AZERBAIJAN MEDICAL UNIVERSITY
DEPARTMENT OF MEDICAL MICROBIOLOGY and IMMUNOLOGY

Pathogenic anaerobes (genus of clostridium and bacteroides).

The causative agents of zoonotic bacterial infections (brucellosis, anthrax, listeriosis, plague, tularemia)

Lecture plan:

1. Anaerobic bacteria:

- General characteristics of the genus Clostridium.
- The causative agents of gaseous anaerobic infection *(C.perfringens, C.novyi, C.septicum, C.histolyticum, C.sordellii)*, morpho-biological characteristics, pathogenicity factors, pathogenesis of diseases caused by them, main clinical symptoms, microbiological diagnosis, specific treatment and prevention principles.
- the causative agent of tetanus, morpho-biological characteristics, pathogenicity factors, pathogenesis of diseases caused by it, main clinical signs, microbiological diagnosis, specific treatment and prevention principles.
- the causative agent of botulism, morpho-biological characteristics, pathogenicity factors, pathogenesis of diseases caused by it, main clinical signs, microbiological diagnosis, specific treatment and prevention principles.
- Clostridium difficile, characteristics. Its role in human pathology, microbiological diagnosis.
- Non-spore forming anaerobes. Genus Bacteroides (bacteroids), morpho-biological characteristics, role in pathology, principles of microbiological diagnosis.

2. The causative agents of zoonotic infections:

- Brucella. Classification, morpho-biological characteristics, pathogenicity factors. Pathogenesis. Principles of microbiological diagnosis, specific treatment and prevention.
- The causative agent of antrax. Morpho-biological features, pathogenicity factors, pathogenesis, clinical forms. Principles of microbiological diagnosis, specific treatment and prevention.
- The causative agent of listeriosis, morpho-biological characteristics, pathogenicity factors, pathogenesis of the disease, microbiological diagnosis.
- Yersinia. The causative agent of plague, morpho-biological characteristics, pathogenicity factors, diseases caused by it. Pathogenesis, microbiological diagnostics. Principles of specific treatment and prevention. Causes, characteristics, microbiological diagnosis of intestinal yersiniosis and pseudotuberculosis.
- The causative agent of tularemia. Morpho-biological characteristics, pathogenicity factors. The main clinical forms of the disease in humans. Microbiological diagnostics. Principles of specific treatment and prevention.

DEFINITIONS

- **OBLIGAETE ANAEROBE**
  - **Lack superoxide dismutase and/or catalase**
  - **toxic radicals formed by oxidative enzymes kill organisms**
- **AERO-TOLERANT ANAEROBES**
  - **survive in presence of oxygen**
  - **Do not use oxygen for energy requirements**
- **FACULTATIVE ANAEROBES**

Gram-negative anaerobes

- *Bactericides* (the most commonly found anaerobes in cultures; intra-abdominal infections, rectal abscesses, soft tissue infections, liver infection)
- *Fusobacterium* (abscesses, wound infections, pulmonary and intracranial infections)
- *Porphyromonas* (aspiration pneumonia, periodontitis)
- *Prevotella* (intra-abdominal infections, soft tissue infections)

Gram-positive anaerobes

- *Actinomyces* (head, neck, pelvic infections; aspiration pneumonia)
- *Bifid bacterium* (ear infections, abdominal infections)
- *Clostridium* (gas, gangrene, food poisoning, tetanus, pseudomembranous colitis)
- *Peptostreptococcus* (oral, respiratory, and intra-abdominal infections)
- *Propionibacterium* (shunt infections)

CLOSTRIDIUM-introduction

- **Clostridia**:
  - are strictly anaerobic to aerotolerant sporeforming bacilli found in soil as well as in normal intestinal flora of man and animals.
  - There are both gram-positive and gram-negative species, although the majority of isolates are gram-positive.
  - Exotoxin(s) play an important role in disease pathogenesis.
  - motile -- peritrichous flagella
    (exception: *C. perfringens*—nonmotile)
  - the sporangia– swollen
  - typical clinical symptoms

CLOSTRIDIA

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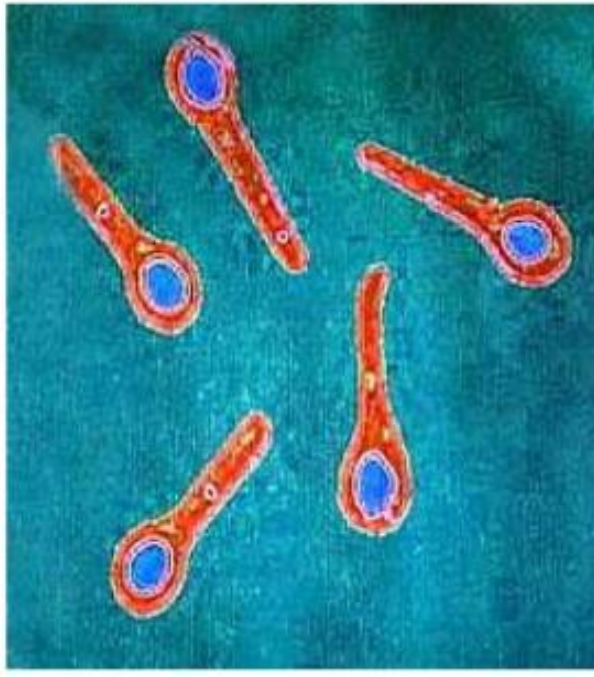
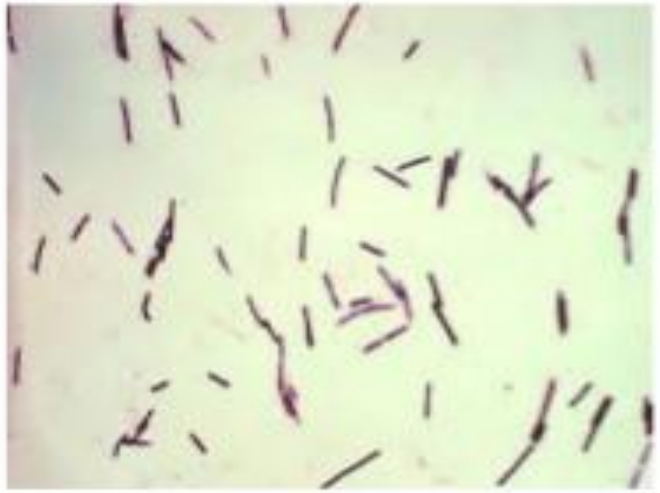
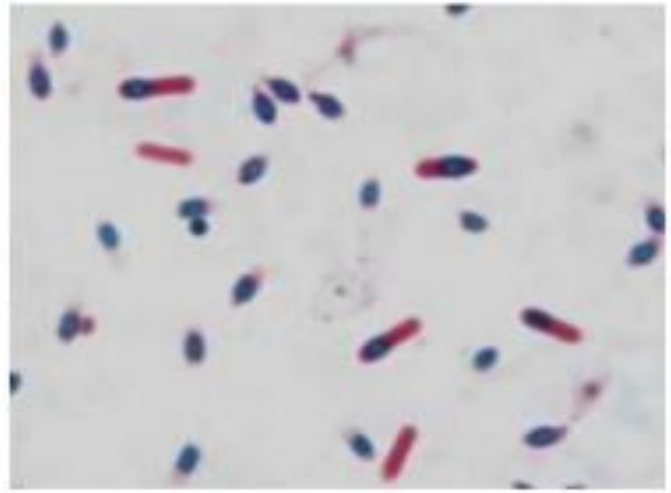
- Gram positive spore forming bacilli
- ubiquitous
  - intestines of man and animals
  - animal and human faeces contaminated soil and water
- Several species associated with human disease

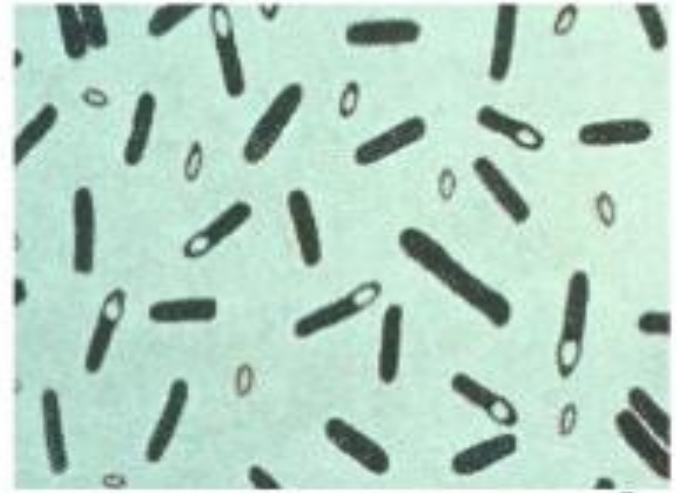


Genus Clostridium

- The genus consists of G+ve, anaerobic, Spore forming bacilli.
- Spores are wider than bacillary body, giving bacillus a swollen appearance resembling spindle; hence named so (Kolster meaning spindle).
- Highly pleomorphic, straight or slightly curved rods with slightly curved ends.
- G +ve , 3-8 x 0.4-1.2 µm in size
- Motile (except *Cl. tetani* Type VI & *Cl. perfringens*)
- *Cl. perfringens* & *Cl. butyricum* are capsulated;
- Others are non-capsulated



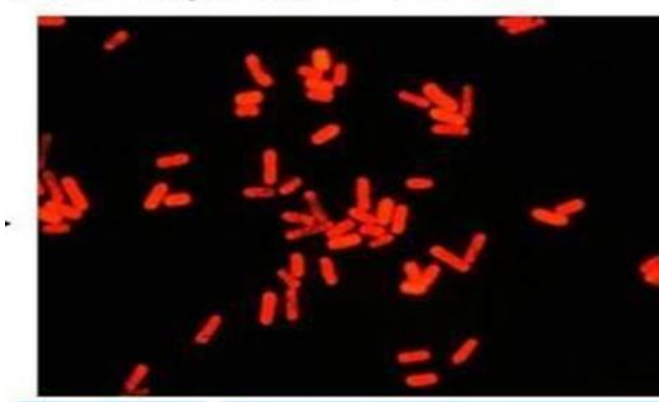




Microbiological diagnosis of anaerobic infections:

- **Examination material -** wound exudate, edema fluid, necrotic tissue, cadaver material, blood, feces, suspicious product, etc.

- **Examination methods:**
- Microscopic
- Bacteriological
- Biological
- Serological (IFR, ELISA, PHAR)
- Molecular-genetic



Species belonging to the genus Clostridium:

Clostridium tetani

C. botulinum

C. perfiringens

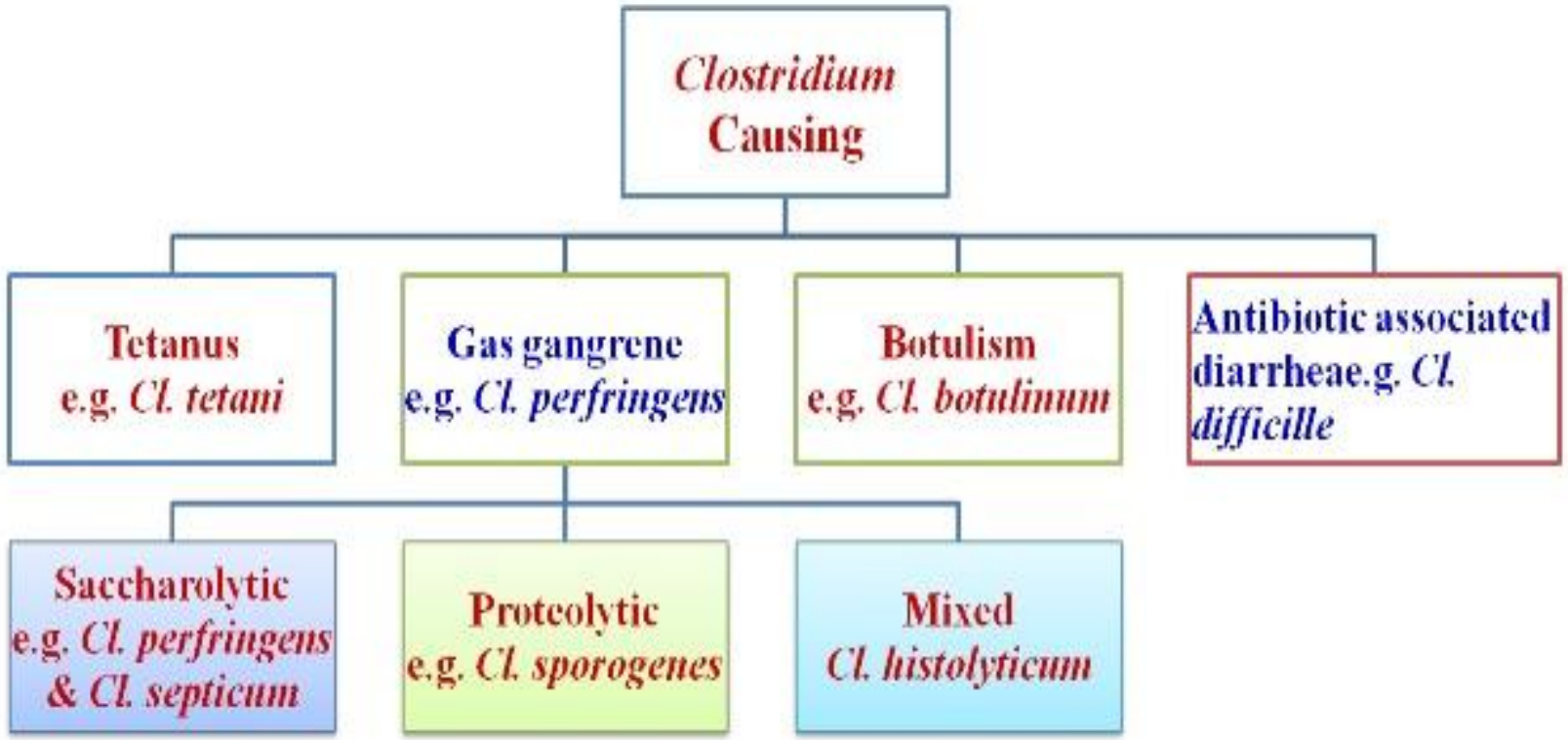
C. bifermentas

C. histolyticum

C. novyi

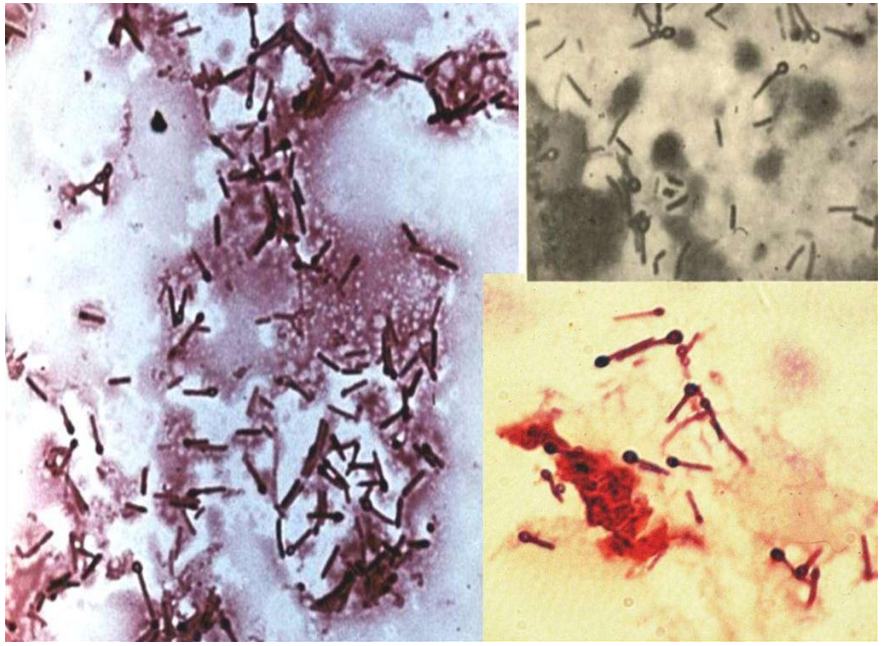
C. septicum

C. difficile

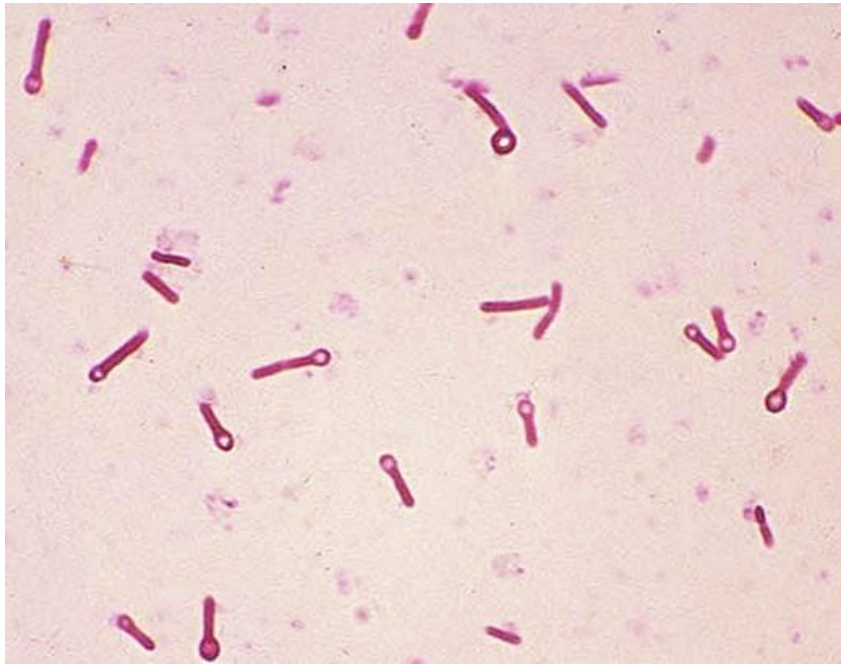


Clostridium tetani
(morpho-biological characteristics)

C.tetani is a large Gram-positive rod-shaped bacterium measuring 4-8x0.4-1.0 μm. It is motile, has peritrichous flagella. Inside, oval-shaped spores are located in the terminal position, giving the bacterial cell a characteristic **"drumstick"** shape.



