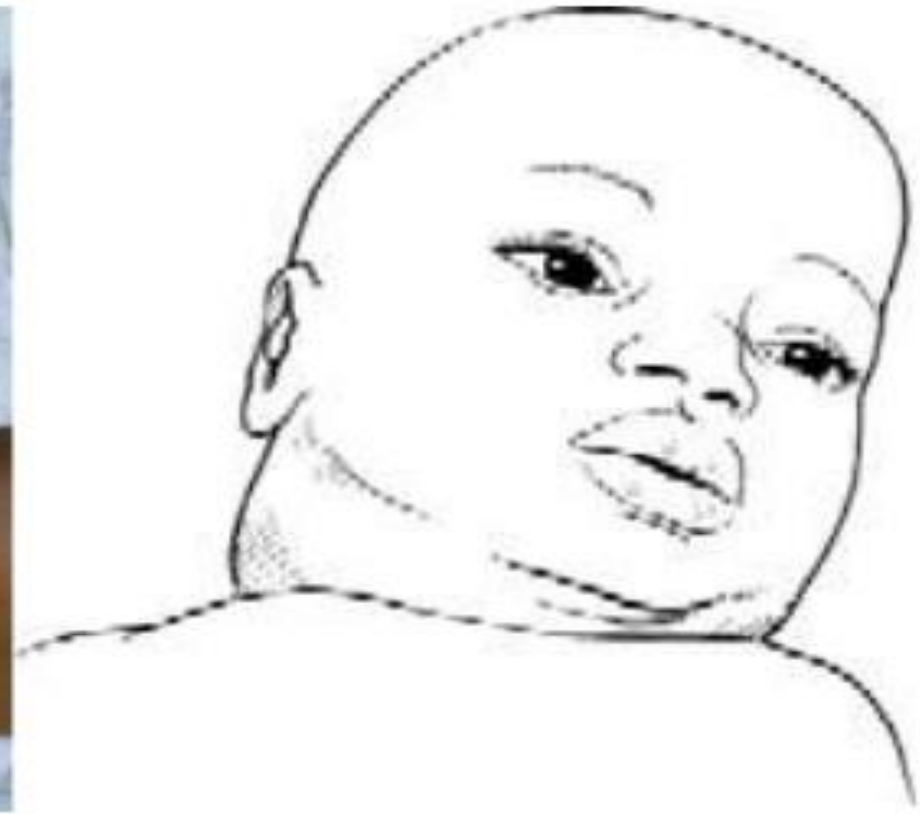
- Characteristic feature is : 'wash –leather' eleveted greyish greeen membrane in the tonsils with a well defined edge surrounded by a zone of inflammation;


Pseudomembrane
'wash –leather' eleveted greyish greeen membrane in the tonsils

CLASSIFICATION BASED ON CLINICAL SEVERITY

- Malignant or hypertoxic:
  - 'Bull neck' due to marked adenitis in neck;
  - Severe toxemia
  - Circulatory failure
  - Death
  - Paralytic squealae in survivors
- Septic : ulceration, cellulitis and gangrene around pseudomembrane;
- Hemorrhagic: bleeding from the edge of pseudomembrane, epistaxis, purpura etc.

Bull neck : due to cervical adenitis and edema of neck



COMPLICATIONS

- Asphyxia : due to mechanical obstruction
  - ➢ Emergency tracheostomy may be necessary;
- Acute circulatory failure
- Myocarditis
- Postdiphtheritic paralysis-
  - ➢ palatine(soft palate) and ciliary (eye muscles) nerves
  - ➢ Recovery – spontaneous and complete
- Septic : pneumonia and otitis media
- Relapse : in about 1% of cases

LABORATORY DIAGNOSIS

- Specimens :
  - Swabs from – nose, throat or other suspected lesions;
- Smear examination: Gram stain
  - shows beaded rods in typical arrangement;
  - Difficult to differentiate from some commensal corynebacteria normally found in throat;
  - Albert's stain or Neisser's stain is useful for demonstrating the granules;

LABORATORY DIAGNOSIS : CULTURE

- If the swabs can not be inoculated promptly, they should be kept moistened with serum;
- Inoculate on :
  - **Loeffler's serum slope**
  - **Tellurite blood agar or Tinsdale medium**
  - **Blood agar (for differentiating Staphylococcal or Streptococcal pharyngitis that simulate diphtheria);**
- *Tellurite medium is particulary useful for isolating the organism from – convalescents, contacts or carriers;*

Schick Test

- Injection of toxin Intradermal route
- Produces redness/erythematic in 2-4 days
- **No reaction** – Protective immunity present.

Prevention

Vaccination: Immunisation with diphtheria toxoid, combined with tetanus and pertussis toxoid (DTP vaccine), should be given to all children at two, three and four months of age. Booster doses are given between the ages of 3 and 5 .

The child is given a further booster vaccine before leaving school and is then considered to be protected for a further 10 years (16 – 18 years).

Treatment

- Antibiotic not useful in Acute infections,
- Antitoxin a must.
- Anti toxin obtained from horse serum
- Mild 20,000 to 40,000
- Moderate 40,000 to 60,000
- Severe 80,000 to 1,00,000
- Commonly used antibiotics,
- Penicillin parentally,
- Oral Erythromycin

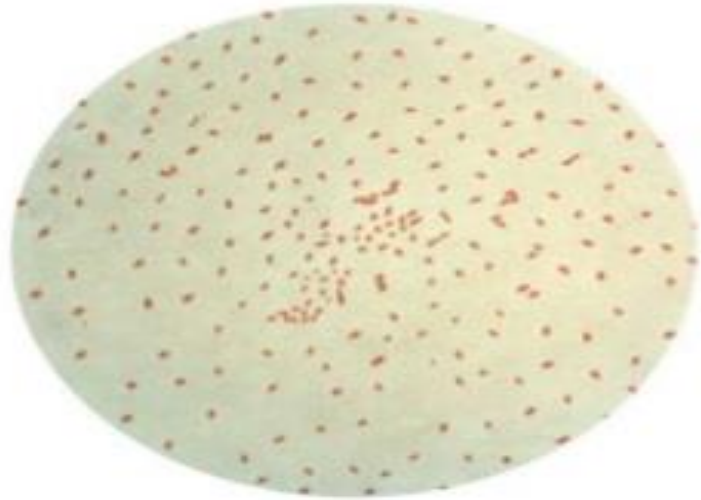
Bordetella

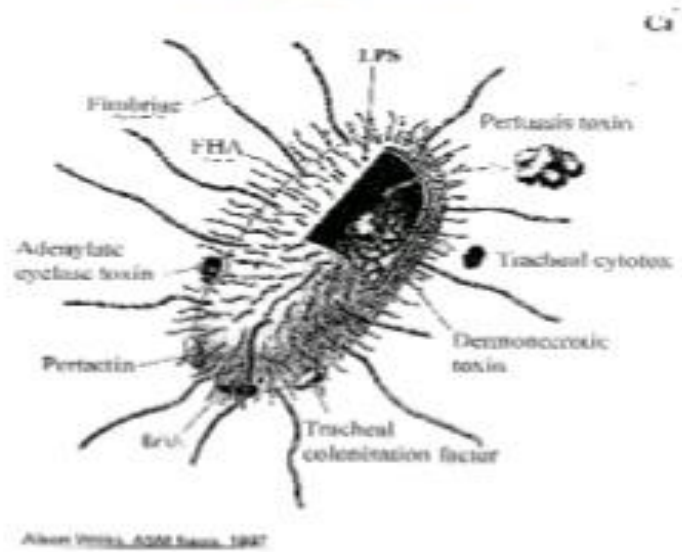
SPECIES

- Bordetella pertussis
- Bordetella parapertussis
- Bordetella bronchiseptica
- Bordetella avium

Morphology

- It is Gram negative.
- It is a small, ovoid coccobacillus (mean length 0.5 µm).
- It is nonmotile and nonsporing.
- It is capsulated.
- Freshly isolated strains of Bord pertussis have fimbriae.