A smear prepared from wound exudate



A smear prepared from a pure culture

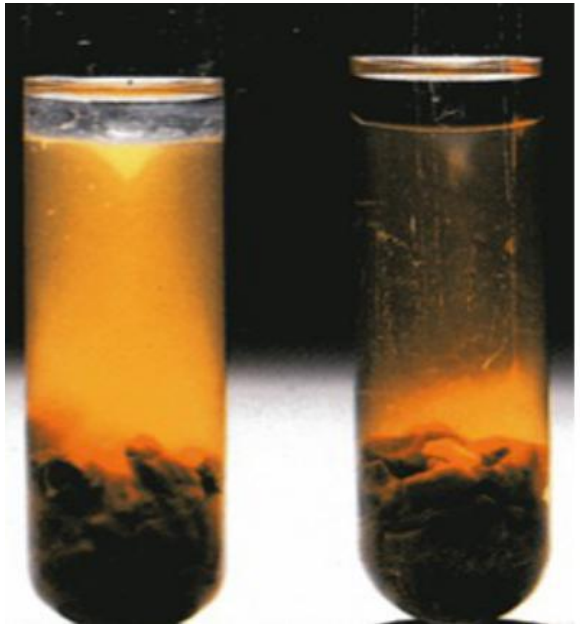
Clostridium tetani
(cultural characteristics)

- It is obligate anaerobic. Cultivated in nutrient media for anaerobes - Kitt-Tarotsi medium, sugar and blood agars at 35-37ºC, pH 6.8-7.4.
- After 3-4 days of incubation in blood-sugar agar, it forms R-colonies with grayish, sometimes transparent, uneven granular surface and protruding edges.
- Deep in the sugar agar column, it forms cotton ball, sometimes pea-like dark colonies.
- In blood agar, a hemolysis area is observed around the colonies.
- It develops by forming turbidity in the Kitt-Tarotsi medium, darkening of the Wilson-Blair medium is observed.

Clostridium tetani

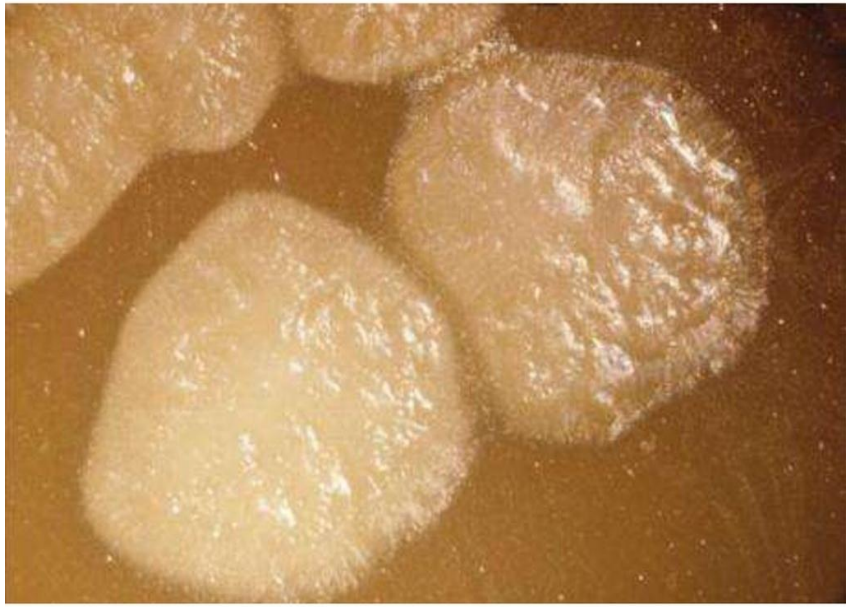
BLOOD AGAR

***Kitt-Tarozzi* medium**
(blurring)



***Wilson-Blair* medium**
(darkening)

Blood sugar agar colonies



Methods of transmission

- *C. tetani* can live for years as spores in animal feces and soil. As soon as it enters the human body through a major or minor wound and the conditions are anaerobic, the spores germinate and release the toxins.
- Tetanus may follow burns, deep puncture wounds, ear or dental infections, animal bites, abortion.
- Only the growing bacteria can produce the toxin.
- It is the only vaccine-preventable disease that is *infectious but not contagious* from person to person.

Methods of transmission

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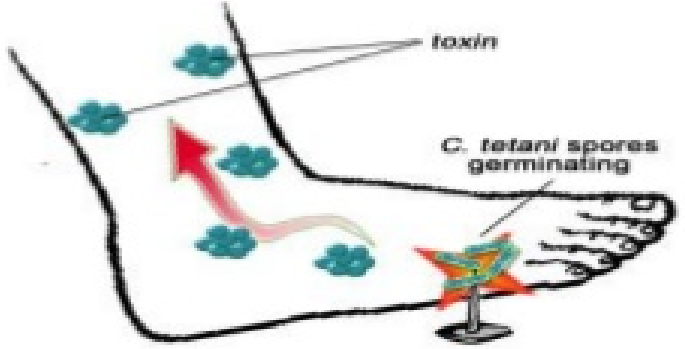
- **It is the only vaccine-preventable disease that is *infectious but not contagious* from person to person.**

C.tetani - *Entry of spores*

- Entry of *C. tetani* into the body usually involves implantation of spores into a wound
- After gaining entry, *C. tetani* spores can persist in the body for months, waiting for the proper low oxygen growth conditions to develop

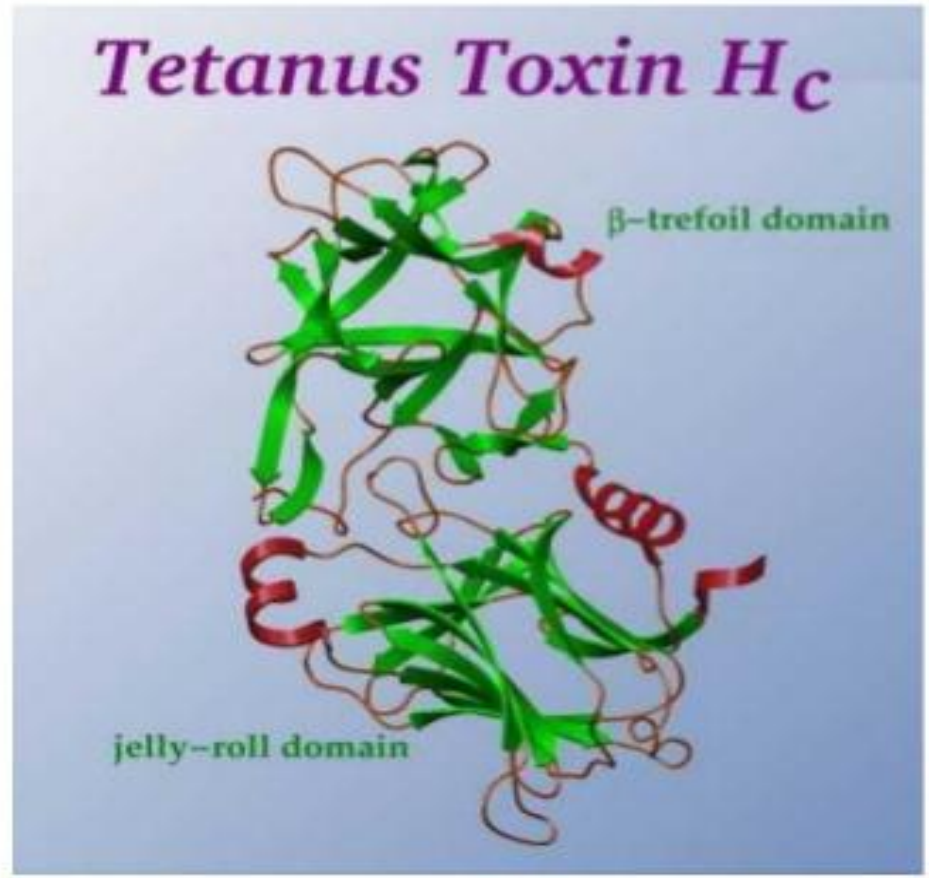


C.tetani produces toxigenic Disease

- *C. tetani* spores enter the body, they are again in an oxygen-free environment where they can germinate. The spores usually enter the body through a deep puncture wound or cut, but animal bites or even a splinter also can allow spore entry. The bacteria then produce tetanus toxins, which circulate in the body. One of the toxins blocks nerve impulses that allow muscles to relax. This toxin is responsible for causing generalized tetanus, the most common form of the disease.

Virulence & Pathogenicity

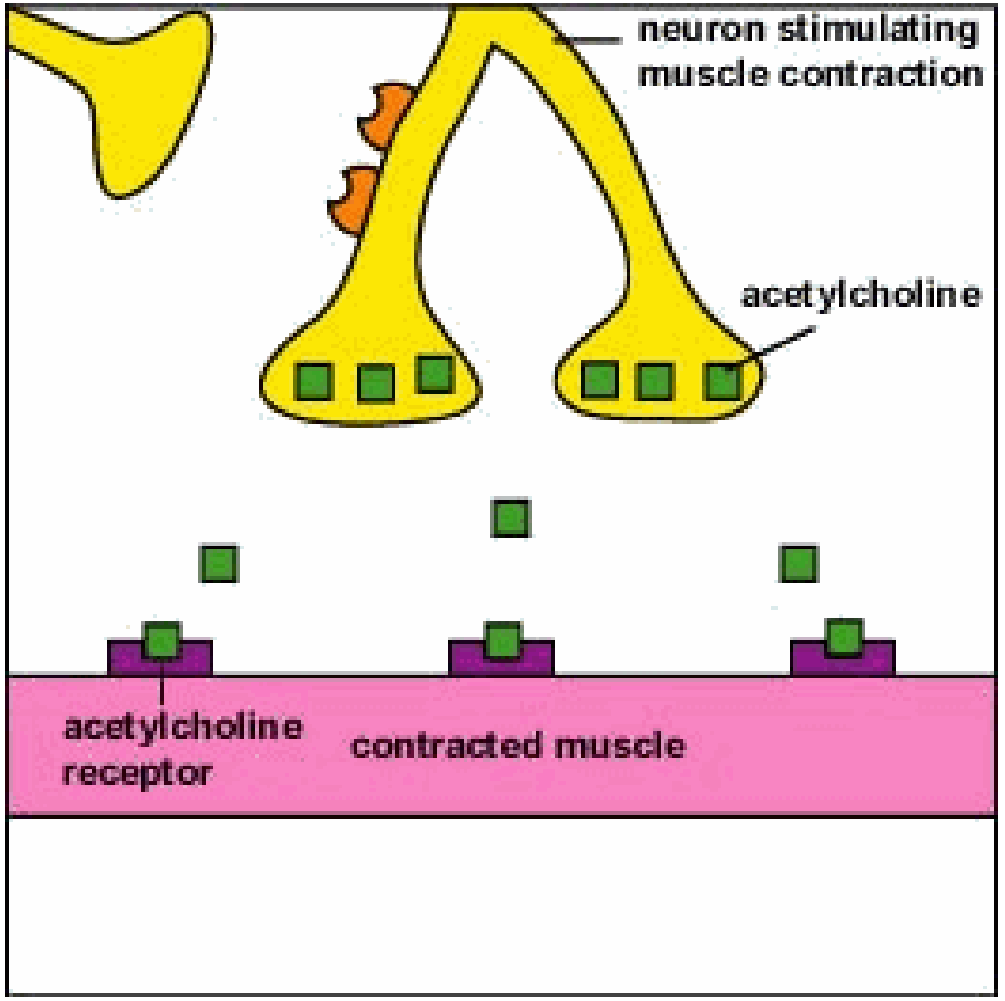
- **Not pathogenic to humans and animals by invasive infection but by the production of a potent protein toxin**
  - **tetanus toxin or tetanospasmin**
  - **The second exotoxin produced is tetanolysin—function not known.**

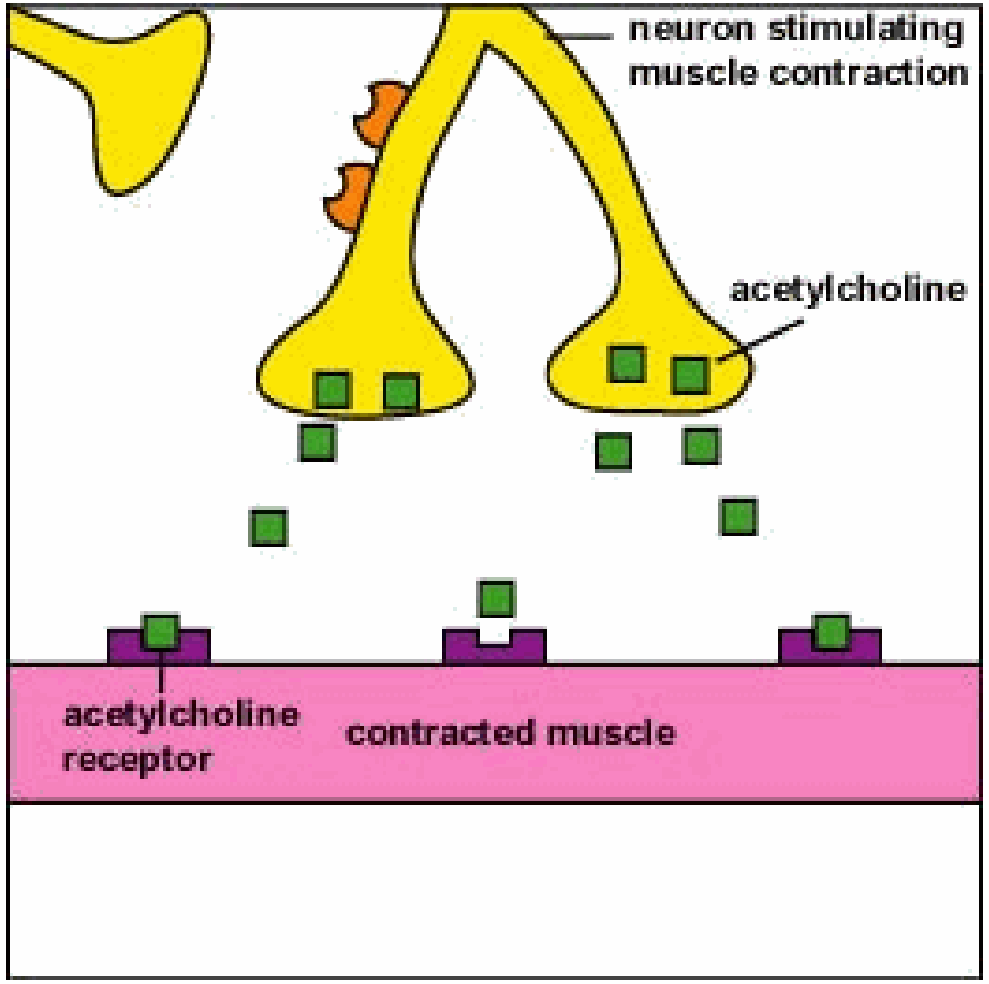


Toxin and C.tetani

- Tetanospasmin (exotoxin) produced locally , released into bloodstream .
- Binds to peripheral motor neuron terminals & nerve cells of ant.horn of spinal cord
- The toxin after entering axon , transported to nerve cell body in brain stem & spinal cord – retrograde intraneuronal transport
- Toxin – migrates across synapse – presynaptic terminals- blocks the release of Glycine & GABA from vesicles.

Mechanism of toxin





Tetanus: *Pathogenesis and clinical findings*

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Notes
- *C. tetani (Clostridium tetani)* is an obligate anaerobe found in soil and the gut of mammals
- Localized and cephalic tetanus may eventually progress to generalized tetanus

C. tetani spores invade damaged human tissue

→ *C. tetani* transforms into a vegetative bacterium & produces tetanus toxin (TT)

→ Retrograde axonal transport of TT to the spinal cord and brain stem

→ TT binds receptors and blocks neurotransmission on membrane proteins involved in neuroexocytosis

→ ↓ modulation of excitatory impulses from motor cortex, anterior horn cell and autonomic neurons

→ **Tetanus**
Nervous system disorder characterized by muscle spasm and rigidity

→ **Neonatal Tetanus**

→ Umbilical cord contamination with *C. tetani* after unsanitary delivery

→ **Poor feeding, irritability**

→ **Cephalic tetanus**

→ Penetrating head and/or neck injury

→ *C. tentani* invasion of cranial nerve VII and occasionally III, IV, VI, XII

→ **Cranial nerve palsies**

→ **Localized tetanus**

→ Dysfunction in the local muscle interneurons that inhibit the alpha motor neurons

→ **Rigidity in muscle group near infected site**

→ **Generalized tetanus**

→ ↓ control of catecholamine release

→ Hypersympathetic state

→ **Diaphoresis, tachycardia, hypertension**

→ ↑ in resting firing rate of motor neurons and ↓ inhibition of reflex motor responses to afferent sensory stimuli

→ **Generalized muscle spasms and rigidity**

→ **Exhaustion**

→ **Respiratory failure**

→ **Aspiration**

Legend: Pathophysiology | Mechanism | Sign/Symptom/Lab Finding | Complications

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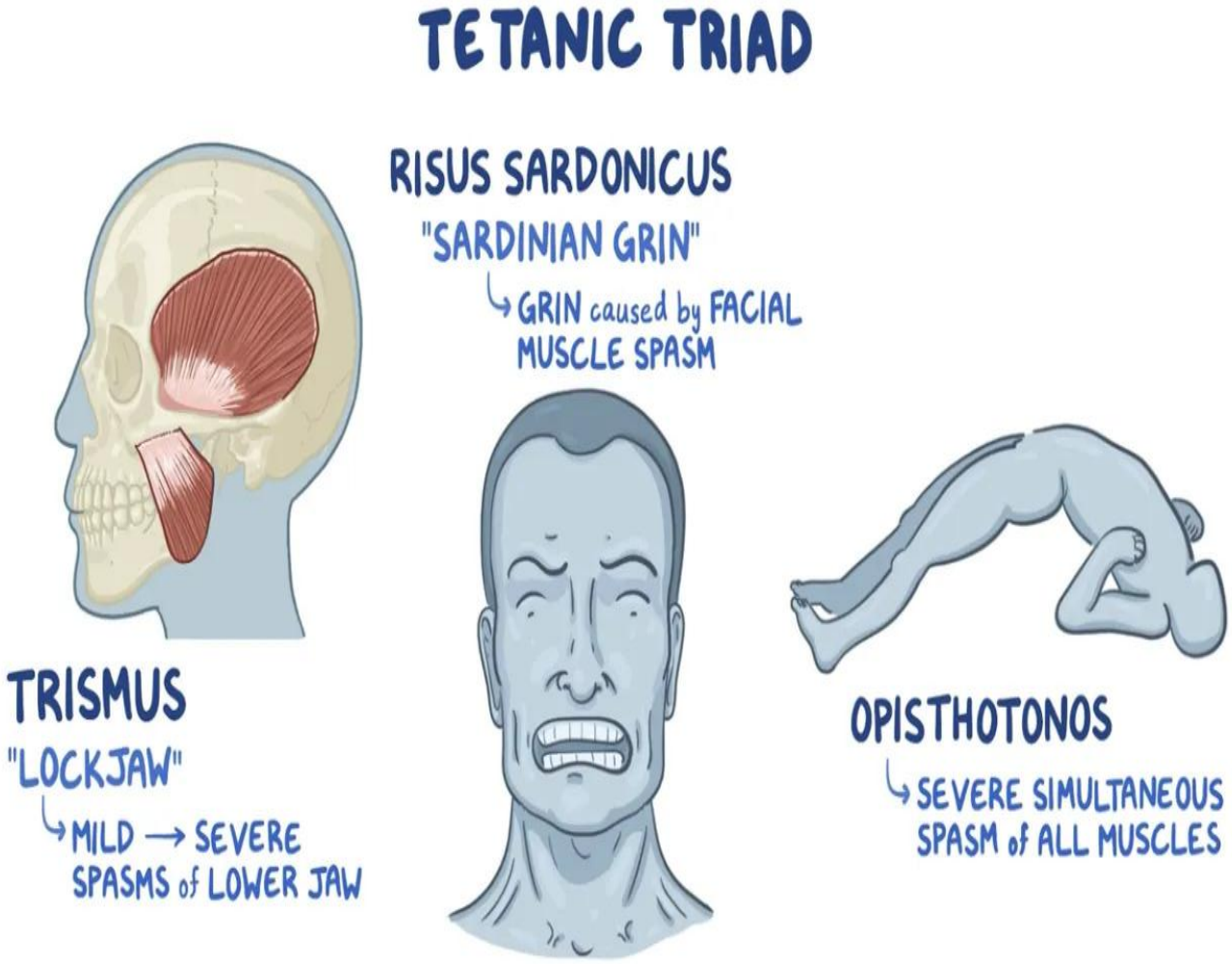
Tetanus - Pathogenesis

Сейчас не удается отобразить рисунок.

Tetanus – clinical finding



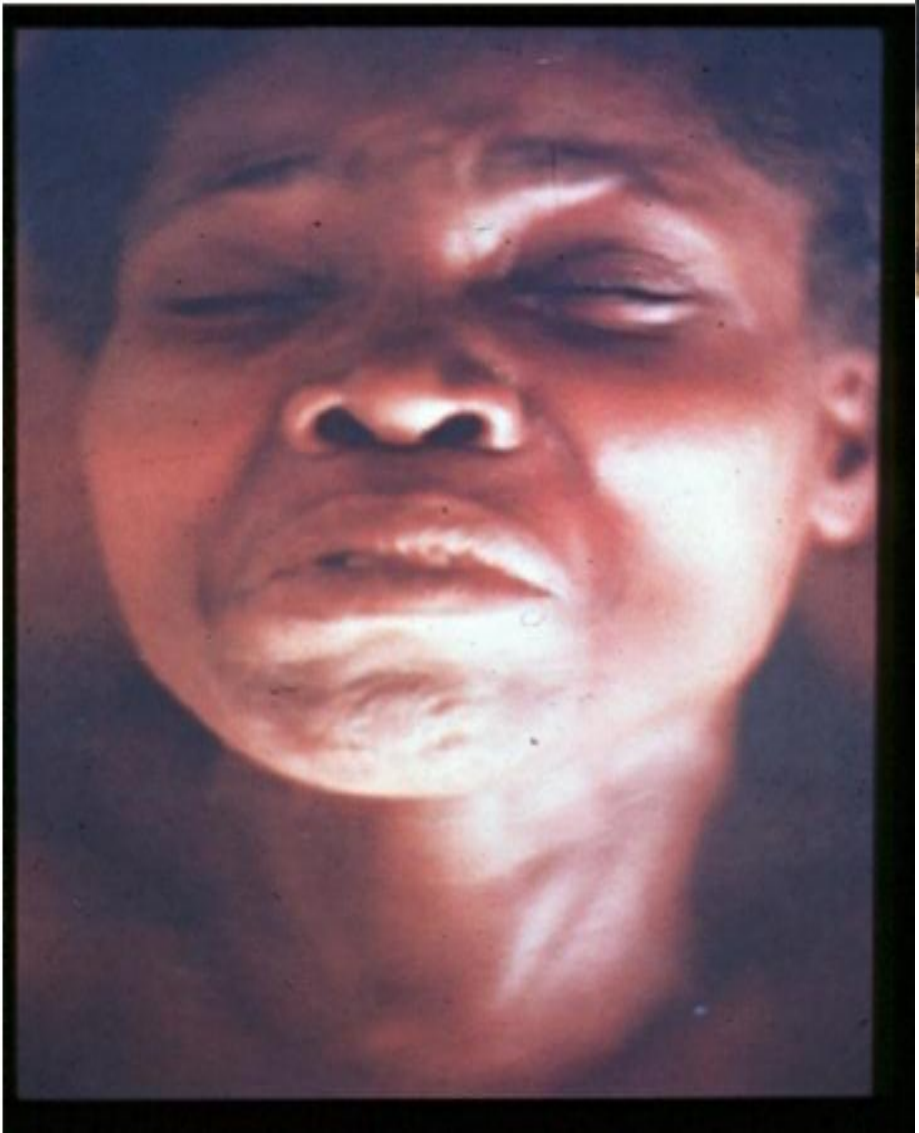
Symptoms

- **Tetanic seizures (painful, powerful bursts of muscle contraction)**
  - if the muscle spasms affect the larynx or chest wall, they may cause asphyxiation
  - stiffness of jaw (also called lockjaw)
  - stiffness of abdominal and back musc
  - contraction of facial muscles
  - fast pulse
  - fever
  - sweating

Patient Manifests with

- A person suffering from tetanus undergoes convulsive muscle contractions of the jaw--called LOCKJAW
- The contractions by the muscles of the back and extremities may become so violent and strong that bone fractures may occur
- The affected individual is conscious throughout the illness, but cannot stop these contractions

Trismus



The back muscles are more powerful, thus creating the arc backward

"Oposthotonus" by Sir Charles Bell, 1809.

Baby has neonatal tetanus with complete rigidity

Types of tetanus:
local, cephalic, generalized, neonatal

- ***Incubation period: 3-21 days, average 8 days.***

Uncommon types:

- **Local tetanus:** persistent muscle contractions in the same anatomic area as the injury, which will however subside after many weeks; very rarely fatal; milder than generalized tetanus, although it could precede it.

- **Cephalic tetanus:** occurs with ear infections or following injuries of the head; facial muscles contractions.

Most common types:

Generalized tetanus

- descending pattern: lockjaw → stiffness of neck → difficulty swallowing → rigidity of abdominal and back muscles.
- Spasms continue for 3-4 weeks, and recovery can last for months
- Death occurs when spasms interfere with respiration.

Neonatal tetanus:

- **Form of generalized tetanus that occurs in newborn infants born without protective passive immunity because the mother is not immune.**
- **Usually occurs through infection of the unhealed umbilical stump, particularly when the stump is cut with an unsterile instrument.**

Methods of diagnosis

- **Based on the patient's account and physical findings that are characteristic of the disease.**
- Diagnostic studies generally are of little value, as cultures of the wound site are negative for *C. tetani* two-thirds of the time.
  - **When the culture is positive, it confirms the diagnosis of tetanus**

Diagnosis

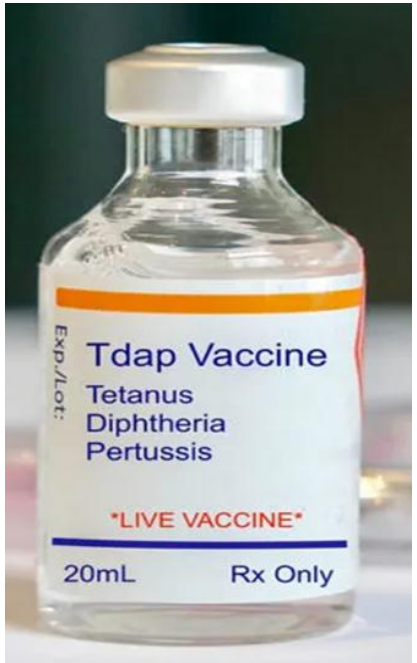
- Tests that may be performed include the following:
  - Culture of the wound site (may be negative even if tetanus is present)
  - Tetanus antibody test
  - Other tests may be used to rule out meningitis, rabies, strychnine poisoning, or other diseases with similar symptoms.

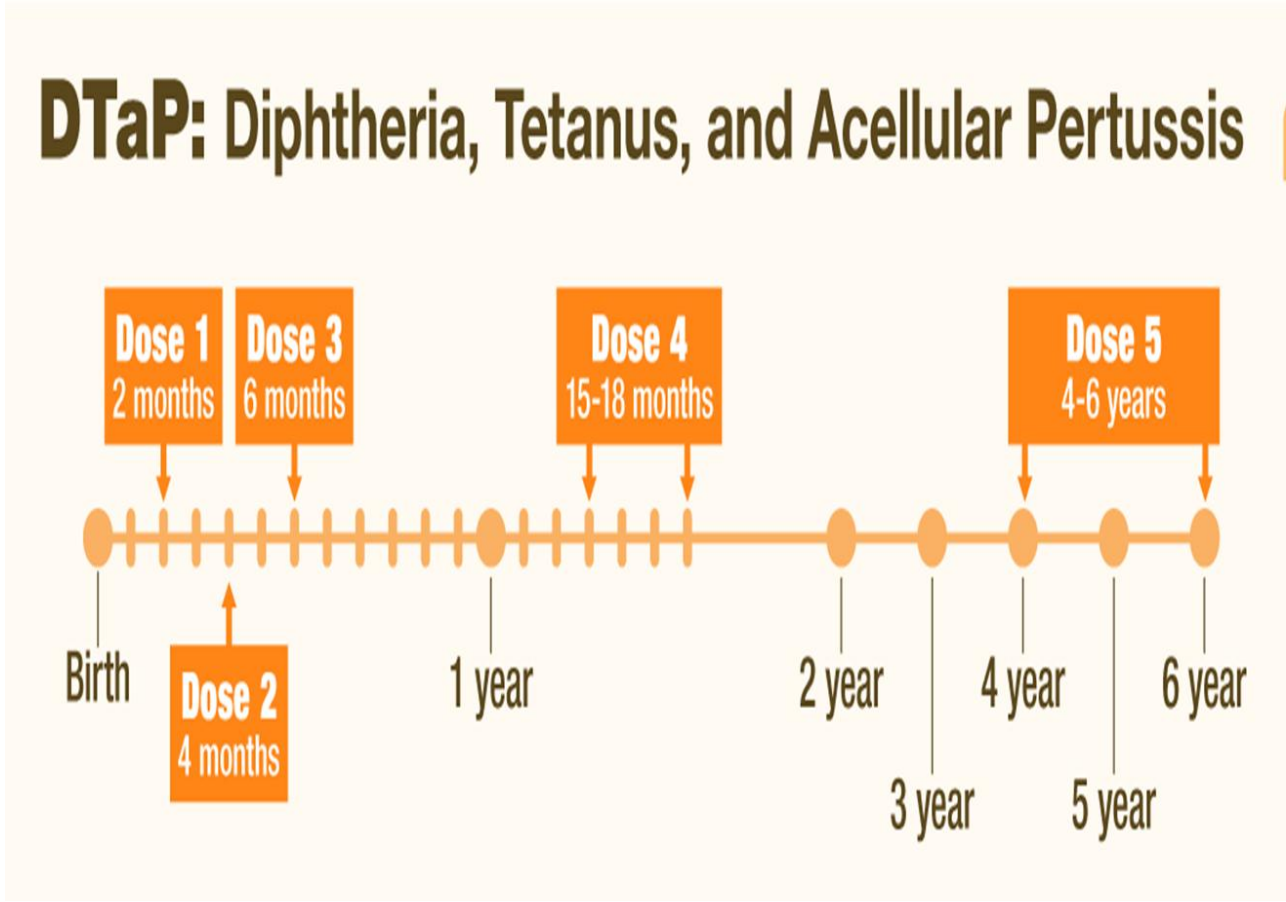
Clinical treatment

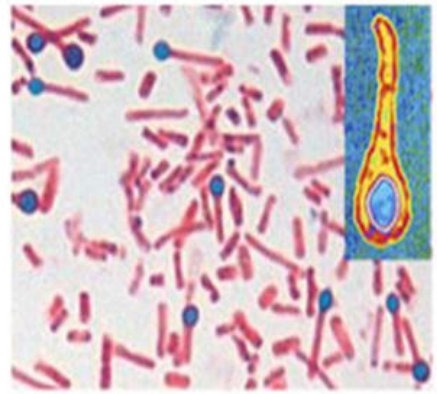
- If treatment is not sought early, the disease is often fatal.
- The **bacteria** are killed with antibiotics, such as penicillin or tetracycline; further toxin production is thus prevented.
- **The toxin is neutralized with shots of tetanus immune globulin, TIG.**
- Other drugs may be given to provide sedation, relax the muscles and relieve pain.
- Due to the extreme potency of the toxin, immunity does not result after the disease.

Method of prevention - Immunization

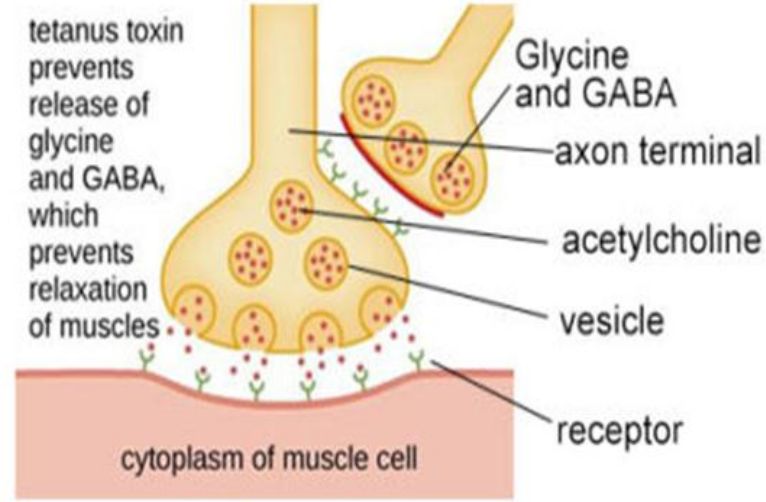
- A person recovering from tetanus should begin active immunization with tetanus toxoid (Td) during convalescence.
- The tetanus toxoid is a formalin-inactivated toxin, with an efficiency of approx. 100%.
- The DTaPe includes tetanus, diphtheria and pertussis toxoids; it is routinely given in the US during childhood. After 7 years of age, only Td needs to be administered.
- Because the antitoxin levels decrease over time, booster immunization shots are needed every 10 years.







'Drum Stick' or
'Tennis Racket' appearance



Mechanism of Tetanus Toxin

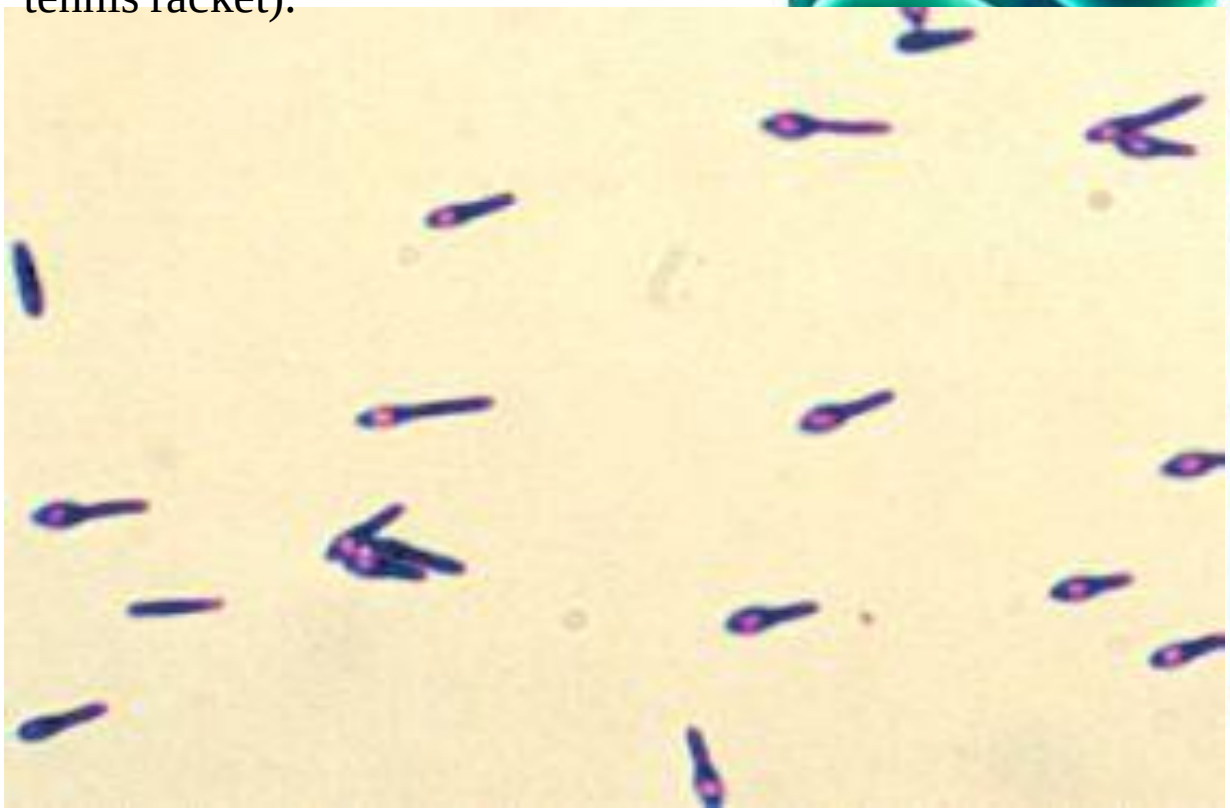
Clostridium tetani

Opisthonus

Risus Sardonicus

Clostridium botulinum ***(morpho-biological characteristics)***

C.botulinum is a 4-9x0.6-1.0 μm size polymorphic rod-shaped bacterium. It is motile, has peritrichous flagella. It does not form a capsule, but under unfavorable conditions produces subterminal spores (similar to a tennis racket).



C.botulinum

Classification:

1.Domain –bacteria

2. Division –firmicutes

3. Class –clostridia

4. Order –clostridiales

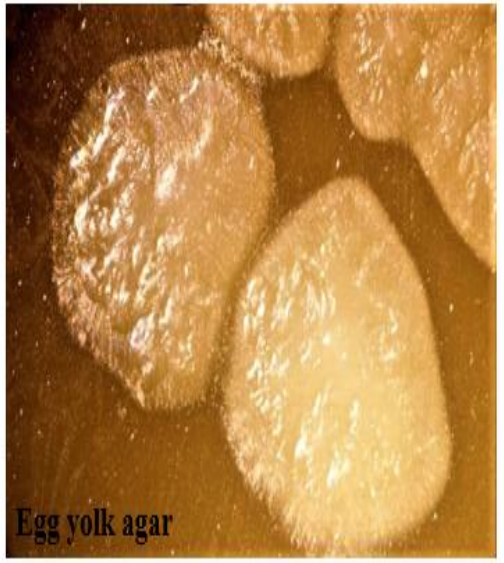
5. Family –closridiacae

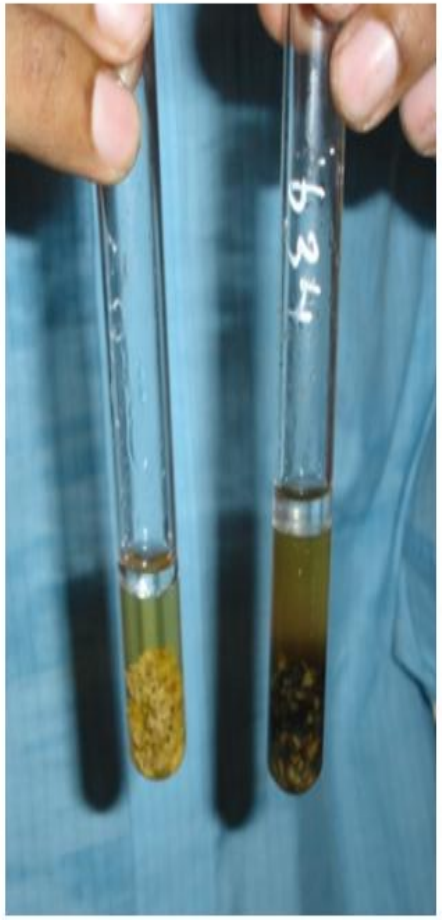
6. Genus –clostridium

7. Species -botuilinium

Clostridium botulinum (cultural characteristics)

- It is obligate anaerobic. Cultivated at 25-37°C pH 7.3-7.6.
- On blood-sugar agar, colonies surrounded by a hemolysis zone with irregularly shaped, thread-like protrusions, on blood agar, they form smooth (S-shaped) with a brigh surface similar to a dew drop, or R-colonies with indented protruding edges.
- In the depth of the sugar agar column, pea-like (S-shaped) sometimes cotton ball-like (R-shaped) colonies are formed.
- It produces turbidity and gas in liquid media (Kitt-Tarozz medium, liver broth, etc.).