Cultural characteristics

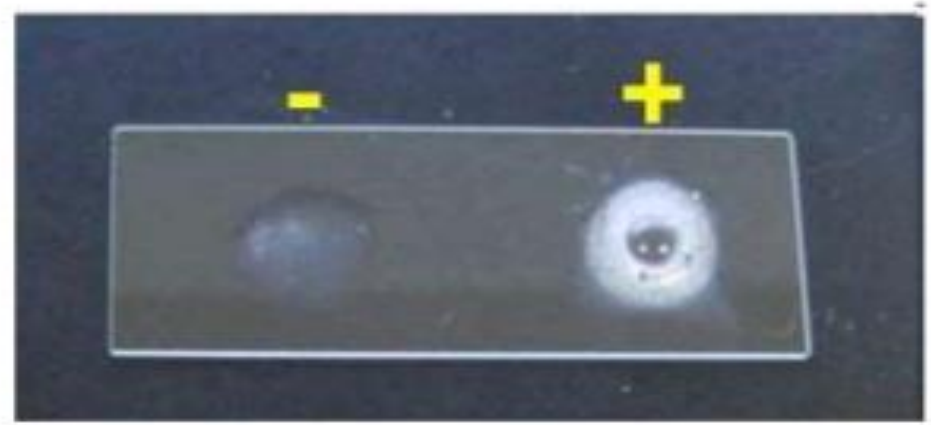
- Aerobic, Not anaerobic
- Grows optimally at 35^0 to 37^0 c
- Complex media are necessary for primary isolation
- Preferred medium – **Bordet Gengou glycerin potato blood agar**
- Blood for neutralizing inhibitory substances formed during bacterial growth.
- Charcoal also serves the same purpose.
- Charcoal blood agar is a useful medium.
- It does not grow on, simple media like nutrient agar.

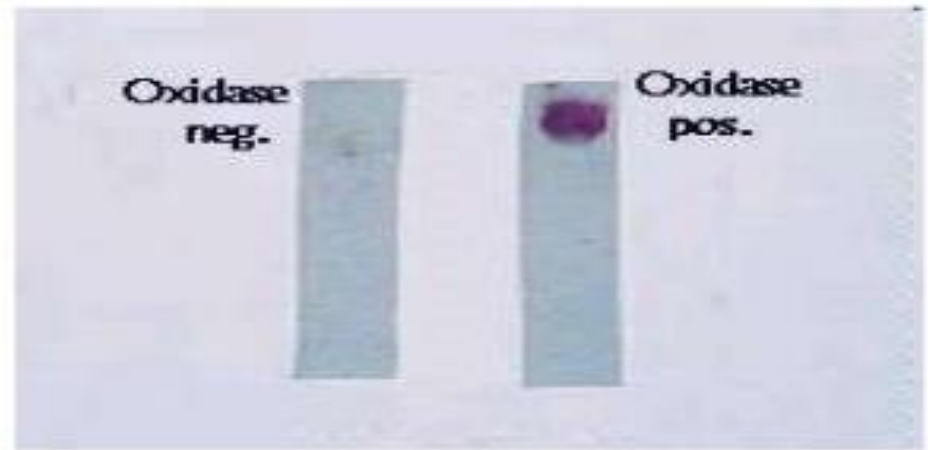
Colonies on Bordet-Gengou medium

- Growth is slow(48-72 hours).
- Colonies are small, dome-shaped, smooth, opaque, viscid, greyish white, refractile and glistening, resembling 'bisected pearls' or 'mercury drops'.
- Surrounded by a hazy zone of hemolysis.
- Confluent growth presents an 'aluminium paint' appearance.

Biochemical reactions

- Do not ferment sugars
- Indole test +
- Nitrates +
- Citrates +
- Urease +
- **Catalase +**
- **Oxidase +**





Resistance

- It is a delicate organism, being killed readily by heat (55°C for 30 minutes), drying and disinfectants.
- Outside the body, Bord pertussis in dried droplets is said to survive for five days on glass, three days on cloth and a few hours on paper.

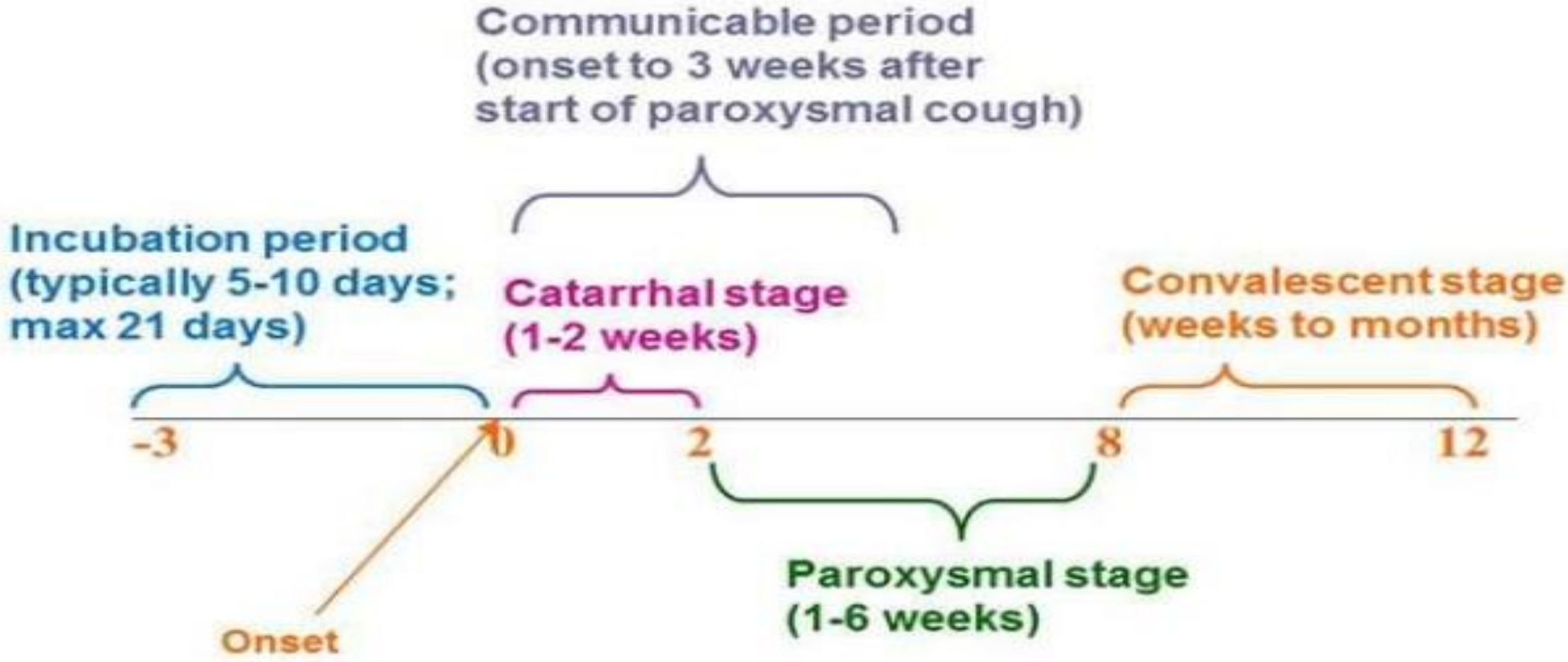
Clinical Manifestations of Pertussis

- Incubation period 3-12 days (up to 21 days)
- Insidious onset, similar to minor upper respiratory infection with nonspecific cough
- Fever usually minimal throughout course
- Apnea & Cyanosis in infant

STAGES

- 1st Stage- Catarrhal Stage
- 2nd Stage- Paroxysmal Stage
- 3rd Stage- Covalescent Stage

Clinical Course (in weeks)



Laboratory Diagnosis

- Microscopy – Demonstration of Bacilli in respiratory secretions.
- Florescent Antibody methods.
- Specimen Collection:
- Cough Plate Method:
- Culture plate held at 10-15 cm infront of the mouth when the patient is coughing spontaneously or induced cough
- Droplets of respiratory exhaled impinge on the media.
- Helpful as bed side investigation

Cough Plate Method

Prophylaxis

- Preventing the spread of infection by isolation of cases is seldom practicable, as infectivity is highest in the earliest stage of the disease when clinical diagnosis is not easy.
- Specific immunisation with killed Bord pertussis vaccine has been found very effective.
- The alum absorbed vaccine produces better and more sustained protection and less reaction than the plain vaccines.
- Pertussis vaccine is usually administered in combination with diphtheria and tetanus toxoid (triple vaccine)(DTwP/DTaP).
- Not only is this more convenient but Bord pertussis also acts as an adjuvant for the toxoids, producing better antibody response.

Bordetella parapertussis

- This is an infrequent cause of whoopIng cough.
- The disease is mild.
- The pertussis vaccine does not protect against Bord parapertussis infection.

Bordetella bronchiseptica

- This is motile by peritrichate flagella.
- It is antigenically related to Bord pertusis and Brucella aburtus.
- It occurs naturally in the respiratory tract of several species of animals.
- It has been found to cause a very small proportion (0.1 per cent) of cases of whooping cough.