Kitt-Tarozzi medium
(blurring)

TOXIN

- **Virulence factor**—botulinum toxin
  - neurotoxin
  - relatively heat-labile and resistant to protease
  - types: A, B, C, D, E, F, G
  - the most potent toxic material known

10,000 times

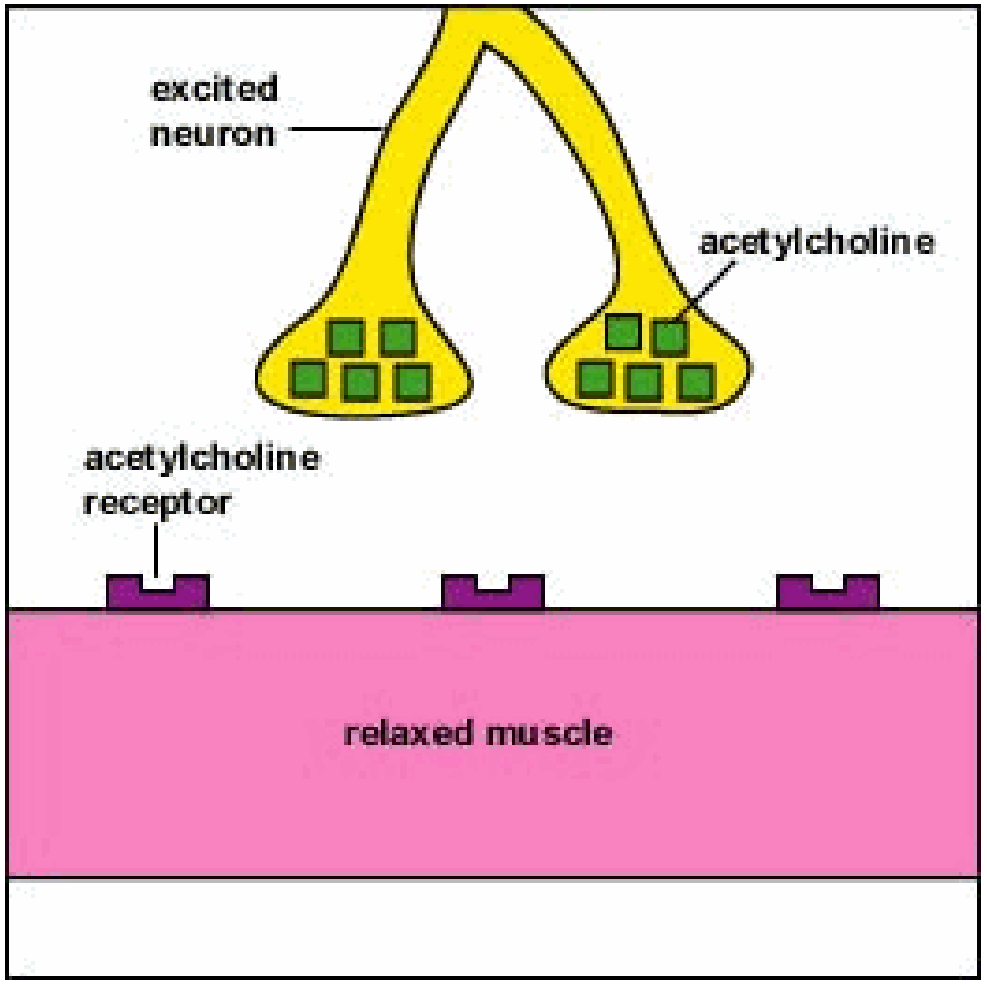
- mechanism of action

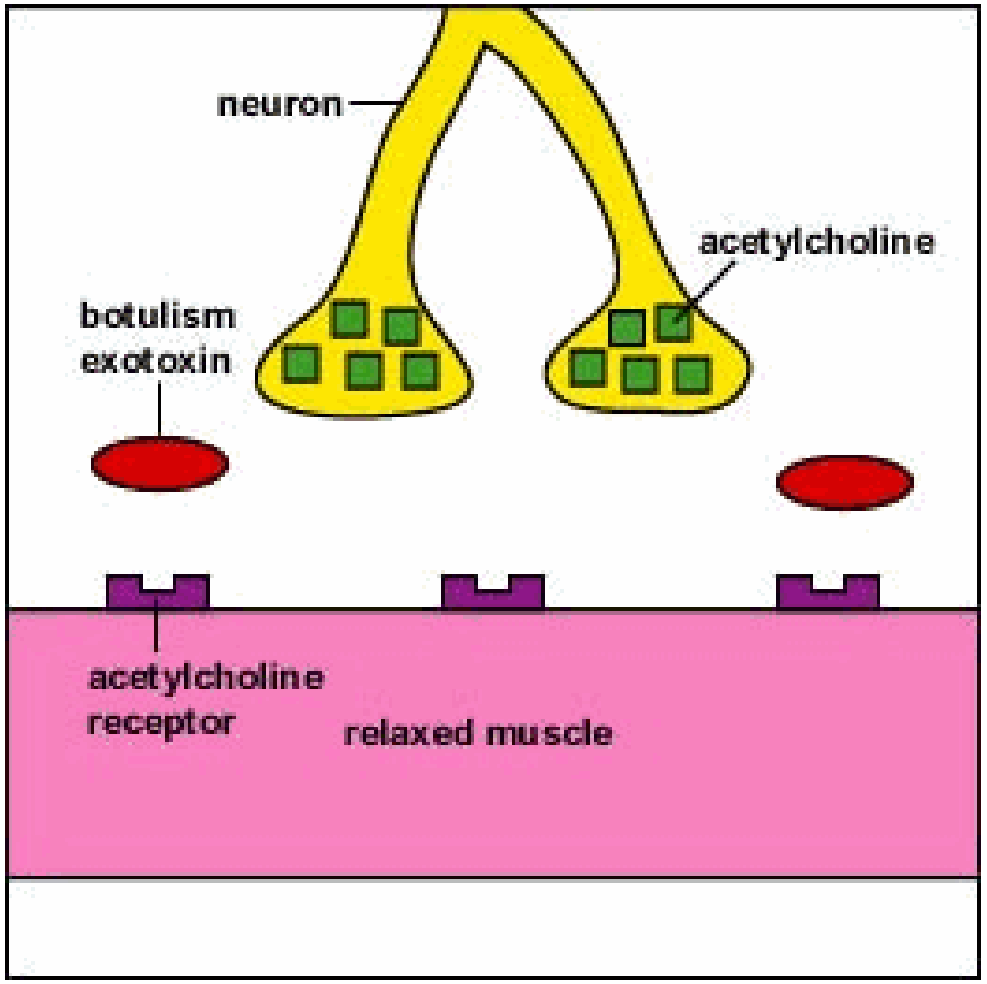
Toxin → gut → blood → cholinergic synapses → block the release of exciting neurotransmitter, e.g., acetylcholine → flaccid paralysis

MECHANISM OF TOXIN.

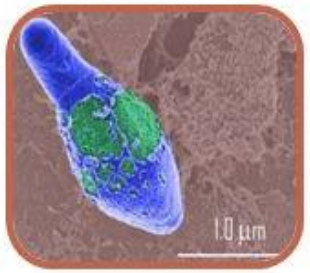
- **Botulinum toxin:**
  - absorbed from the gut
  - Binds to receptors of presynaptic membranes of motor neurons of the peripheral nervous system and cranial nerves.
  - Proteolysis-by the light chain of botulinum toxin of the target SNARE proteins in the neurons inhibits the release of acetylcholine at the synapse, resulting in lack of muscle contraction and paralysis
  - SNARE proteins are-synaptobrevin, SNAP 25, syntaxin.
  - Type A and E toxin cleaves-SNAP 25
  - Type B toxin cleaves synaptobrevin

Mechanism of toxin

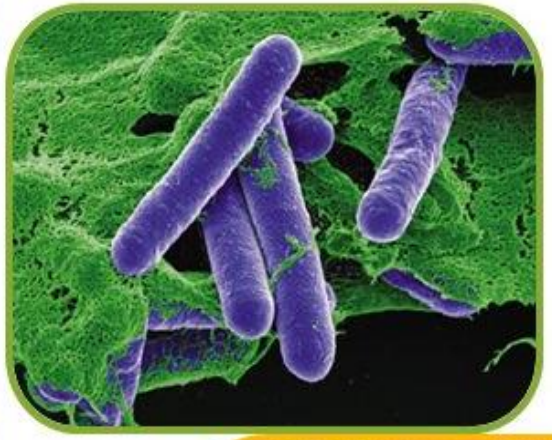


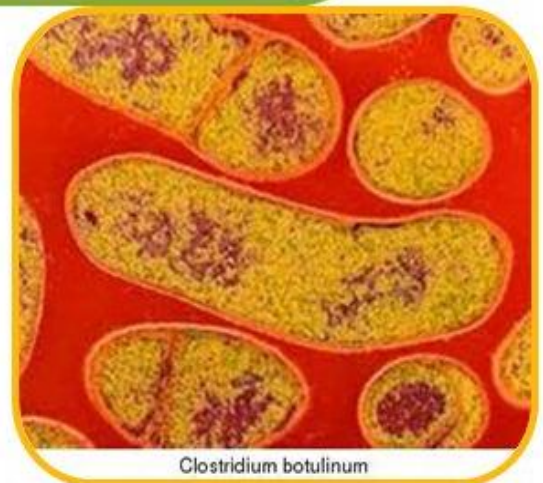


Botulism



- Latin: *botulus* = sausage
- Is a rare but serious paralytic illness caused by botulinum toxin, which is produced by the bacterium *Clostridium botulinium* under anaerobic conditions




Clostridium botulinum

-Gram-positive Bacillus

-Spores

-Found in soil, vegetables, fruits and animal feces

Canned meat, fish and vegetable products can cause botulism

Pathogenesis

- **Transmitted in three ways:**
  - **Food or water toxin contamination.**
  - **Wound infected with *C. Botulinum.***
  - **Ingestion of C. botulinum.**
- **Most common contaminated food: Vacuum packed, or canned alkaline food.**

  E.g. fish, green beans, any home-canned food.
- **Foods eaten without cooking.**

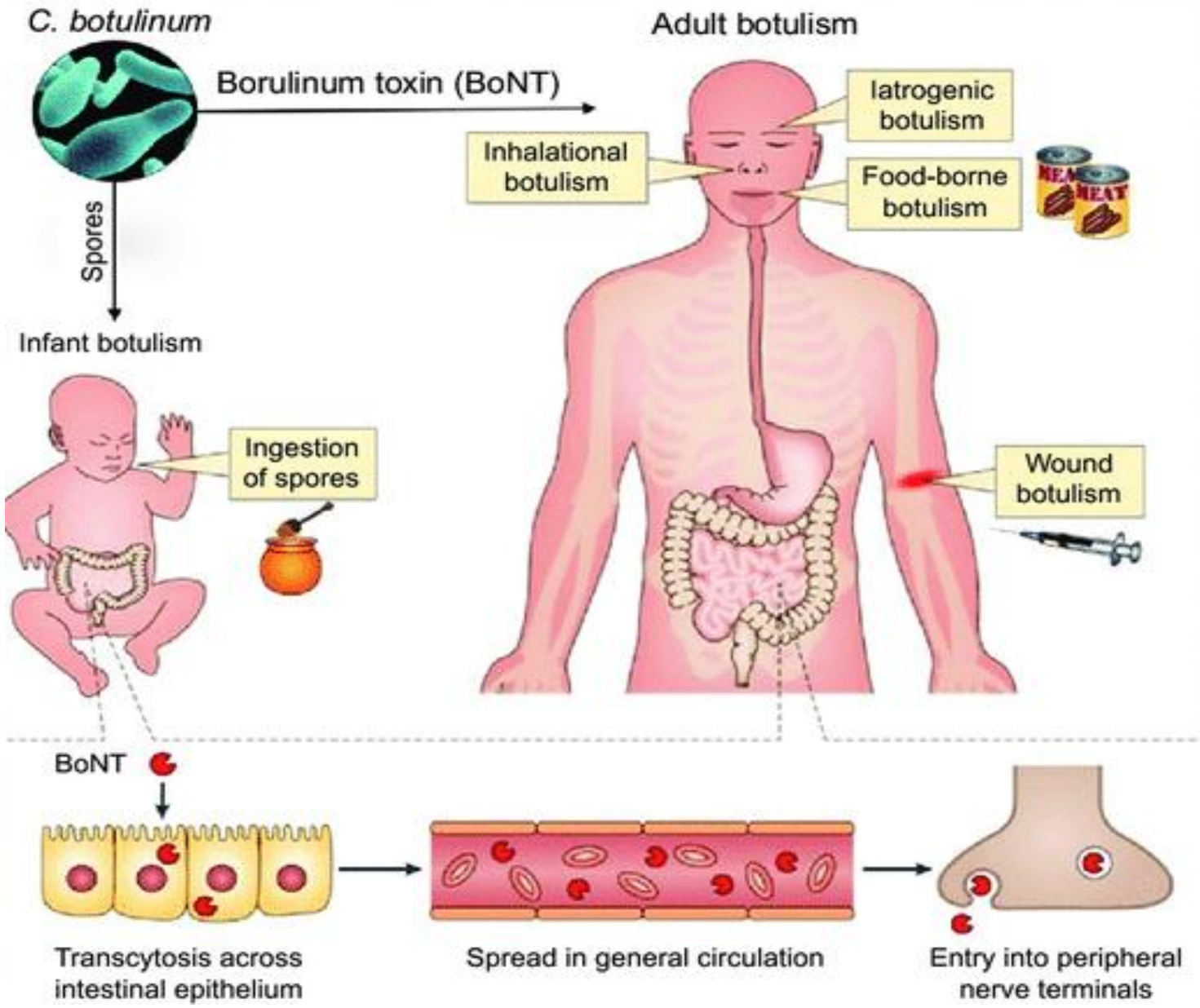
Pathogenesis

- Toxin must enter body
  - Direct toxin absorption from mucosal surface
    - Gut – foodborne
    - Lungs – inhalational
  - Via toxin produced by infection with *C.botulinum*
    - Skin breaks – wound botulism after trauma, IV drugs
    - Gut – intestinal botulism
    - Would not be seen in BT event, as toxin would be used
- Does not penetrate intact skin

Botulism – pathogenesis

Pathogenesis of Infant Botulism

- Cause :infection by C. botulinum.
- Age: 5 - 20 weeks of age.
- Characterized by constipation and weak sucking.
- It cause "sudden infant death syndrome-SIDS".
- found in the stool.



CLNICAL MANIFESTATIONS

- **Adult botulism:**

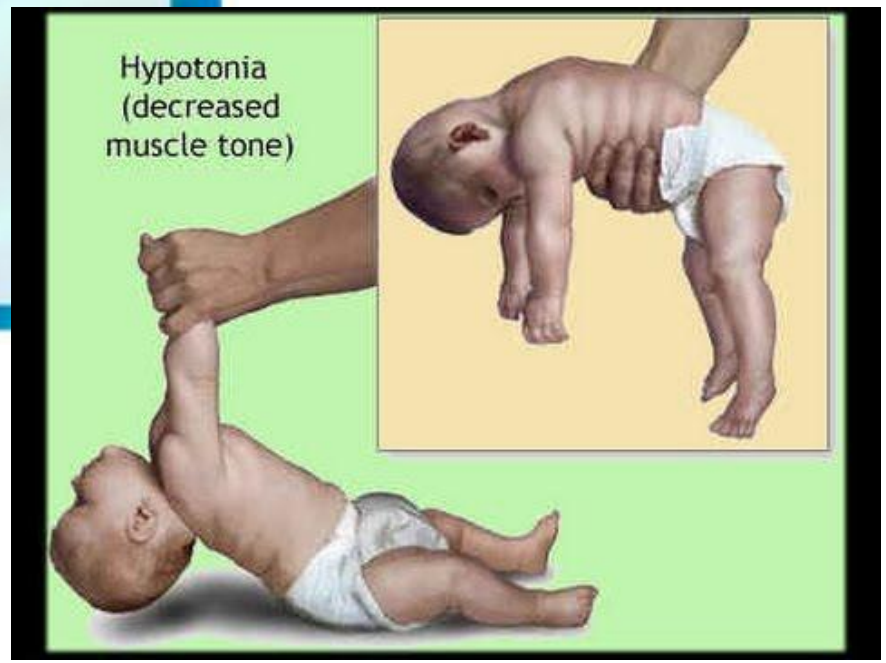
  flaccid paralysis: double vision, dysphagia, difficulty in breathing & speaking ,rare gastrointestinal symptoms .cause of death: respiratory failure

- **Infant botulism:**

  – manifestation: constipation, poor feeding, difficulty in sucking and swallowing, weak cry, loss of head control.

  Floppy baby

  prevention: free of honey



Symptoms

- **Appear in 18-24 hrs.**
- **include :**

  Blurry vision, Double vision.

  Dry mouth.

  Trouble swallowing.

  Trouble breathing.

  Muscle weakness.

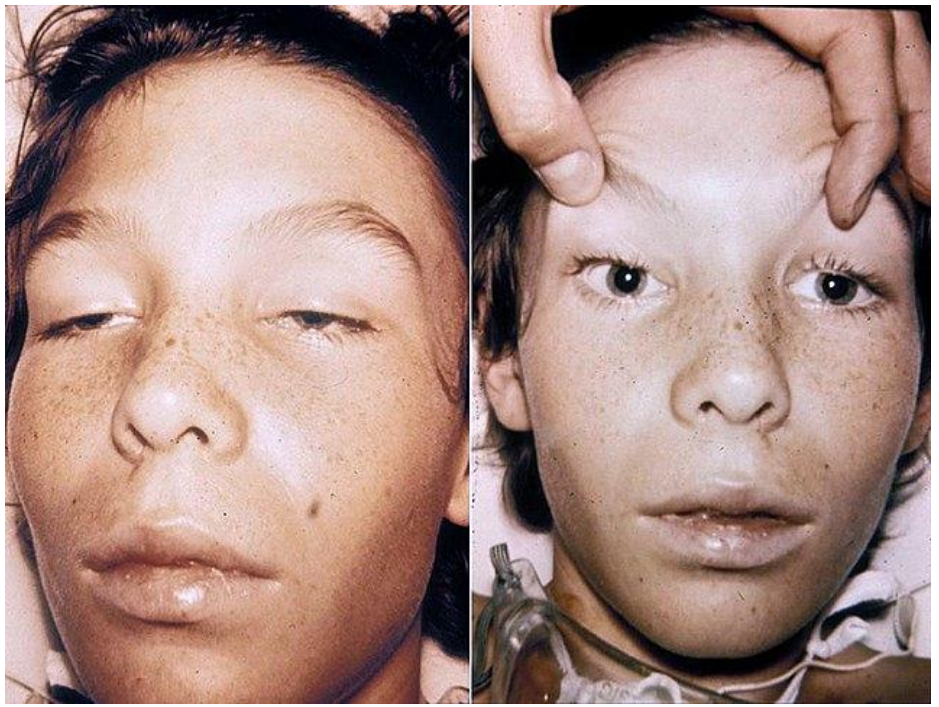
Fever is not a symptom of botulism

Clinical Features

- Classic Triad
  - Symmetric, descending flaccid paralysis with prominent bulbar palsies
  - Afebrile
  - Clear sensorium
- Bulbar palsies summarized as "4 Ds"
  - Diplopia, dysarthria, dysphonia, dysphagia

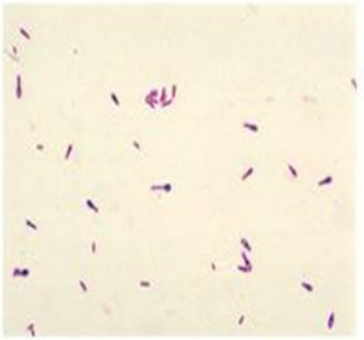
Botulism symptoms: Characteristic Triad

- **Symmetric, descending (cranial nerves first, then upper extremities, then respiratory muscles, and lower extremities) flaccid paralysis with prominent bulbar palsies, particularly:**
  - **Diplopia – double vision**
  - **Dysarthria – difficulty in speech articulation**
  - **Dysphonia – difficulty in voice production**
  - **Dysphagia – difficulty in swallowing**
- **Patient is afebrile (although fever may be present in wound botulism)**
- **Patient's sensibilities intact; cognitive functions unaffected**



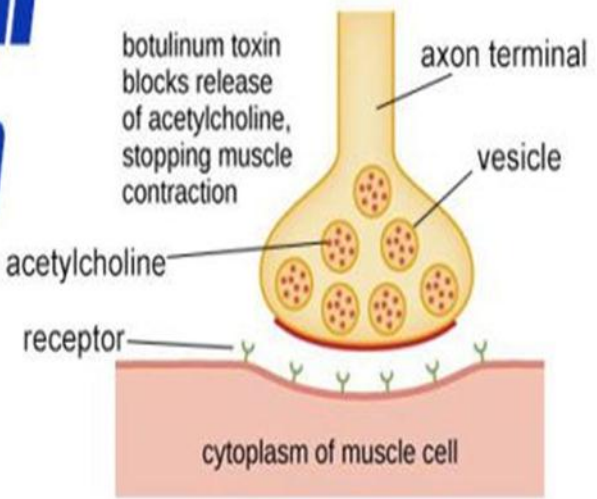
Diagnosis

- **Clinical diagnosis**
- Diagnostic tests help confirm
  - Toxin neutralization mouse bioassay
    - Serum, stool, or suspect foods
  - Infant botulism
    - *C botulinum* organism or toxin in feces
    - Testing is done in Reference laboratories under Biosafety regulations



Gram-positive bacilli
with 'Tennis Racket' appearance

Clostridium botulinum

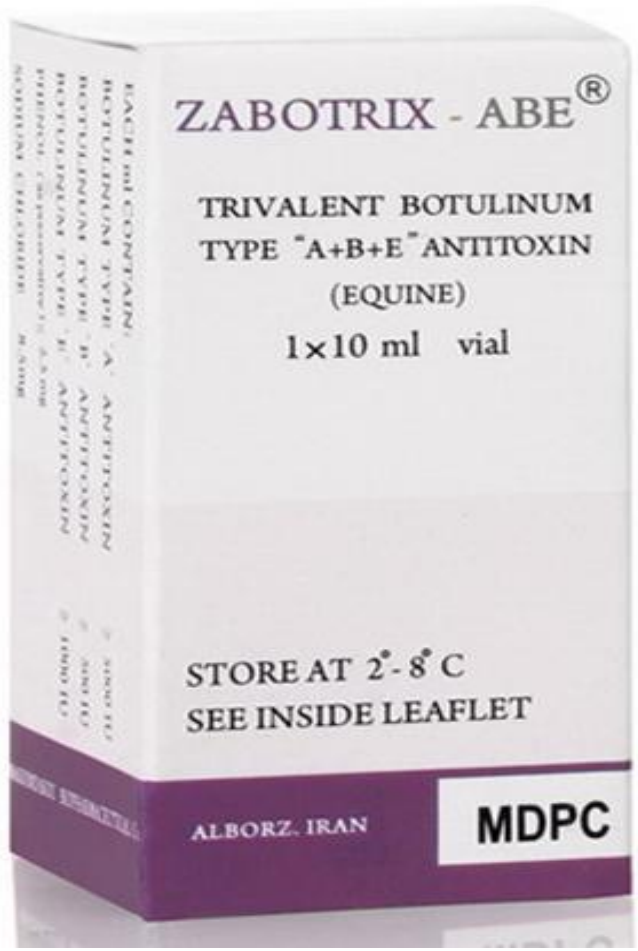


Mechanism of Botulinum Toxin

microbeonline

Treatment

- **Trivalent (A,B,E) antitoxin must administrated intravenously (recovery takes several weeks)**
- **Mechanical respirator is administrated if necessary.**



- **Antitoxin action**
  - Food-borne botulism
    - Neutralizing antibody levels exceed toxin levels
    - Single dose adequate
  - Large exposure (e.g. biological weapon)
    - can confirm adequacy of neutralization
      - recheck toxin levels after treatment
- Antitoxin adverse effects
  - Serum sickness (2-9%), anaphylaxis (2%)

Clostridium perfringens

- Large rectangular Gram positive bacillus
- Spores seldom seen in vivo or in vitro
- non motile
- Produces several toxins
  - **alpha (lecithinase), beta, epsilon .....**
  - **enterotoxin**
- Causes a spectrum of human diseases
  - **Bacteraemia**
  - **Myonecrosis**
  - **food poisoning**
  - **enteritis necrotica (pig bel)**

Causes of gas gangrene:

C.perfiringens (90-100%)	C.septicum (4-12%) (spontan)
C.novyi (25-30%)	C.bifermentas
C.histolyticum (0,1 %)	C.sporogenes
C.sordellii	

1.Clostridium perfringens(Cl.welchii)

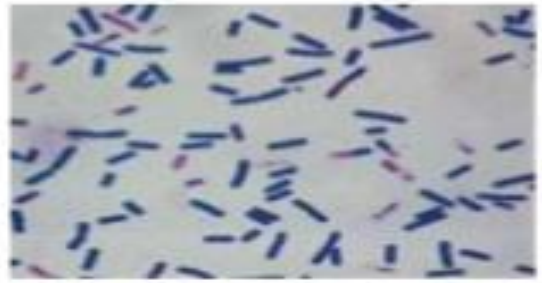
Morphology

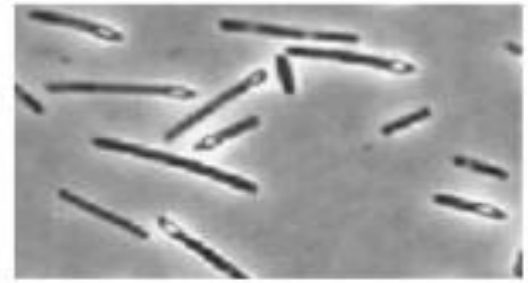
-Large Gram-positive bacilli with straight, parallel sides & slightly rounded ends.

-Measure 4-6x1µm in size,occuring singly or in chains

-Pleomorphic,capsulated & non-motile.

-Spores are **central or sub terminal**. **Spores are rarely seen in culture media or material from pathogenic lesions**, a characteristic morphologic feature





CULTURAL CHARACTERISTICS

-**Robertson's cooked meat broth** is ideal; meat is turned **pink but not digested** with sour odor.

-**Stormy fermentation** of lactose in litmus milk; the acid coagulates casein-**acid clot.**

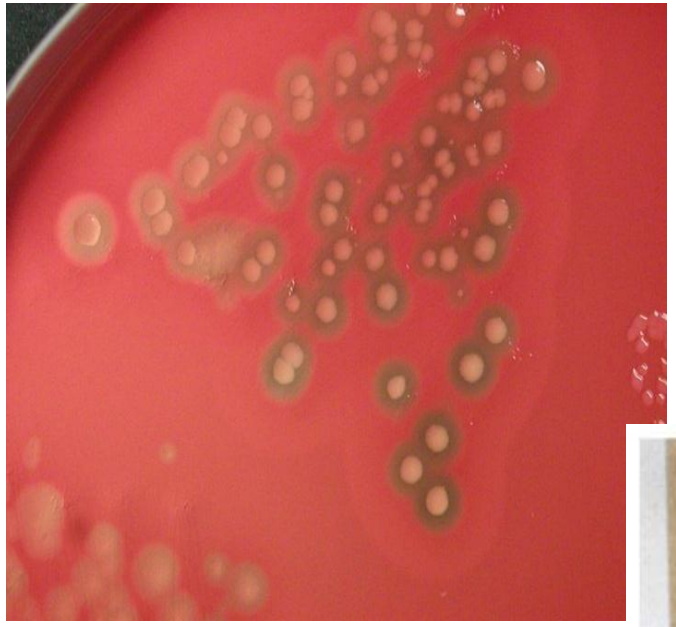
-**On BAM**: Target haemolysis

BIOCHEMICAL REACTIONS:

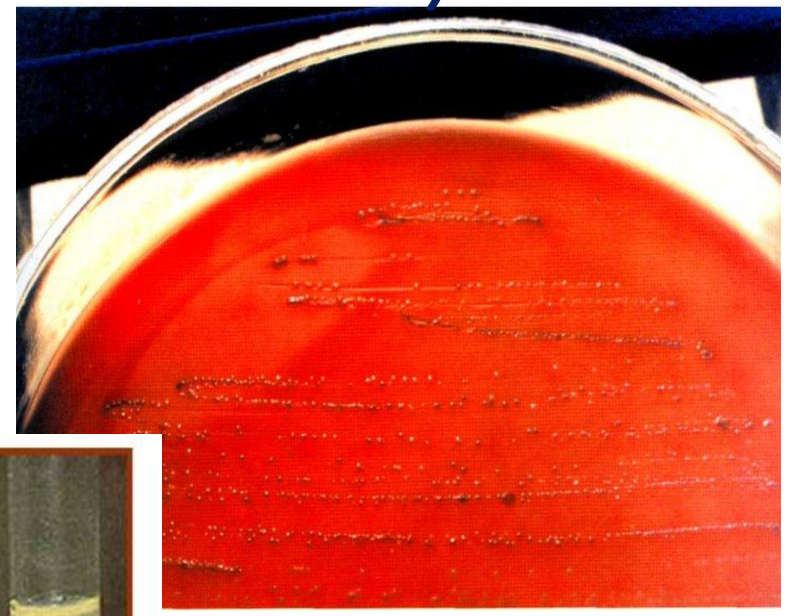
Glucose]		Indole	-ve
Lactose]	Fermented with	MR	+ve
Maltose]	A & G production	VP	-ve

-H_2S prodn. test & Nitrate redn. test - +ve

Clostridium perfringens (cultural characteristics)

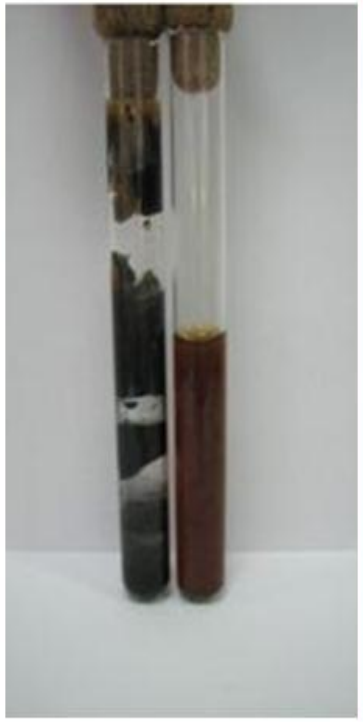


Blood agar



Sessler agar

Kitt-Tarozzi medium



Wilson-Blair medium

Sugar agar due to gas formation
agar column digestion

TABLE 29-2 Virulence factors of *Clostridium perfringens*

Virulence factors	Biological functions
α (Alpha) toxin	Lethal, dermonecrotic, hemolytic, and is a lecithinase; causes toxemia, increases vascular permeability of blood vessels, leading to tissue destruction. Main cause of toxaemia associated with gas gangrene
β (Beta) toxin	Lethal toxin; causes necrotic lesions in necrotizing enteritis
ε (Epsilon) toxin	Lethal protoxin; increases vascular permeability of the wall of the gastrointestinal tract
ι (Iota) toxin	Lethal toxin; causes necrotic lesions and increases vascular permeability
δ (Delta) toxin	Hemolytic
θ (Theta) toxin	Oxygen-labile hemolytic and cytolytic toxin
κ (Kappa) toxin	Collagenase
λ (Lambda) toxin	Proteinase and gelatinase
μ (Mu) toxin	Hyaluronidase
ν (Nu) toxin	Deoxyribonuclease
Enterotoxin	Enterotoxic and hemolytic; alters permeability of the gastrointestinal membrane
Neuraminidase	Alters cell surface ganglioside receptors and promotes capillary permeability
Bursting factor	Causes typical muscle lesions in gas gangrene
Circulating factor	Increases adrenaline sensitivity of the capillary membrane
Hyaluronidase	Breaks down intercellular cement substance and promotes the spread of infection along tissue planes

PATHOGENICITY

-**Three Clinical conditions** produced include;

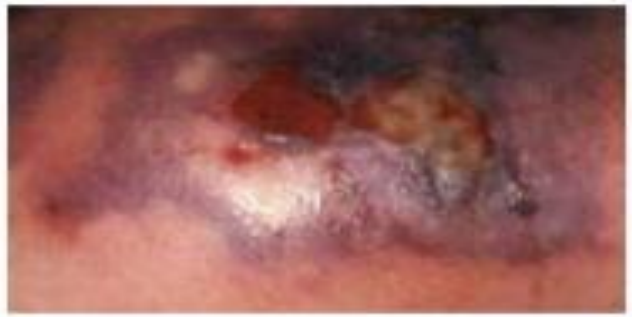
1.Simple wound contamination: Slow wound healing

2.Anaerobic or clostridial cellulitis:

-Clostridiae invade fascial planes(fasciitis) with minimal toxin production but no invasion of muscle tissue.

-Lesions vary from limited '**gas abscess**' to extensive involvement of limbs.

-Seropurulent discharges with offensive odor produced



3.Anaerobic myositis or myonecrosis or gas gangrene

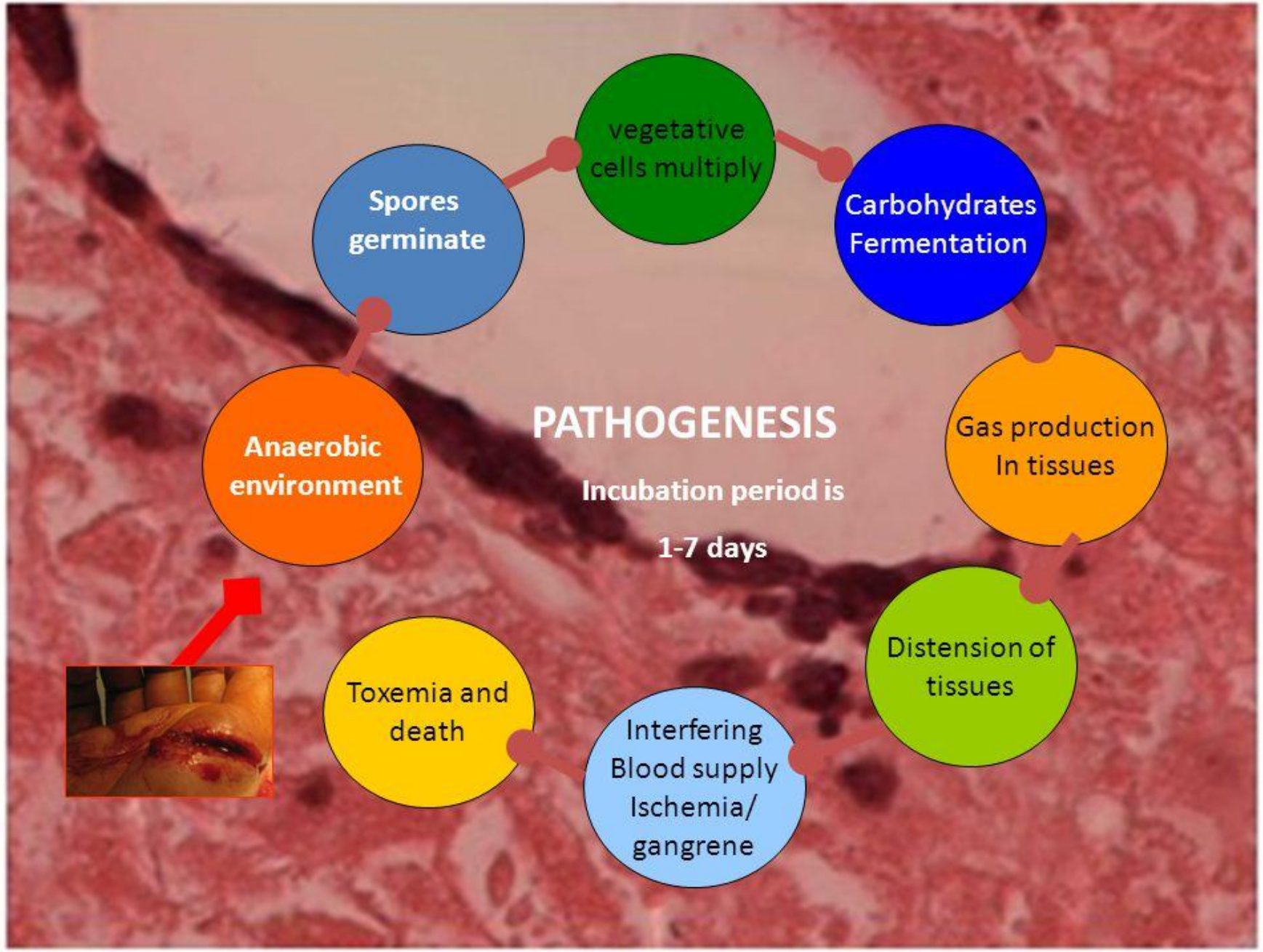
-Most serious complication of clostridial invasion of healthy muscle tissue .

-Abundant formation of exotoxin & production of gas.