Choice of regimen

- Erythromycin
- Azithromycin
- Clarithromycin

Haemophilus

Scientific classification

Kingdom: Bacteria
Phylum: Proteobacteria
Class: Gamma Proteobacteria
Order: Pasteurellales
Family: Pasteurellaceae
Genus: Haemophilus
Species: influenzae
Binomial name: *Haemophilus influenzae*

Haemophilus species of clinical importance

1. ***H. influenzae***

-**type b is an important human pathogen**

2. ***H. ducreyi***

-**sexually transmitted pathogen (chancroid)**

3. Other *Haemophilus are normal flora*

- *H. parainfluenzae – Pneumonia & endocarditis*
- *H. aphrophilus – Pneumonia & endocarditis*
- *H. aegyptius – Pink eye (purulent conjunctivitis)*

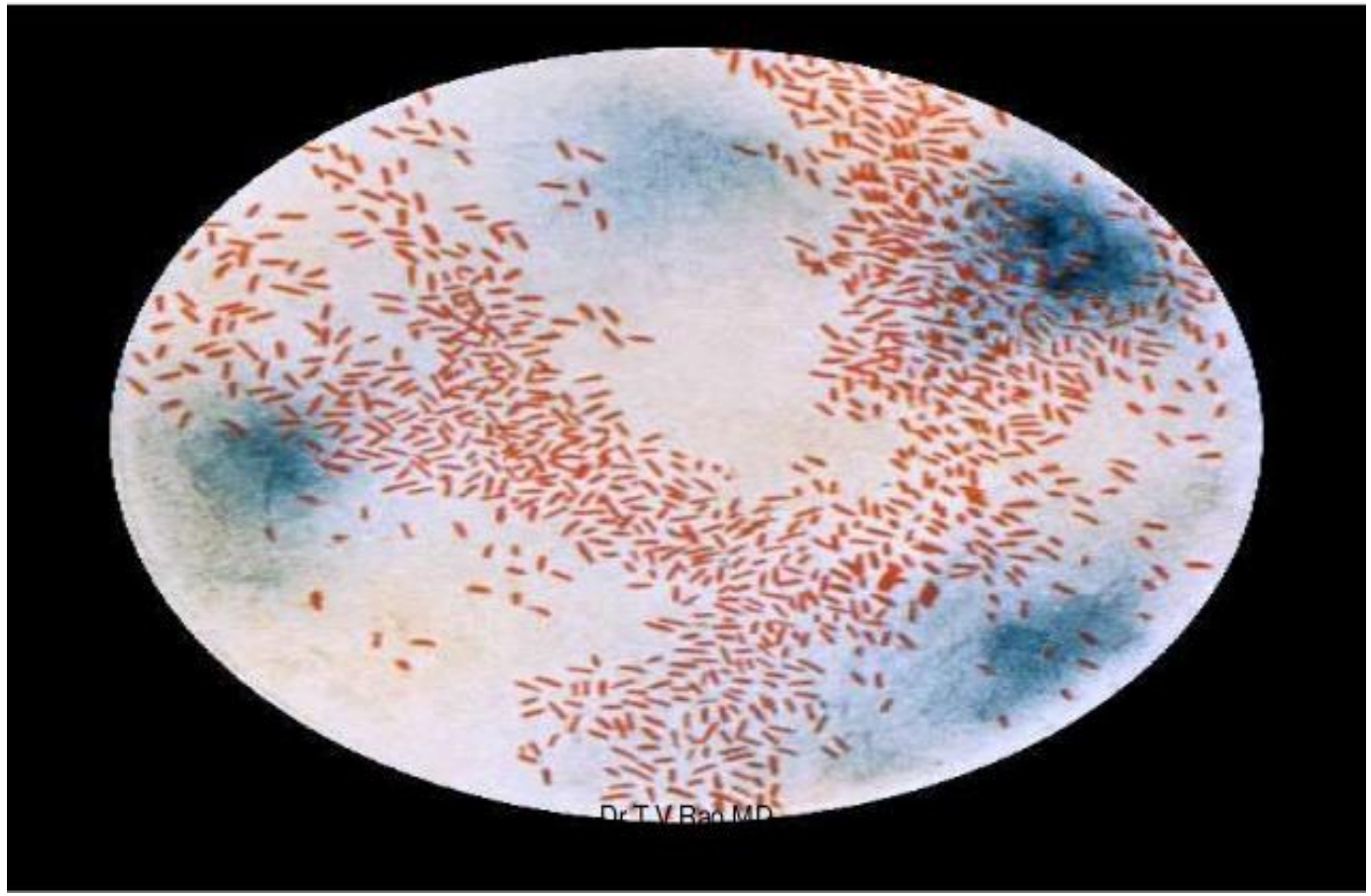
Haemophilus Influenza

- Aerobic gram-negative bacteria
- Polysaccharide capsule
- Six different serotypes (a-f) of polysaccharide capsule
- 95% of invasive disease caused by type b (Hib)

Morphology

- Size is (1-2 X 0.3 – 0.5 microns)
- Non motile,
- Non sporing
- Gram negative rod or coccobacillus
- Pleomorphic (old culture)
- Appear as clusters of Coccobacillary forms in infected Sputum
- Long bacillary and filamentous form in infected CSF (Meningitis)

Haemophilus influenza



Gram staining

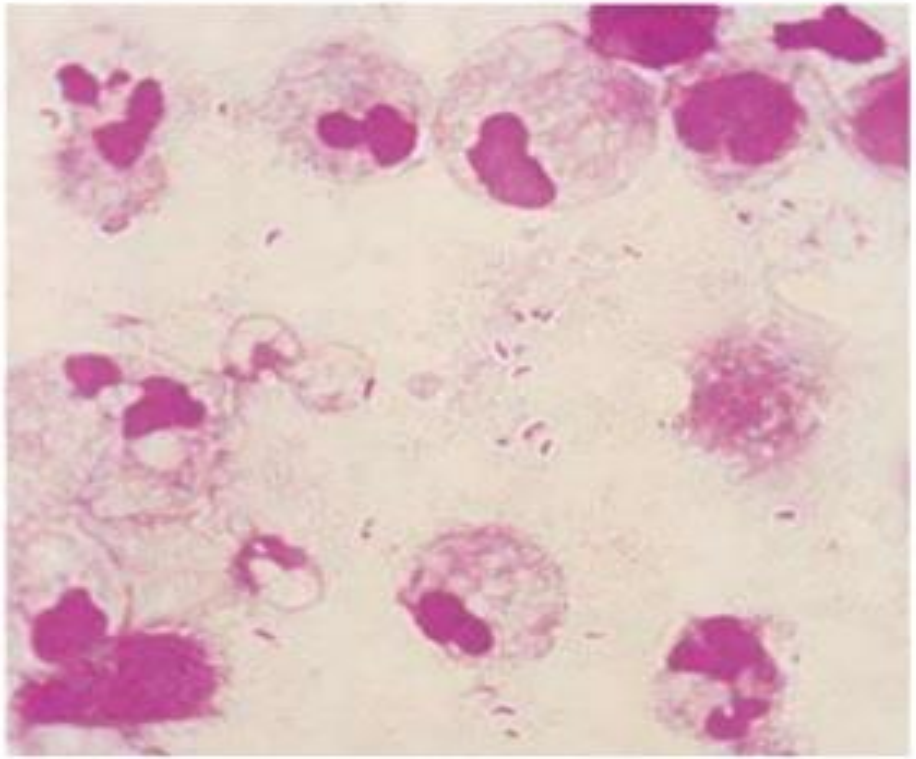


Fig: Gram-stained CSF sediment preparation.
Fine, Gram-negative rods surrounded by a capsule (serovar b).
Clinical diagnosis: purulent meningitis

Culture characteristics

- Fastidious growth requirements
- Factors X and V are essential for growth
- X is Hemin heat stable
- Porphyrins for synthesis of Cytochromes
- V-factor (NAD): Heat- labile, coenzyme I, nicotinamide adenine dinucleotide, found in blood – oxidation
- Aerobic 37 dg C

Haemophilus Species, cont.