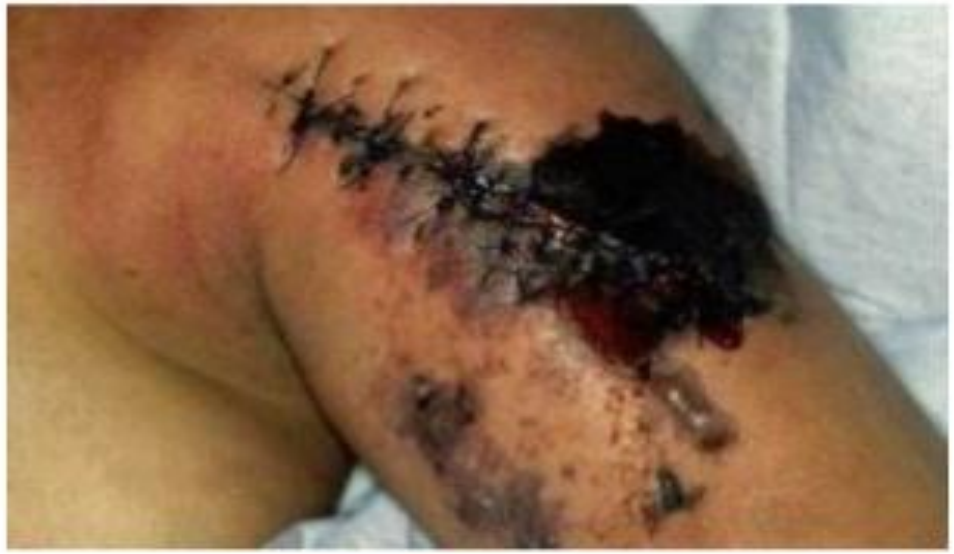
-GG is disease of war. In civilian life it follows road accidents or injuries with crushing of muscle mass.

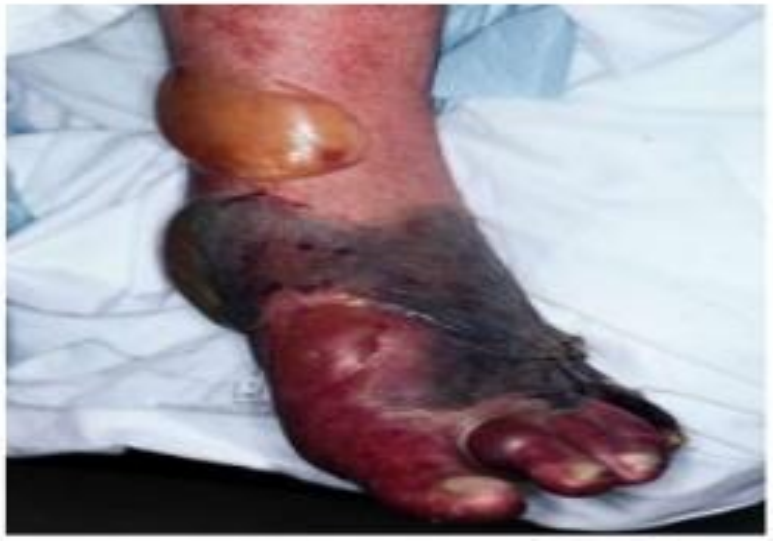
-GG is rarely infection of single clostridium; several species found in association with anaerobic streptococci & facultative anaerobes (*E.coli,Stap,Proteus*)

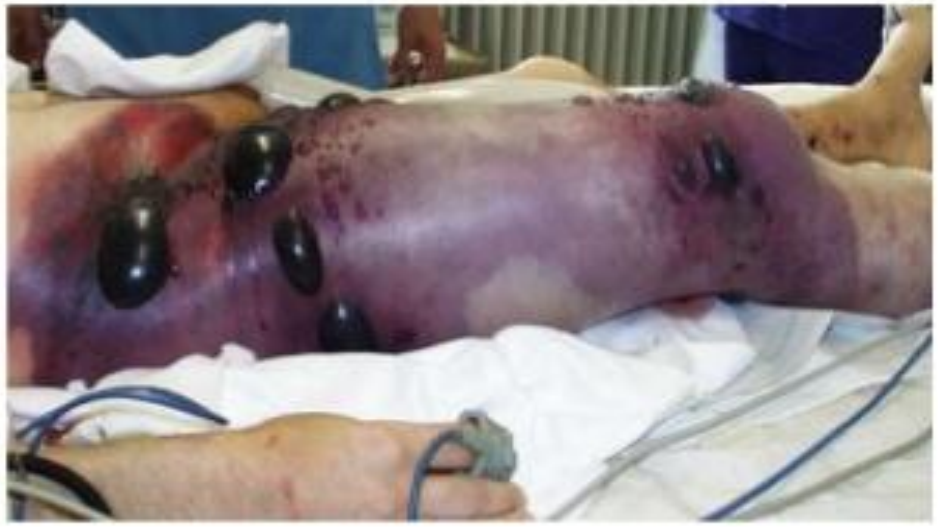
-Among pathogenic clostridiae,*Cl.perfringens* is most frequently encountered(60%) followed by *Cl.Novyi* & *Cl.septicum*(20-40%).

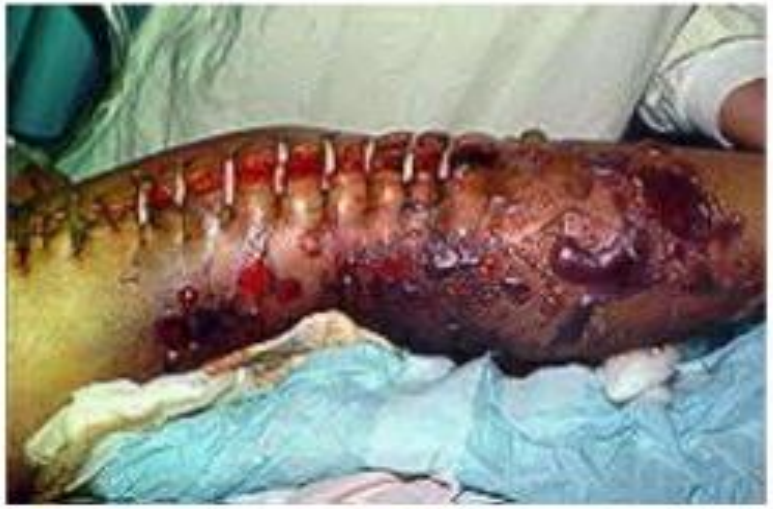

Spores germinate
vegetative cells multiply
Carbohydrates Fermentation
Gas production In tissues
Distension of tissues
Interfering Blood supply Ischemia/ gangrene
Toxemia and death
Anaerobic environment
PATHOGENESIS
Incubation period is
1-7 days

Gas gangrene:









OTHER INFECTIONS:

-**Food poisoning**: usually caused by **Type A** strains

-**Gangrenous appendicitis:** *Cl.perfringens* **Type A**
& occasionally by **Type D**

-**Necrotizing enteritis**: caused by **Type C** strains

-**Biliary tract infection**: Rare but serious -EC & PCS

-**Endogenous gas gangrene of intra-abdominal origin**

-**Brain abscess & meningitis:** Rare

-**Panophthalmitis:** Rare

-**Thoracic infections**

-**Urogenital infections-** usually follow UT surgery

Necrotizing Enteritis

- Rare, acute necrotizing process in the jejunum
- Abdominal pain, bloody diarrhea, shock, and peritonitis
- Mortality: 50%
- Beta-toxin-producing *C. perfringens* type C

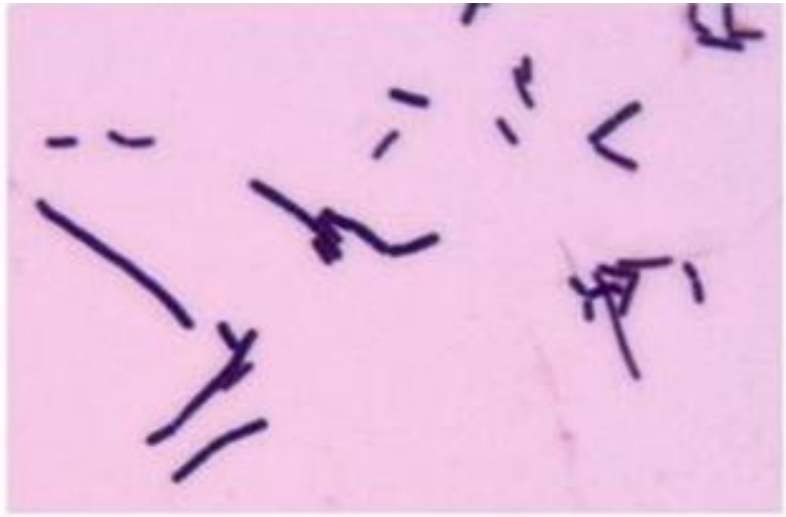
Septicemia

Other causative agents of gas gangrene:

- ***Clostridium novyi* -**
- ***Clostridium septicum* -**
- ***Clostridium histolyticum* -**
- ***Clostridium sordellii* -**

Clostridium novyi
morpho-biological characteristics:

- Gram-positive is a large, straight or slightly bent rod-shaped bacterium measuring 4-22x1-2 μm.
- Unlike C.perfiringens, it is motile, has peritrix flagella, does not form a capsule.
- Spores are located in the subterminal position.



C.novyi

Clostridium novyi
cultural characteristics:

- Obligate anaerobic, very sensitive to oxygen.
- It develops in anaerobic conditions at 37-43ºC, pH 7.4-7.6 in casein, carbohydrate, meat-peptone environments.
- On blood-sugar agar, it forms semi-transparent colonies with granular and fringed edges.
- In the depth of the sugar agar column, it forms colonies with a compact center similar to cotton balls or snowflakes.
- Most strains produce hemolysis on blood agar.
- When it develops in liquid nutrient media (Kitt-Tarotsi, Wilson-Blair), turbidity is observed along with the formation of gas, and then sedimentation.

Clostridium novyi
Blood agar

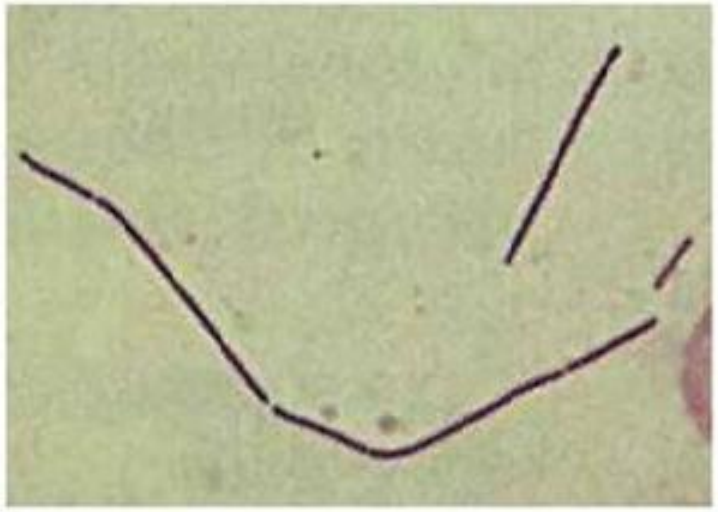
Clostridium novyi
antigen structure and virulence factors:

- According to the antigenic properties of the exotoxins it synthesizes, it is divided into serotypes A, B, C and D.
- C.novyi secretes a toxin with a complex composition and broad biological activity. The toxin consists of 8 factors with lethal, necrotic and hemolytic effects. They are denoted by Greek letters alpha (α), beta (β), gamma (γ), delta (δ), epsilon (ε), and mi (μ).
- Toxins lead to the formation of jelly-like edema by increasing the permeability of blood vessels.

Clostridium septicum

morpho-biological characteristics:

- It is a Gram positive 3-4x1.0-1.5 μm polymorphic rod-shaped bacterium. Sometimes the forms of filomentus up to 50 μm in length are found.
- Unlike C.perfiringens, it is motile and does not form a capsule.
- Spores are subterminal, sometimes central.



C. septicum

Clostridium septicum

cultural characteristics:

- It is obligate anaerobic.
- It grows under anaerobic conditions at 37-43ºC, pH 7.4-7.6 in casein and meat-peptone media supplemented with 0.5% glucose.
- On glucose-blood agar, it forms colonies surrounded by a delicate zone of hemolysis, reminiscent of "woven threads".
- In the depth of the sugar agar column, they form colonies with a compact center with a fringe around the edges.
- When it develops in liquid nutrient media (Kitt-Tarozzi, Wilson-Blair), turbidity is observed along with the formation of gas, and then sedimentation.

Clostridium septicum
glucose-blood agar

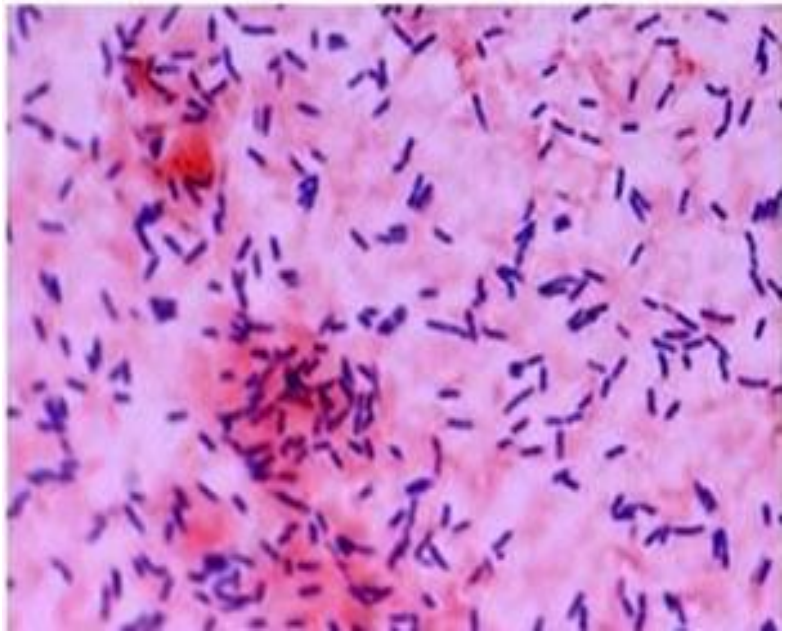
Clostridium septicum

virulence factors:

- *C.septicum* secretes 4 exotoxins with lethal, necrotic and hemolytic effects, denoted by Greek letters:
- α -toxin (lecithinase)
- β-toxin (DNA-ase)
- γ-toxin (hyaluronidase)
- δ-toxin (oxygen-sensitive hemolysin)

Clostridium histolyticum - "tissue-dissolving bacillus" morpho-biological characteristics:

- It is a Gram-positive rod-shaped bacterium measuring 2-3x0.5-1.0 μm. They are found in pairs or in chains.
- Unlike *C.perfiringens*, it is motile and does not form a capsule.
- Spores are located in the subterminal position.



Clostridium histolyticum

Clostridium histolyticum
cultural characteristics:

- It is an aerotolerant anaerobe.
- It develops in anaerobic conditions in casen and meat-peptone environments.
- On blood agar, it forms transparent, convex, shiny colonies with a diameter of 0.5-1 mm, surrounded by a delicate zone of hemolysis.
- In the depth of the sugar agar column, it forms pea-like or fringed colonies with a compact center.
- As a result of proteolysis of pieces of meat and liver in the Kitt-Tarozzi medium, it causes complete turbidity of the medium.

Clostridium histolyticum
Blood agar

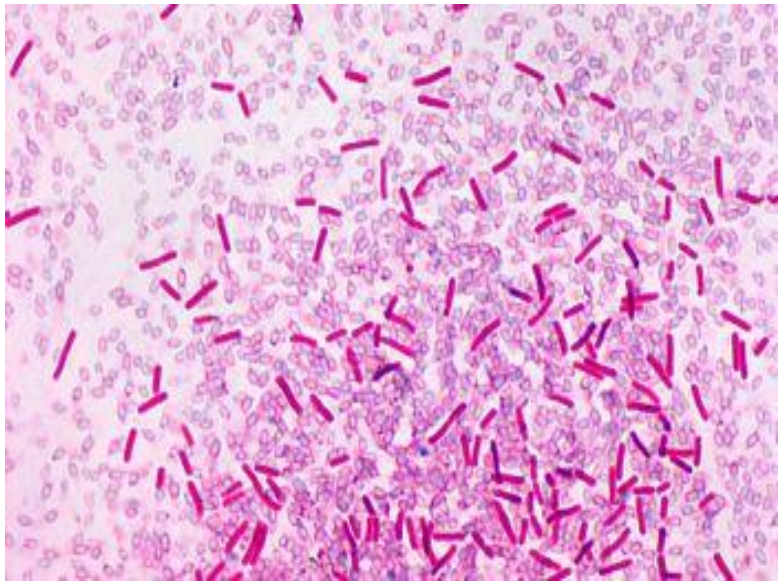
Clostridium histolyticum

virulence factors:

- *C.histolyticum* synthesizes a lethal, necrotic and hemolytic toxin with high biological activity:
- α -toxin (lecithinase)
- β-toxin (collagenase)
- γ-toxin (proteinase)
- δ-toxin (elastase)
- ε-toxin (oxygen-sensitive hemolysin, similar to streptococcal O-streptolysin)

Clostridium sordellii
morpho-biological and cultural characteristics: :

- It is a Gram positive 2-4x0.6-1.0 μm, motile rod-shaped bacterium.
- It is a facultative anaerob.
- After 1-2 days of incubation in blood-sugar agar, they form convex, grayish colonies with rough edges.
- Causes hemolysis in blood agar.
- Development in liquid nutrient media (Kitt-Tarozzi, Wilson-Blair) is accompanied by the formation of mucus.



Clostridium sordellii

LABORATORY DIAGNOSIS

A. Hematological investigation: Not significant

B. Bacteriological Investigation:

Specimen: Wound swabs,necrosed tissue, muscle fragments, exudates from active parts etc.

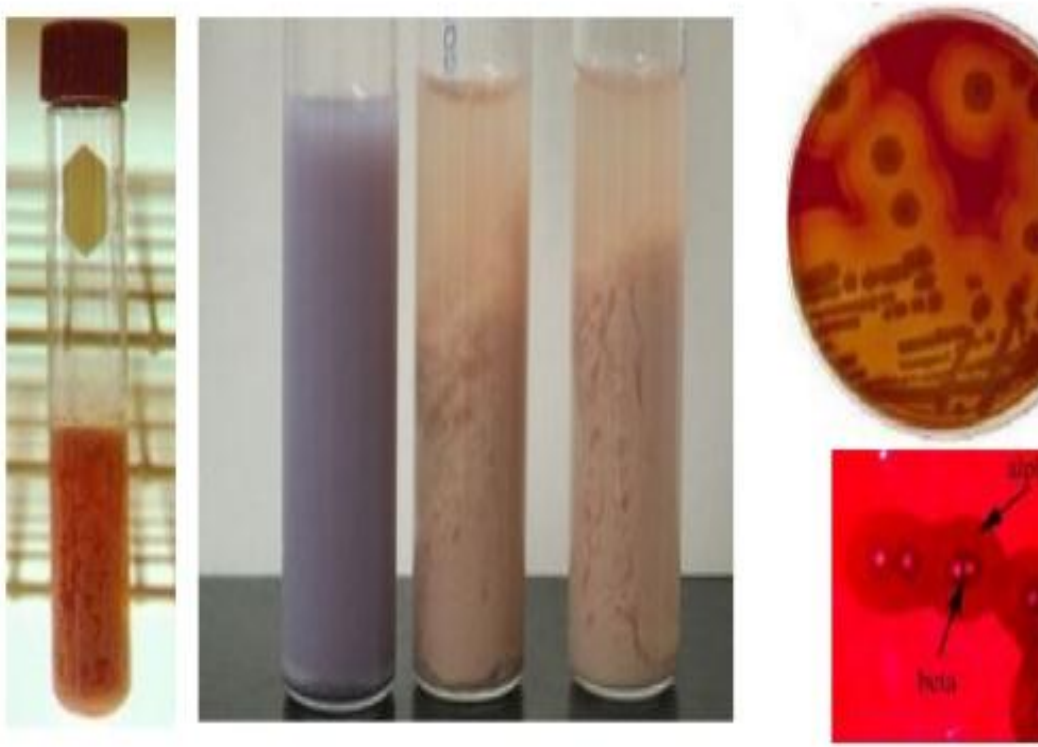
1.Microscopy: Gram +ve, non-motile, capsulated bacilli.

-Spores are rarely observed in *Cl.perfringens*

2.Culture:

- **On RCM**→ meat turned pink but not digested
- **On blood agar** → target hemolysis

3. Biochemical reactions: As discussed above



Left to right:

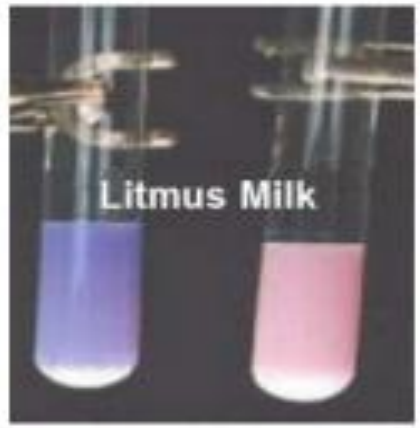
a.RCM: Meat turned pink but not digested

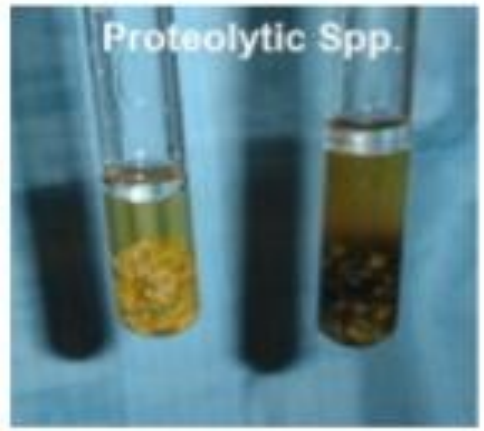
b.Litmus Milk: Stormy fermentation & acid clot in Litmus

c.BAM: Target hemolysis

CULTURAL CHARACTERISTICS

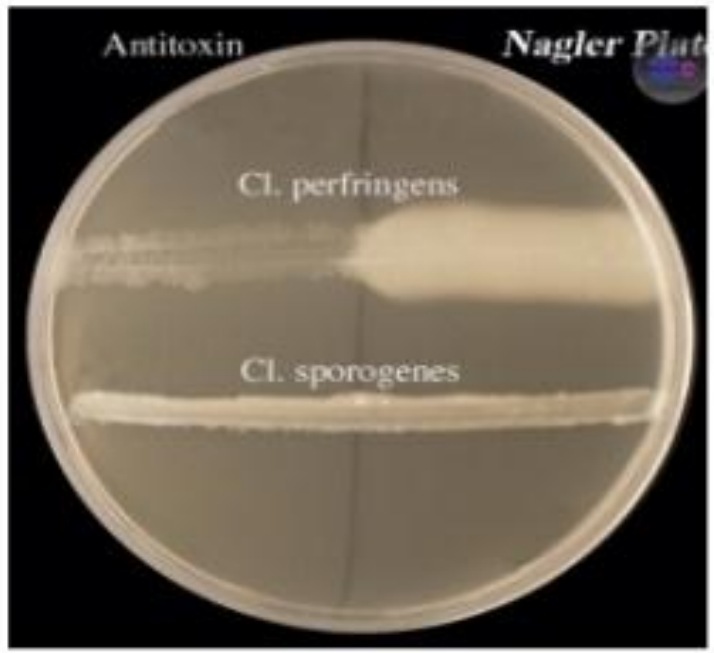
- Clostridia are anaerobic.
- Optimum temp. for growth is 37°C; pH 7-7.4.
- Robertson's cooked meat broth is useful medium.
- Most species produce gas in this medium
- Saccharolytic species turn meat pink.
- Proteolytic species turn meat black with foul smell.
- Robertson's cooked meat broth is ideal; meat is turned pink but not digested with sour odor.
- Stormy fermentation of lactose in litmus milk; the acid coagulates casein-acid clot.
- On BAM: Target haemolysis

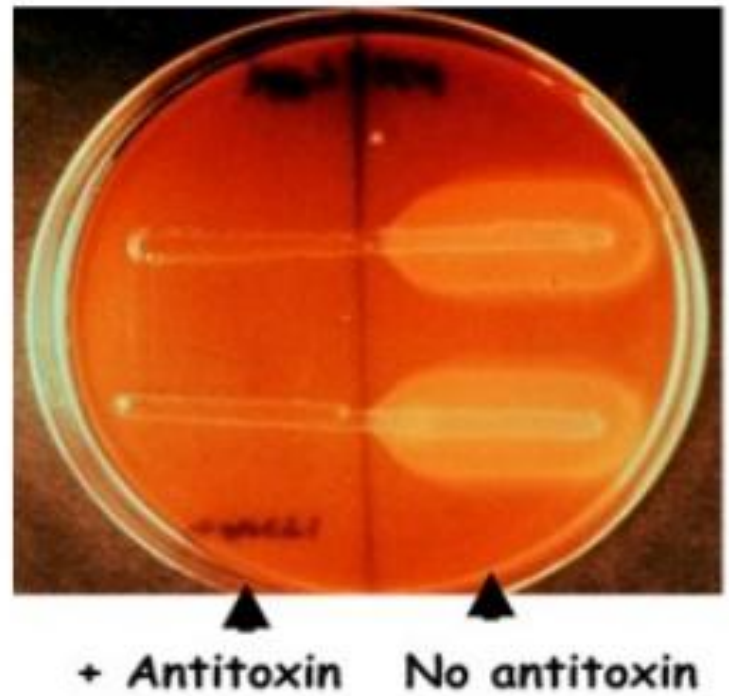




4. Nagler's Reaction

- Rapid detection of *Cl. perfringens* from clinical sample
- Done to detect the lecithinase activity of alpha toxin
- Characteristics opalescence is produced around colonies in +ve test due to breakdown of lipoprotein complex in the medium





PROPHYLAXIS & TREATMENT

1.Surgery: All damaged tissue should be removed, wounds irrigated to remove clots, necrotic tissue & foreign materials, **excision** of affected parts in EGG.

2.Antibiotics: Metronidazole given **intravenously** before surgery & repeated **8 hourly for 24 hrs**.

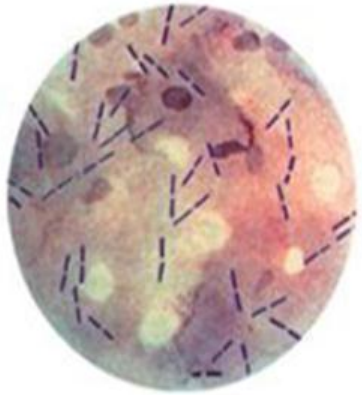
-Broad spectrum antibiotics in combinations (like metronidazole+gentamycin+amoxicillin)are effective.

3.Antitoxins: Passive immunization with **AGS**

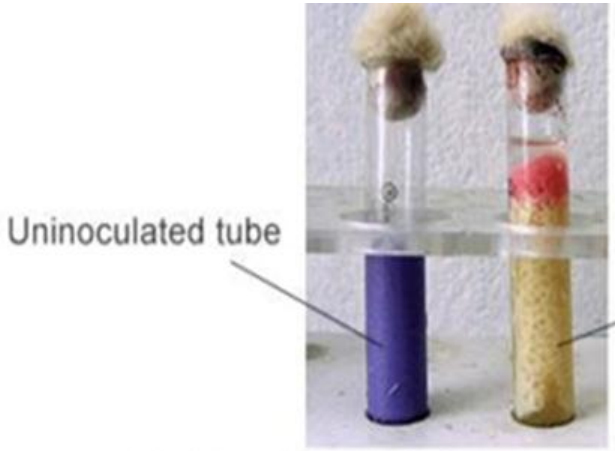
3 doses- **1 intravenous** dose followed by

2 intramuscular doses at **6hrs**. interval

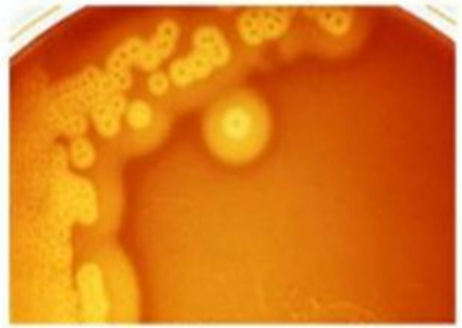
Clostridium Perfringens



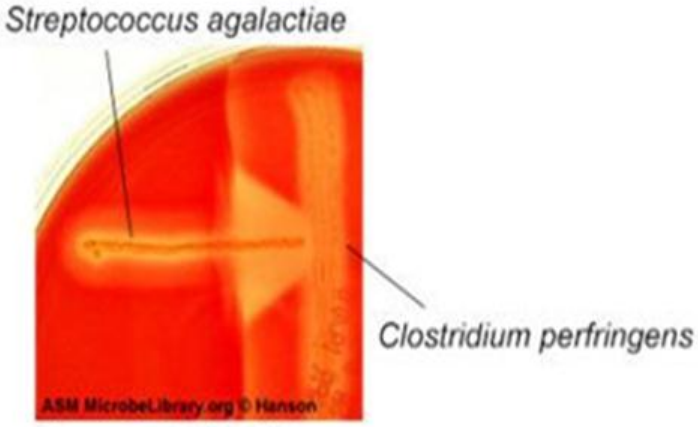
Gram-positive
'boxcar shaped' bacilli



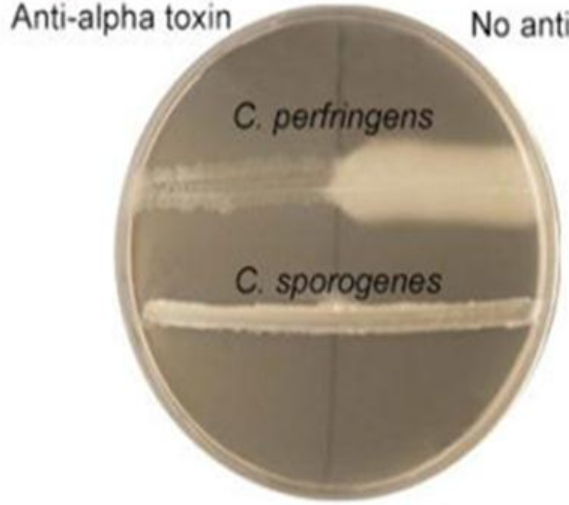
Litmus milk test with 'stormy clot reaction'



Target hemolysis
(double zone of hemolysis)



Reverse CAMP test

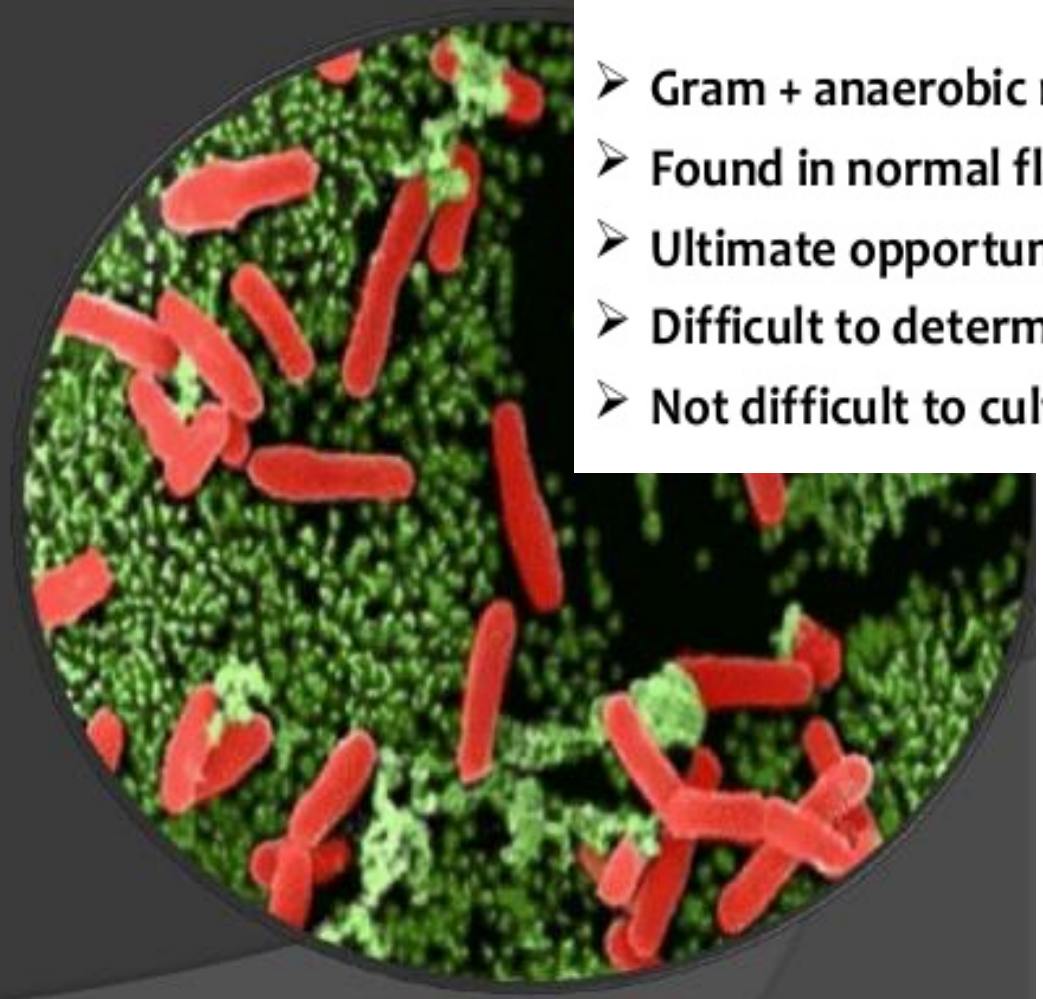


Nagler Test

Gas gangrene

Clostridium difficile

Clostridium difficile (Greek *kloster* (κλωστήρ), spindle, and Latin *difficile* difficult), also known as "CDF/cdf", or "C. diff", is a species of Gram-positive bacteria of the genus *Clostridium* that causes diarrhea and other intestinal disease when competing bacteria are wiped out by antibiotics.

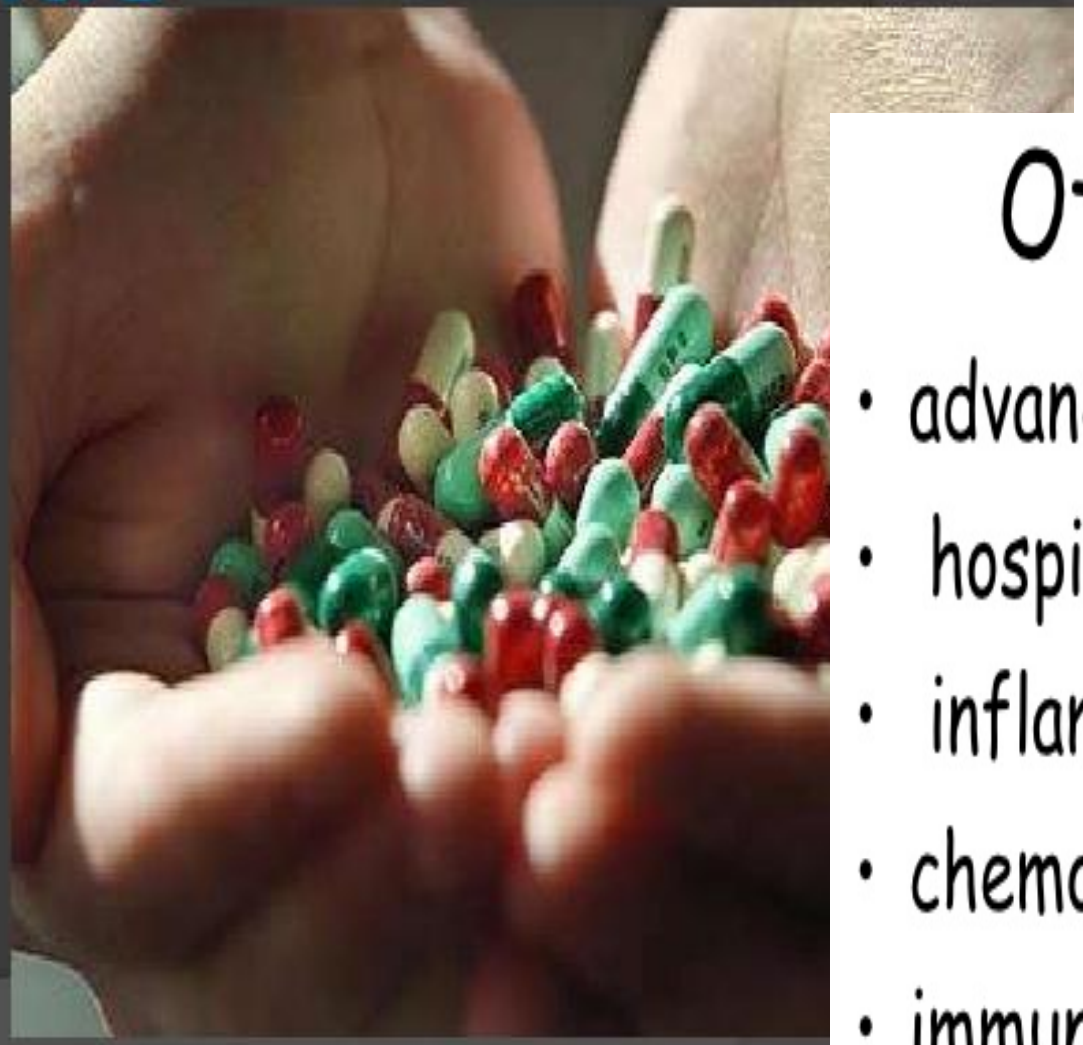


C. difficile

- Gram + anaerobic rod.
- Found in normal flora
- Ultimate opportunistic pathogen
- Difficult to determine cause as the organism is ubiquitou
- Not difficult to culture

Several Antibiotics cause pseudomembraneous colitis

Nearly all antibiotics can cause antibiotic-associated diarrhea, colitis or pseudomembraneous colitis. The antibiotics most commonly linked to antibiotic-associated diarrhea :



Other risk factors

- advanced age,
- hospitalization,
- inflammatory bowel disease,
- chemotherapy, and
- immunosuppression.