Species	X	V	Hemolysis
►H. influenzae (H. aegyptius)	+	+	-
►H. parainfluenzae	-	+	-
►H. ducreyi	+	-	-
►H. haemolyticus	+	+	+
►H. parahaemolyticus	-	+	+
►H. aphrophilus	-	-	-

Culture characteristic

- On Chocolate agar, flat, grayish-brown colonies, 1-2 mm in diameter present after 24 hrs
- Colonies of staphylococci on sheep Blood agar cause the release of NAD, yielding satellite growth phenomenon

Satellite growth

When *Staph aureus* is streaked across plate of Blood agar with a species containing *H Influenzae*

Biochemical reaction

- Catalase +ve
- Oxidase +ve
- Reduces nitrite to nitrate
- Ferment glucose and galactose
- Can’t ferment sucrose, lactose and mannitol

Antigenic Properties

- Contains 3 Major surface antigens

1 Capsular polysaccharide

2 Outer membrane proteins (OMP)

3 Lipopolysaccharides (LPS)

Virulence factor of H. influnzae

- Polysaccharid capsule
- Fimbriae
- LPS- lipid A
- All virulence strain produce Neuraminidase (bioflim) and IgA protease.
- No exotoxin

Haemophilus Influenza

Mode of 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

Pathogenesis

- Type b *H influenzae* colonizes the nasopharynx, and may penetrate the epithelium and capillary endothelium to cause bacteremia
- Meningitis may result from direct spread via lymphatic drainage or from hematogenous spread.
- Nontypable *H influenzae* colonizes the nasopharynx and, to a lesser extent, the trachea and bronchi and may infect mucosa damaged by viral disease.
- Lipooligosaccharide is largely responsible for inflammation

Clinical Presentation

Pneumonia

Septic Arthritis

Epiglottitis

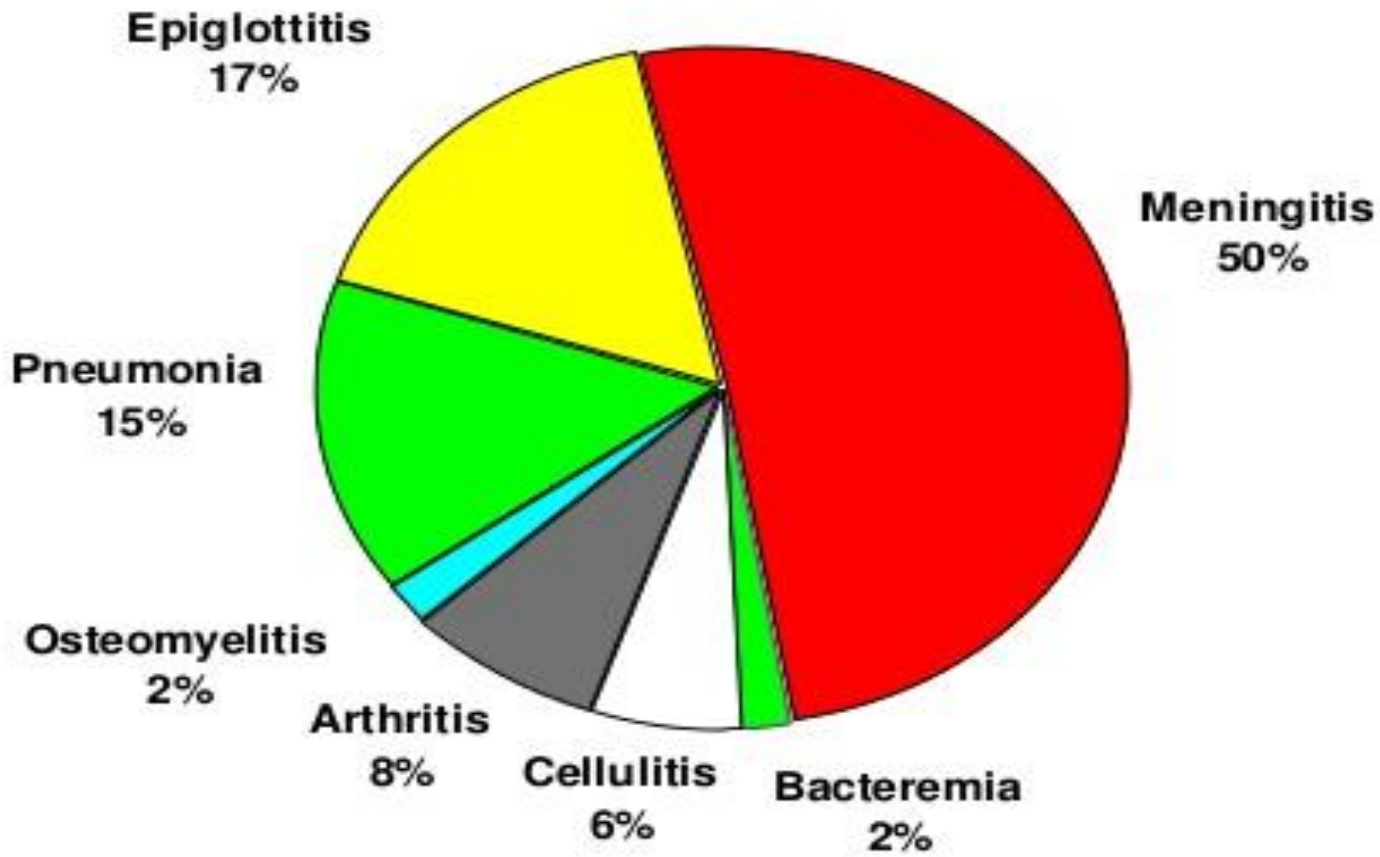
Meningitis

Invasion infection

Secondary infection

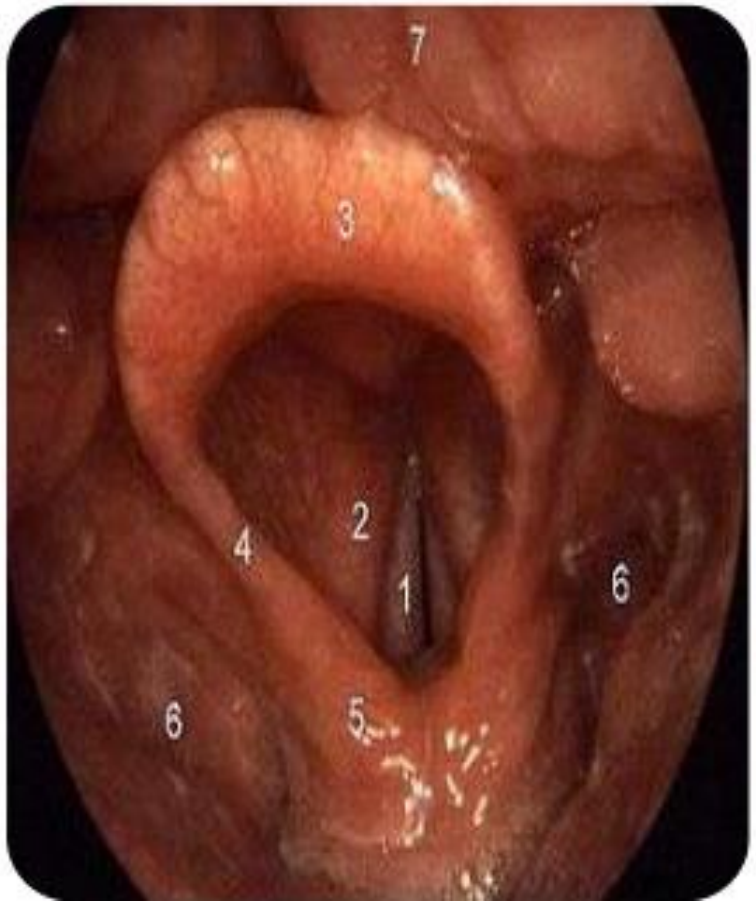
- Respiratory tract infections
- Otitis media
- Sinusitis
- Chronic Bronchitis

Haemophilus influenzae type b
Clinical Features*



Laryngo epiglottitis

- Causes Epiglottis
- Obstructive Laryngitis
- > 2 years children are vulnerable
- Can be fatal in 2 hours



Treatment

- Cefotaxime
- Ceftazidime
- Ampicicillin, Contrimixazole
- Plasmid born resistance set in Ampicillin
- Amoxycillin with Clavulanate
- Clarithromycin
- Treatment with an effective 3rd generation cephalosporin, or chloramphenicol plus ampicillin

❑ Ampicillin-resistant strains

Current Vaccines

- Haemophilus B conjugate vaccine
- Wide spread use of

  H influenza type b vaccine has reduced H influenza type b meningitis in children by 95%

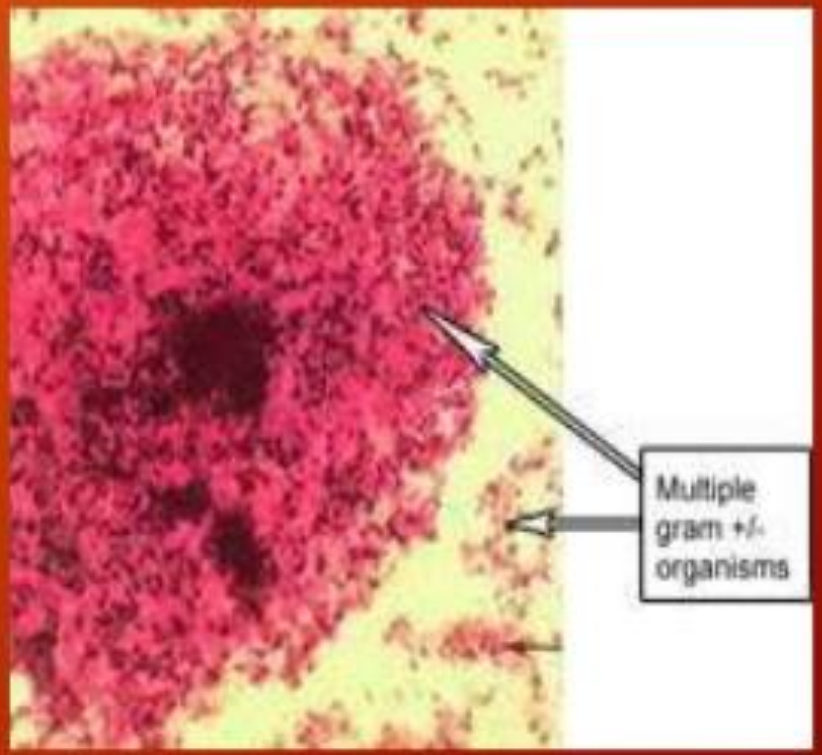
Public Health Aspect of other *Haemophilus* strains

- ***H. ducreyi***
  - **Sexually transmitted disease - chancroid**
- ***H. influenzae* biogroup *aegyptius***
  - **Brazilian Purpuric Fever**
- ***H. aegyptius***
  - **"pink eye" (purulent conjunctivitis)**
- ***H. aphrophilus***
  - **pneumonia**
  - **Infective endocarditis**

Bacterial Vaginosis
(Gardnerella Vaginitis)

Morphology

- Small, Gram negative, on motile
- Pleomorphic rod which shows metachromatic granules
- Presence of Clue cells



Gardnerella Vaginitis

- Gram-variable-staining rod, facultative anaerobic bacteria (actually has a Gram-positive cell wall, but because the cell wall is so thin it can appear either Gram-positive or Gram-negative under the microscope).
- Small (1-1.5 µm diameter) non-spore forming, non-motile coccobacilli.
- Previously classified as Haemophilus vaginalis and afterwards as Corynebacterium vaginalis.

Culturing

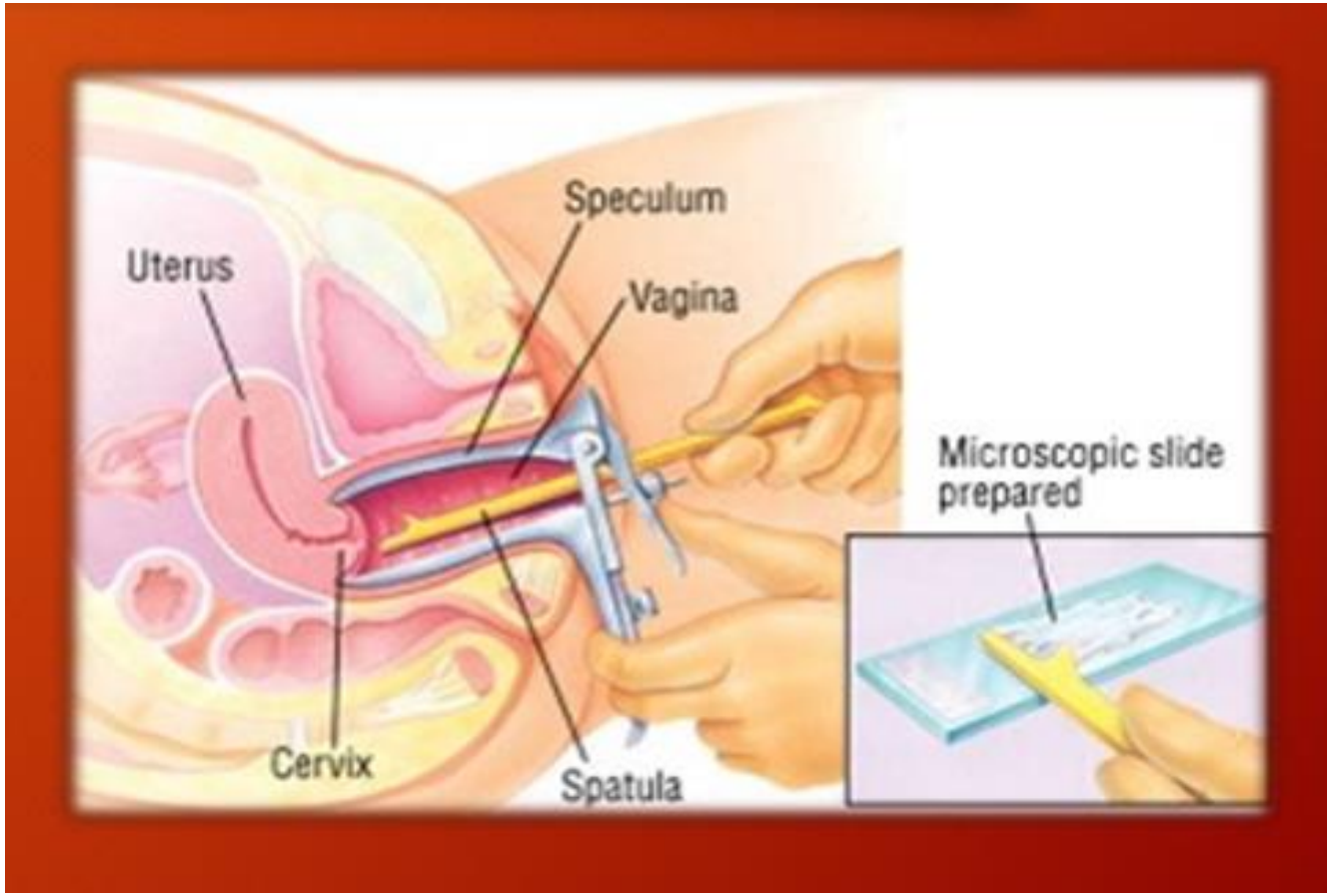
- Grows on Blood and Chocolate Agar
- Hemolytic colonies on Human and Rabbit blood agar,
- Catalase -
- Oxidase -

Symptoms

- Up to 50% of women diagnosed with bacterial vaginosis do not have symptoms. In others, it causes an unpleasant "fishy" vaginal odor and a yellow or white vaginal discharge. For some women, these symptoms are especially bothersome during or after intercourse.

Diagnosis

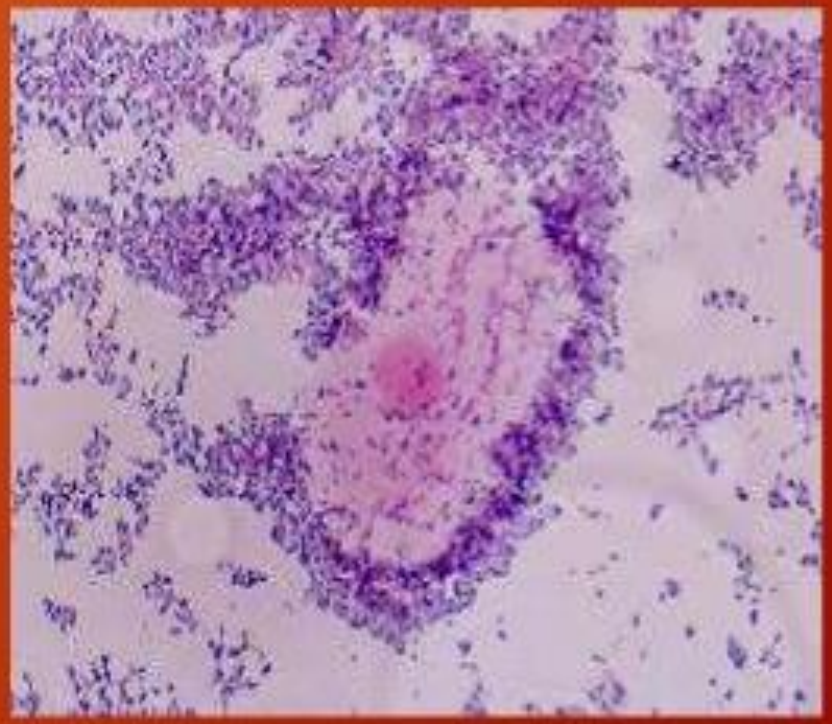
- 1 White, thin, coating on your vaginal walls during the pelvic exam



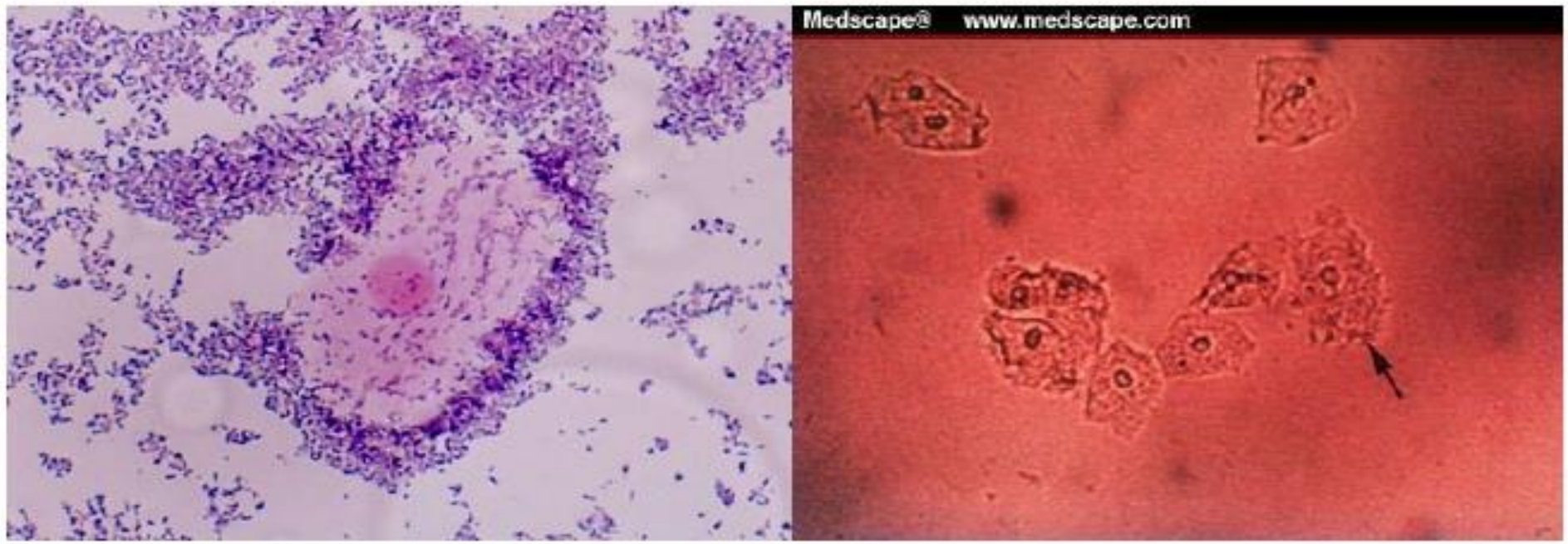
- 2 pH test of vaginal discharge that shows low acidity (pH greater than 4.5)
- 3 Fishy odor when a sample of vaginal discharge is combined with a drop of potassium hydroxide on a glass slide (the "whiff test")

Clue cells

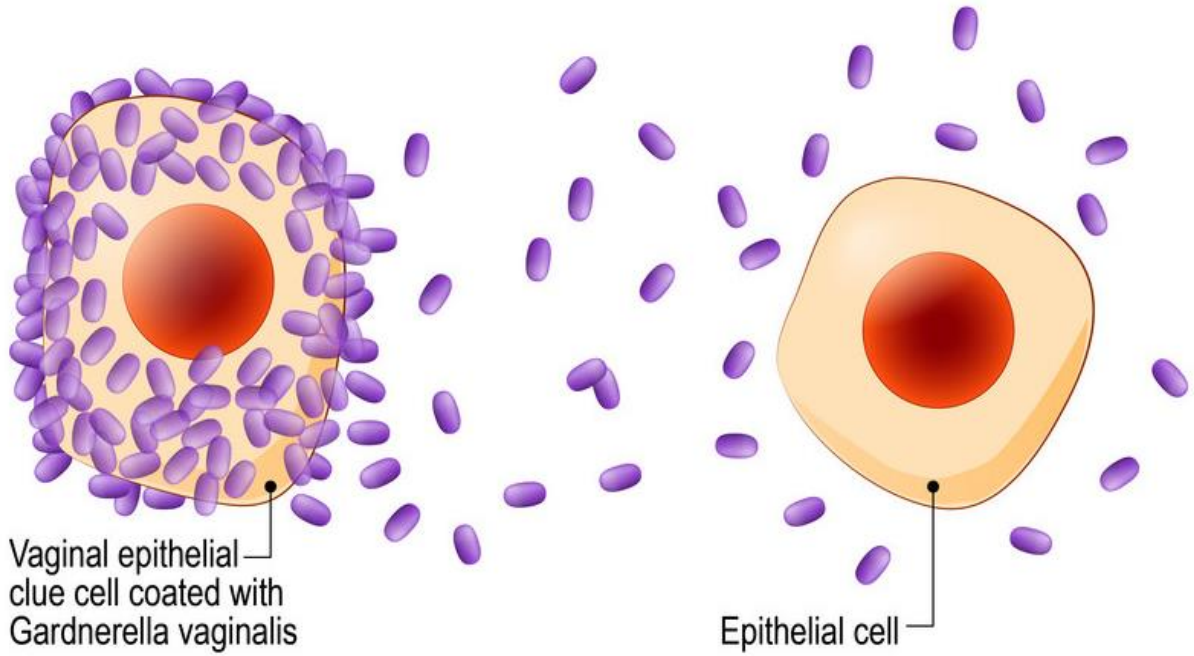
- 4 Clue cells (vaginal skin cells that are coated with bacteria) visible on microscopic exam of vaginal fluid

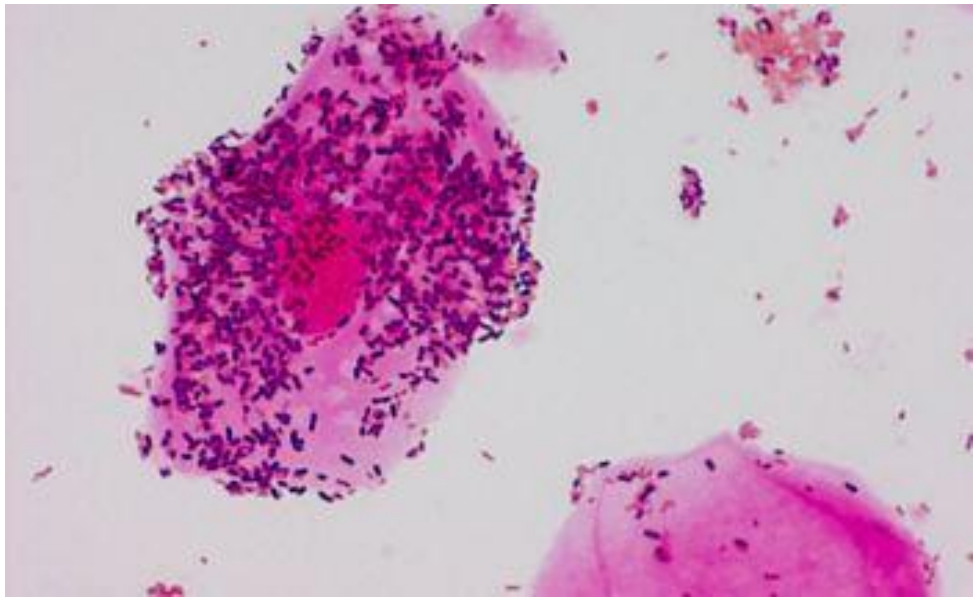


Clue cell



Clue cell on Gram stain and saline wet mount of vaginal discharge (on >20% cells)
Bacteria adhered to epithelial cells; most reliable single indicator


Vaginal epithelial
clue cell coated with
Gardnerella vaginalis
Epithelial cell



Treatment

- Studies show that a seven-day treatment with oral metronidazole or a five-day treatment with metronidazole vaginal gel is equally effective in non-pregnant women. Clindamycin vaginal cream is slightly less effective than either type of metronidazole.

Legionella pneumophila

Historical Background and Epidemiology

Historical Background

The name legionella originates from a widely publicized outbreak of pneumonia in persons attending an American Legion convention in Philadelphia in 1976.

In a hotel on the occasion of a United States army veterans' meeting (Fraser et al., 1977).

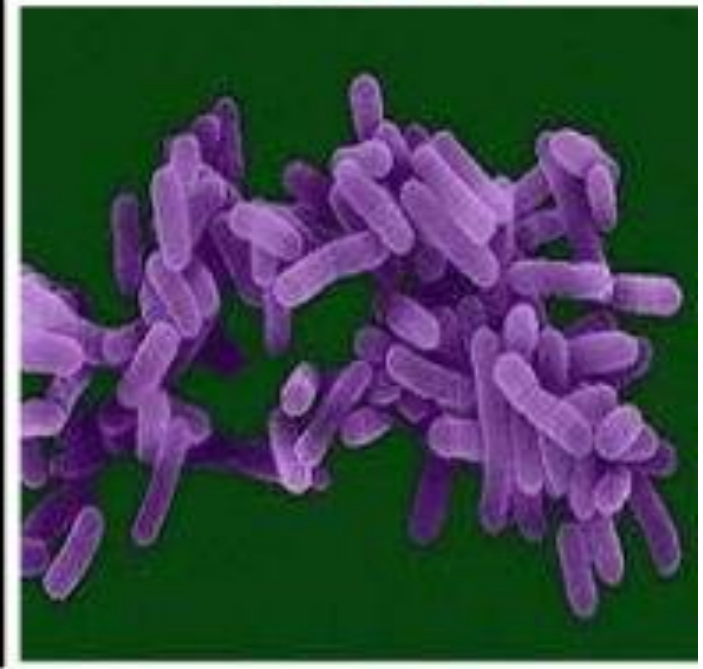
Bacterial Characteristics

Legionellaceae are facultative intracellular parasites that cause primarily respiratory tract infections.

Legionella are :

- gram–negative
- slender rods
- unencapsulated
- fastidious,
- Aerobic
- catalase-positive
- Most produce gelatinase and ß-lactamase
- 0.5–1 m wide and 2–50 m long.
- poorly stained by Gram's stain

This has been attributed to the presence of the branched chain fatty acids that are a major component of the cell walls.



- Motile by means of one or more *polar* or *subpolar flagella*
- grown on complex media such as *buffered charcoal-yeast extract (BCYE) agar* with ;

  ketoglutarate,

  pH of 6.9,

  temperature 35 °C, and

  90% humidity.
- Legionellae grow slowly;

  3 days of incubation with BCYE & ≥2 weeks in blood cultures
- Colonies are round or flat, colorless to iridescent pink or blue

Virulence factors

The Legionellae make:

- proteases,
- phosphatase,
- lipase,
- DNase, and
- Rnase
- A major secretory protein, a ***metalloprotease,*** has hemolytic and cytotoxic activity; however, this protein **has not been shown** to be a required virulence factor.

Pathogenesis

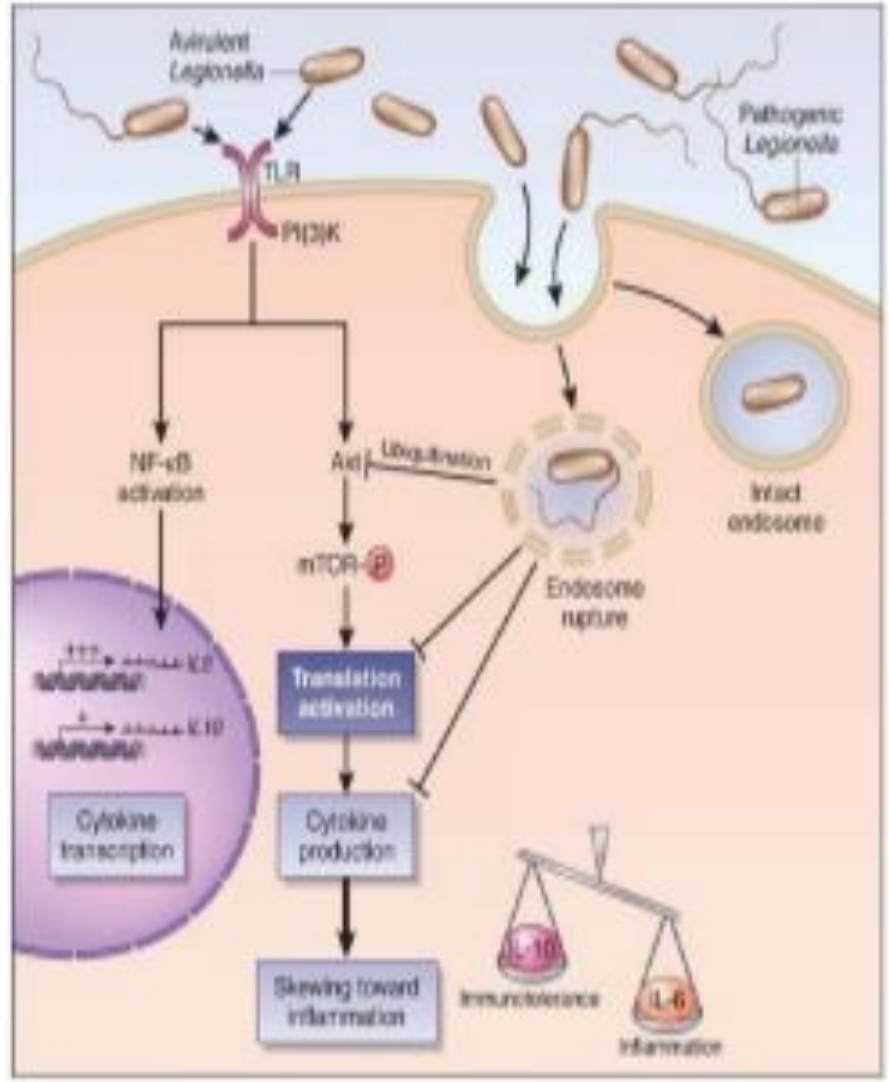
Legionellae are intracellular pathogens of ***macrophages***, by which they are phagocytosed in a process involving ;

1. Both virulent and non-virulent strains are *phagocytosed*
2. Virulent strains can multiply inside the phagocytes and are able to ***inhibit the fusion of phagosomes with lysosomes***

- ❑ non-virulent strains do not multiply

3. The bacteria multiply within the vacuoles until they are numerous,
4. The cells are destroyed, the bacteria are released, and infection of other macrophages then occurs.

(transferrin-iron) is essential for the process of *intracellular growth* of the bacteria, but other factors important to the processes of *growth, cell destruction, and tissue damage* are not well understood.



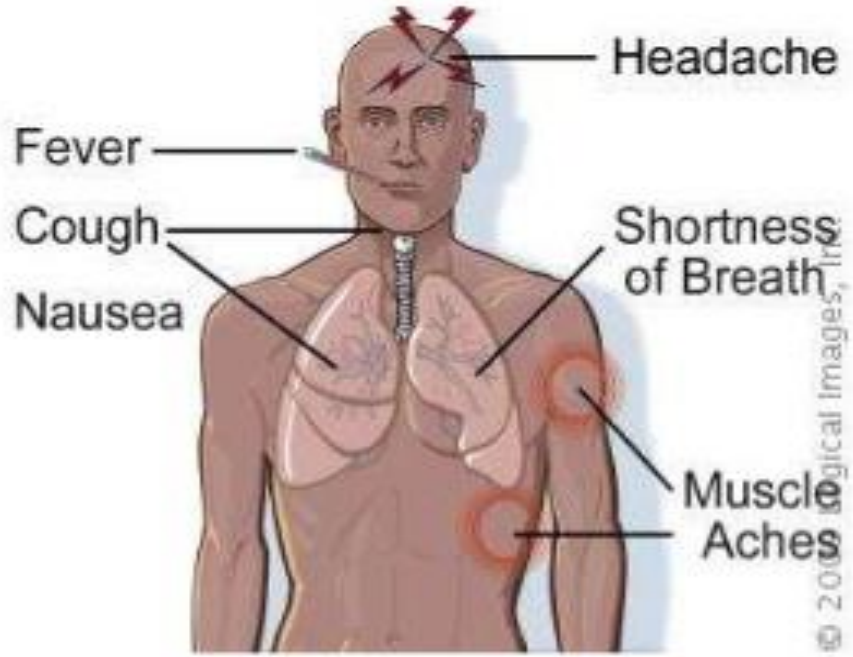
Clinical manifestations

- *L.neumophilia* causes **Legionnaires' disease** can have symptoms like many other forms of pneumonia, so it can be hard to diagnose at first.

Signs of Legionnaires' disease can include:

- Cough
- Shortness of breath
- High fever
- Muscle aches
- Headaches

These symptoms usually begin 2 to 14 days after being exposed to the bacteria.



Potanic fever

- *L .pneumophila* also produces a disease called **"Pontiac fever,"** after the clinical syndrome that occurred in an outbreak in Michigan.

characteristics

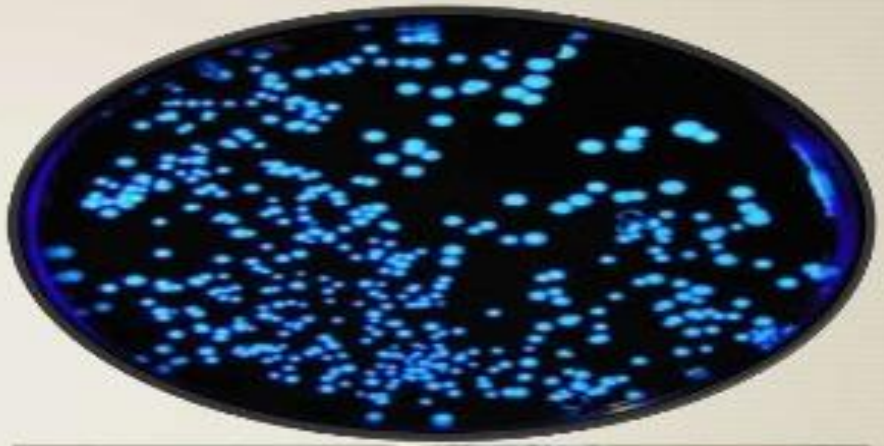
- ❑ fever and chills,
- ❑ myalgia,
- ❑ malaise, and
- ❑ headache ,that develop over *6–12 hours*. Dizziness, photophobia, neck stiffness, and confusion also occur.

- The **symptoms of Pontiac fever** are similar to those of Legionnaires' disease and usually last for 2 to 5 days. Pontiac fever is ***different from Legionnaires' disease*** because the patient **does not** have pneumonia.
- Symptoms go away on their own without treatment.

Laboratory Diagnosis

- Specimens
  - Sputum
  - Bronchial aspirate
  - Lung biopsy
  - Florescent methods

Serology ELISA



Specimens

The organisms can be recovered from:

- bronchial washings
- pleural fluid
- lung biopsy specimens or
- blood

❖ Isolation of legionella from sputum is more difficult because of the predominance of bacteria of the normal flora. Legionella is rarely recovered from other anatomic sites.

Smears

- Legionellae are ***not*** demonstrable in Gram-stain
- *Direct fluorescent antibody tests*, but the test has low sensitivity compared with culture
- *Silver stains* are sometimes used on tissue specimens.

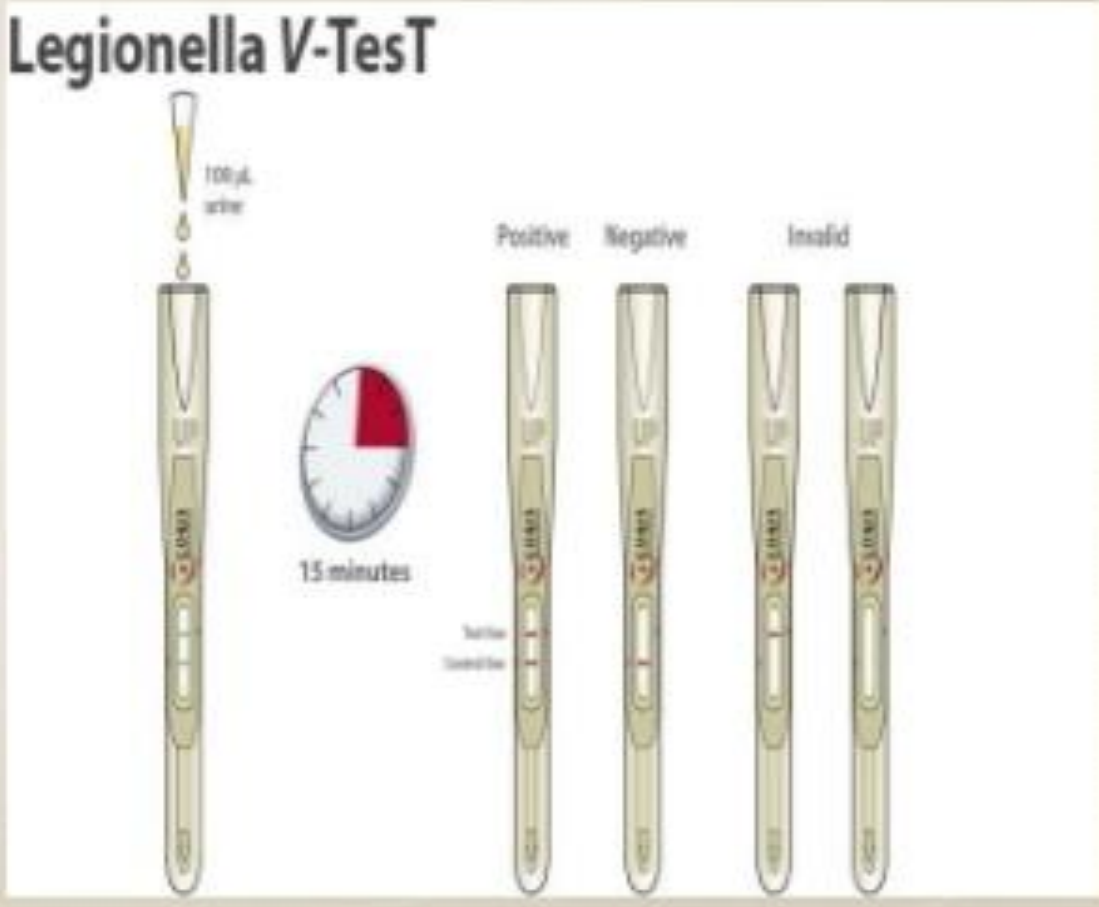
Culture

Specimens are cultured on **BCYE agar** & can be rapidly identified by immunofluorescence staining.

BCYE agar containing antibiotics can be used

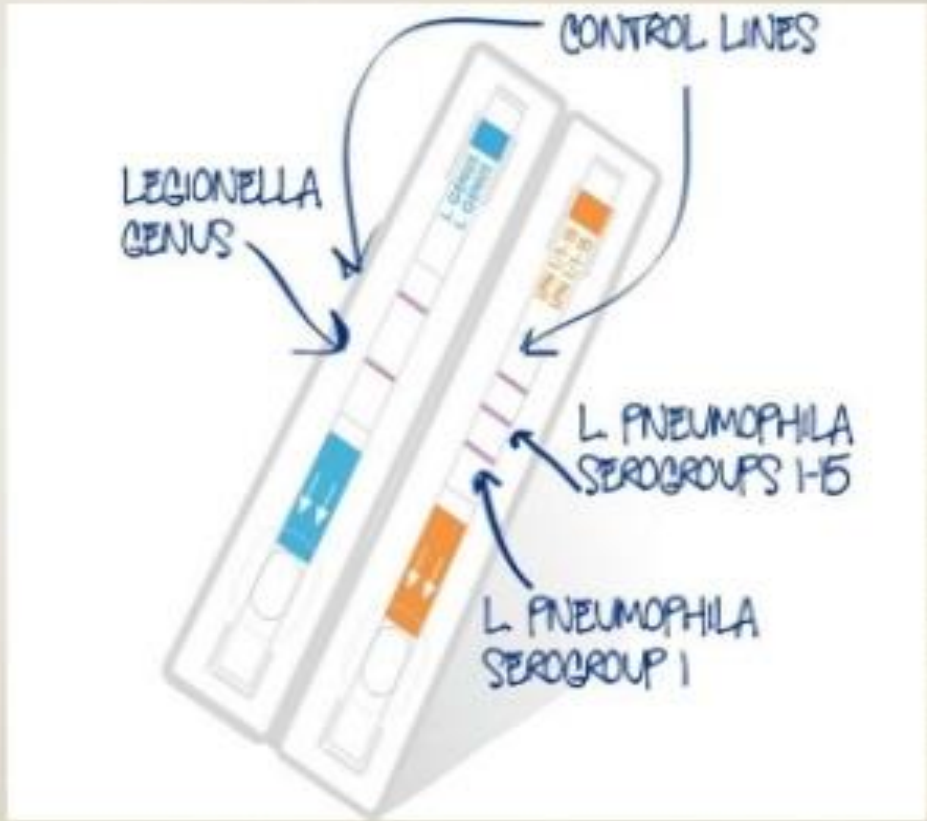
Urine Antigen Test

- The most commonly used laboratory test for diagnosis is the urinary antigen test, which detects a part of the Legionella bacteria in urine (pee). If the patient has pneumonia and the test is positive, then the patient is considered to have Legionnaires' disease.



Blood Specimens Testing the Serum

- Paired sera (blood specimens) that show a four-fold increase in antibody levels when drawn shortly after illness and several weeks following recovery, can also be used to confirm the diagnosis.



Specific Tests

The urine antigen test is specific for *L. pneumophila* serotype 1.

Serologic Tests

Levels of antibodies to legionellae rise slowly during the illness.

Serologic tests have :

- a sensitivity of 60–80% and
- a specificity of 95–99%.
- *Serologic tests are most useful in obtaining a retrospective diagnosis in outbreaks of legionella infections.*

Treatment

* Macrolides
* Ciprofloxacin
* Tetracycline's
* Rifampicin