Pathophysiology

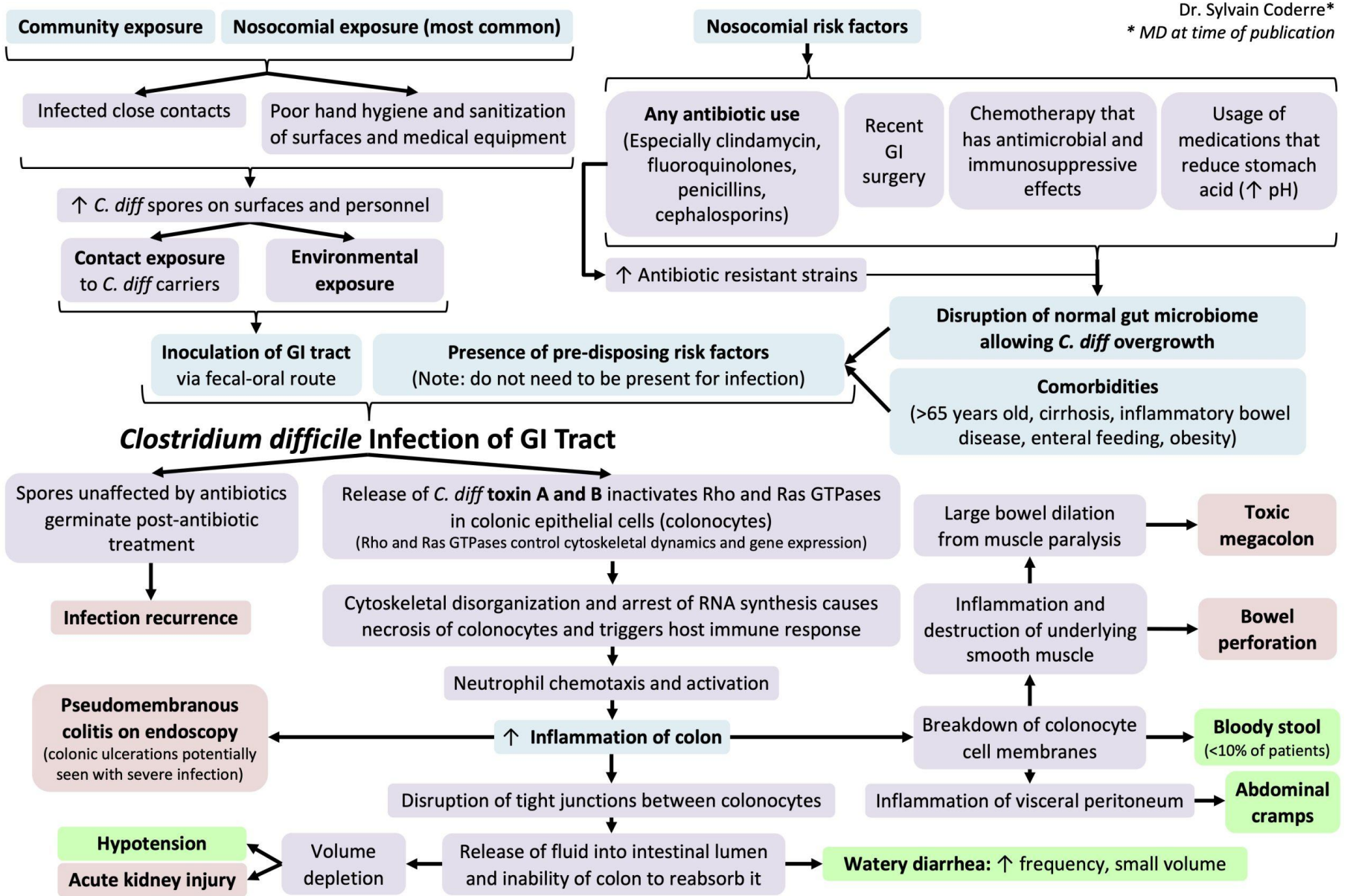
- disruption of the normal bacterial flora of the colon
- colonization with *C. difficile*
- release of toxins
- mucosal damage and inflammation.

C. difficile Virulence Factors

Virulence Factor	Biologic Activity
Enterotoxin (toxin A)	Produces chemotaxis; induces cytokine production with hypersecretion of fluid; produces hemorrhagic necrosis
Cytotoxin (toxin B)	Induces depolymerization of actin with loss of cellular cytoskeleton
Adhesin factor	Mediates binding to human colonic cells
Hyaluronidase	Produces hydrolytic activity
Spore formation	Permits organism's survival for months in hospital environment

Clostridium difficile (C. diff) Infection

Authors: Ryan Brenneis, Sravya Kakumanu
Reviewers: Yoyo Chan, Sean Doherty, Vina Fan, Ben Campbell, Dr. Steve Vaughan*, Dr. Sylvain Coderre*
** MD at time of publication*



Legend: Pathophysiology | Mechanism | Sign/Symptom/Lab Finding | Complications

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C. difficile Diagnosis

- ✓ Cytotoxin - stool
- ✓ Culture
- ✓ *C. difficile* antigen -- latex agglutination

Culture: standard test for Clostridia include: indole, sugars, lecithinase, catalase (usually neg.)

C. difficile: Latex agglutination

Stool buffered and centrifuged

Drop on slide of stool supernatant

add 1 drop latex detection reagent. Latex particles coated with rabbit antibody to *C. difficile* antigen.

In presence of *C. difficile* clumps can be seen by eye.

Culture

Inoculate

- anaerobe blood agar -- 2-3 days
- egg yolk medium -- 2-3 days

Incubation temp. = 30 C except C. *perfringens*

Inoculate cooked meat medium - (broth with meat particles)

Heat to destroy vegetative cells

Alcohol spore selection for heat labile spores.

Clinical syndromes: most serious is Pseudomembranous colitis (PMC)

Pseudomembranous colitis brought about by destruction of the other indigenous intestinal flora.

Ranges from mild to serious.

PMC is self-limiting.

Treatment

- Stop antibiotic causing disease
- ✓ Metronidazole, Vancomycin
- Relapses due to resistant spores.
- ✓ retreatment with same antibiotic
- neutralization with specific antitoxin obtained commercially
- ✓ amount of toxin present can be determined by a dilution series of the stool sample.

ANAEROBIC GRAM NEGATIVE BACILLI

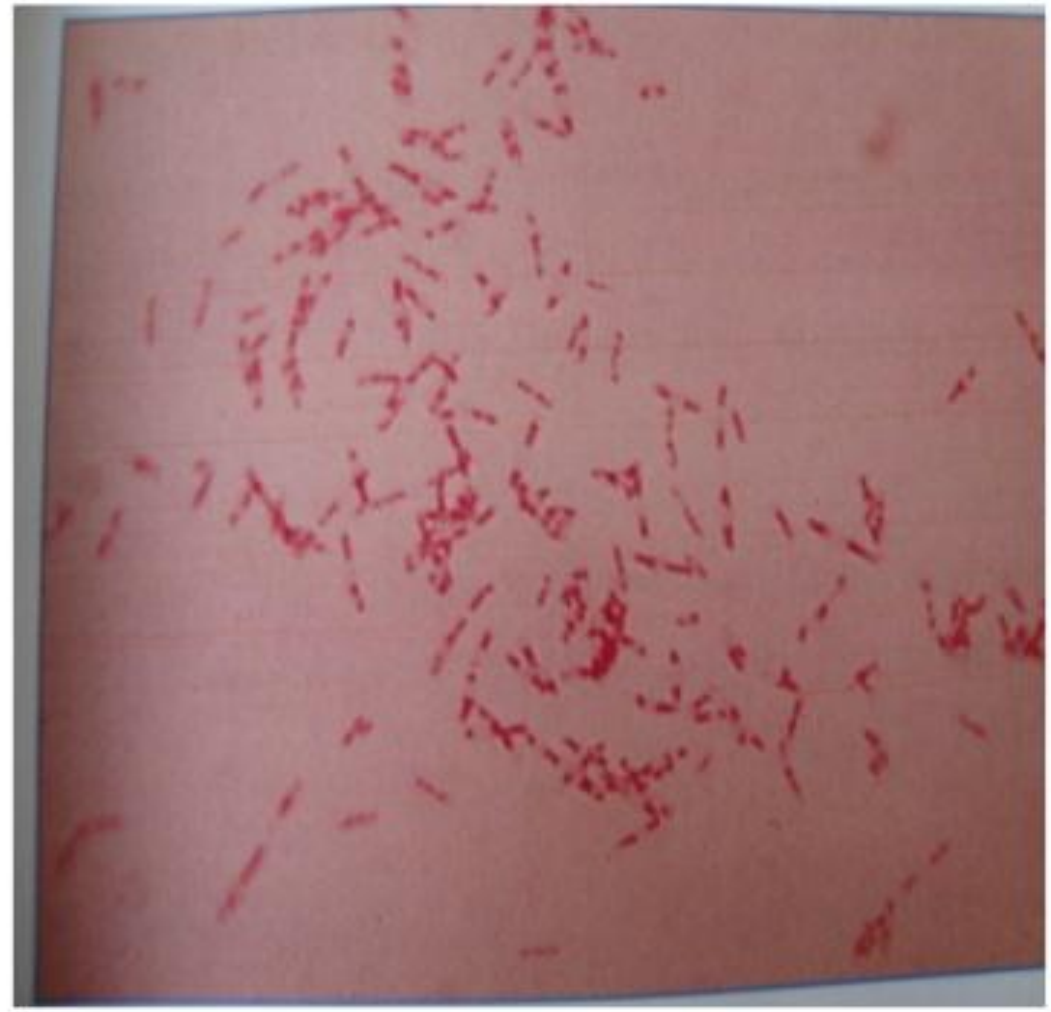
- Bacteroides (most common)
- Prevotella
- Porphyromonas
- Fusobacterium
- Bilophila

All non motile

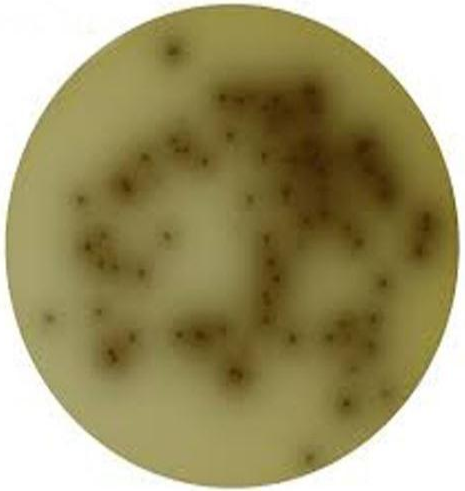
1. Bacteroides group

- B. fragilis (most con
- B.thetaiotaomicron

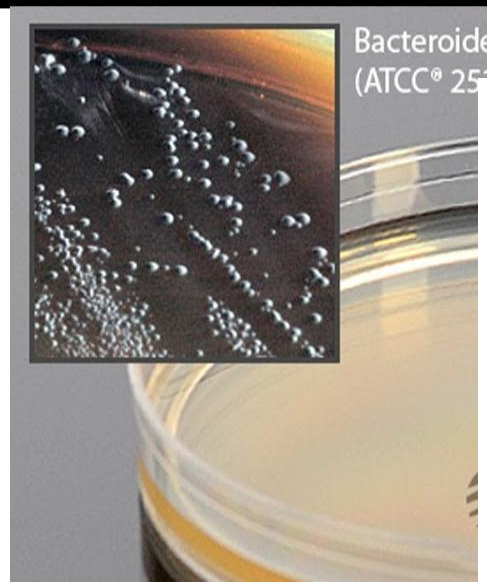
- Gram negative bacilli with **rounded ends**
- 1.5-9 µm vs 0.5-0.8 µm
- Nonmotile
- Broth culture : pleomorphic with vacuoles
- Capsules



Bacteroides Bile Esculin Agar



Bacteroides fragilis



BACTEROIDES FRAGILIS GROUP

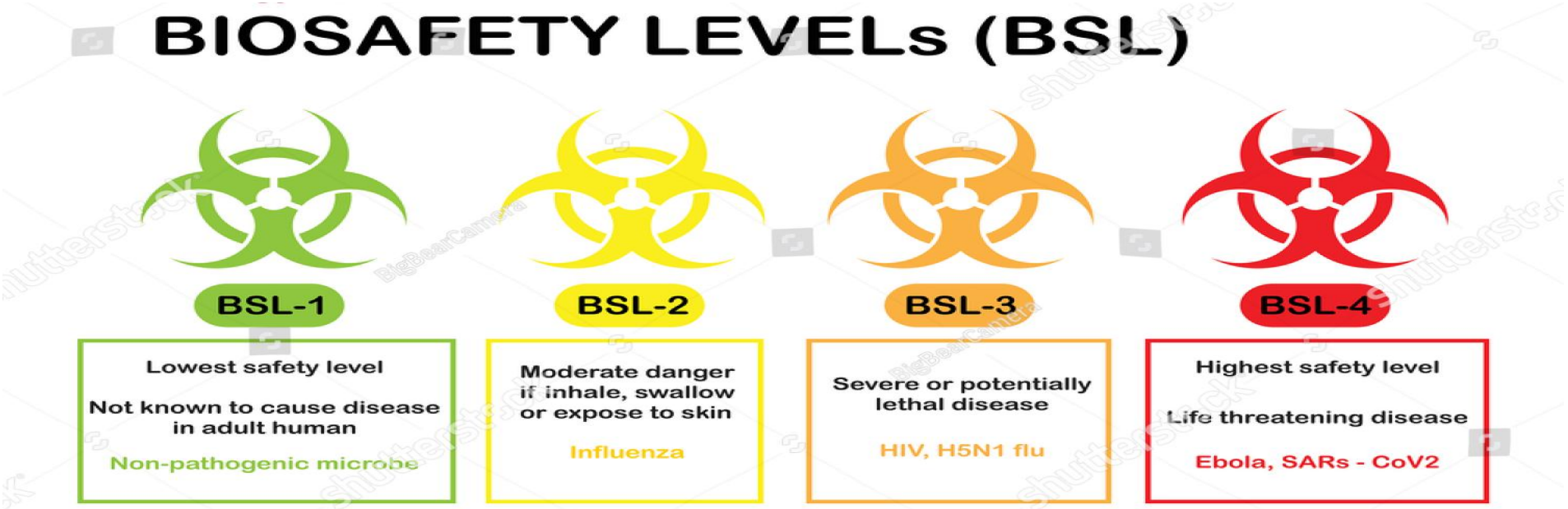
- Non-motile
- On aerobic blood agar, colonies are non-hemolytic and gray with an entire margin and ring-like structures
- Saccharolytic
- Extremely virulent and can cause widespread tissue destruction
- Has a capsule and also produces several enzymes
- No pigment or spore formation

Zoonotic infections and their characteristics

- ***Zoonotic infections*** are a group of infectious and parasitic diseases whose causative agents are transmitted from animals to humans. The causative agents of zoonoses are protozoa, viruses, bacteria, fungi, helminths, parasitic mites.
- Zoonoses such as brucellosis, anthrax, listeriosis, plague, and tularemia are transmitted from sick animals to humans in a variety of ways. Humans have a non-specific host for the causative agents of zoonoses. The human body becomes a biological dead end for these pathogens and cannot be a reservoir.

Understanding of particularly dangerous infections, rules of working with their causative agents:

- Particularly dangerous infections are infectious diseases with a high epidemiological risk. Such infections have a severe clinical course and high lethality. These include cholera, plague, tularemia, anthrax, brucellosis, yellow fever, hemorrhagic fevers (Ebola, Marburg, Lassa, etc.).
- Examination of the causative agents of particularly dangerous infections is carried out in a special regime laboratory with strict adherence to safety rules.



High Risk Microbes

Low Risk Microbes

BSL-4

Dangerous and exotic, posing a high risk of aerosol-transmitted infections. Infections caused by these microbes are frequently fatal and without treatment or vaccines.

Examples: Ebola virus, smallpox virus

BSL-3

Microbes there can either indigenous or exotic, and they can cause serious or potentially lethal disease through respiratory transmission.

Examples: *Yersinia pestis* (plague), *Mycobacterium tuberculosis*, SARS, rabies virus, West Nile Virus, hantaviruses

BSL-2

Moderate potential hazard to personnel and the environment. Includes bacteria and viruses that cause mild disease to humans, or are difficult to contract via aerosol in a lab setting.

Examples: Hepatitis A virus, *Streptococcus pyogenes*, *Borrelia burgdorferi* (Lyme disease), *Salmonella* species

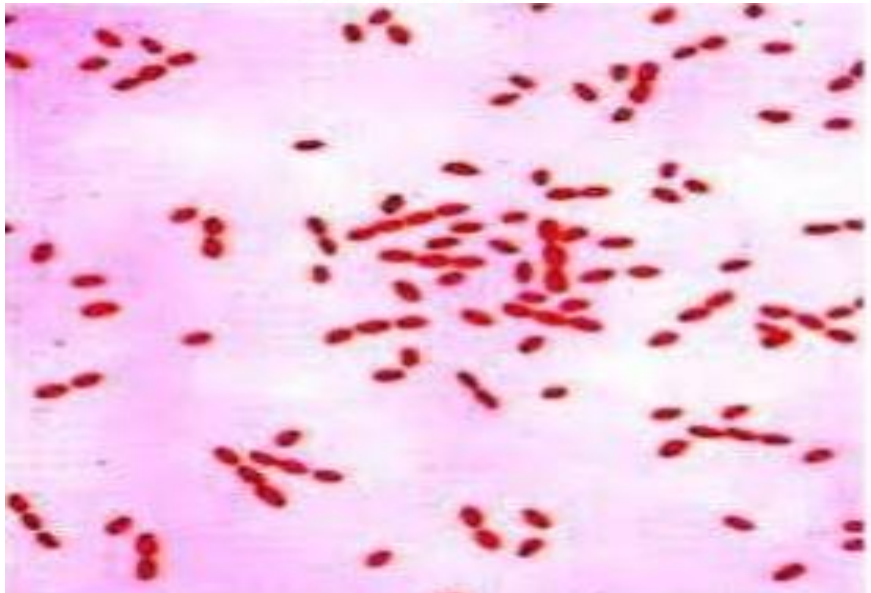
BSL-1

Not known to consistently cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment. **Examples: *Saccharomyces cerevisiae*, *E. coli* K-12, and non-infectious bacteria**

Brucellaceae - Taxonomy

- (Domain): Bacteriae
- (Kingdom): Pseudomanadota
- (Class): Alphaproteobacteria
- (Order): Hyphomicrobiales
- (Family): Brucellaceae
- (Genus): Brucella

- Brucella belongs to family Brucellaceae.
- Genus Brucella encompasses 9 recognized spp—6 terrestrial sp. & 3 marine spp.
- Terrestrial sp. are **B.melitensis, B.abortus, B.suis, B.canis, B. ovis, B.neotamae.**
- Marine spp are **B.delphini, B. pinnipediae, B. cetaceae.**



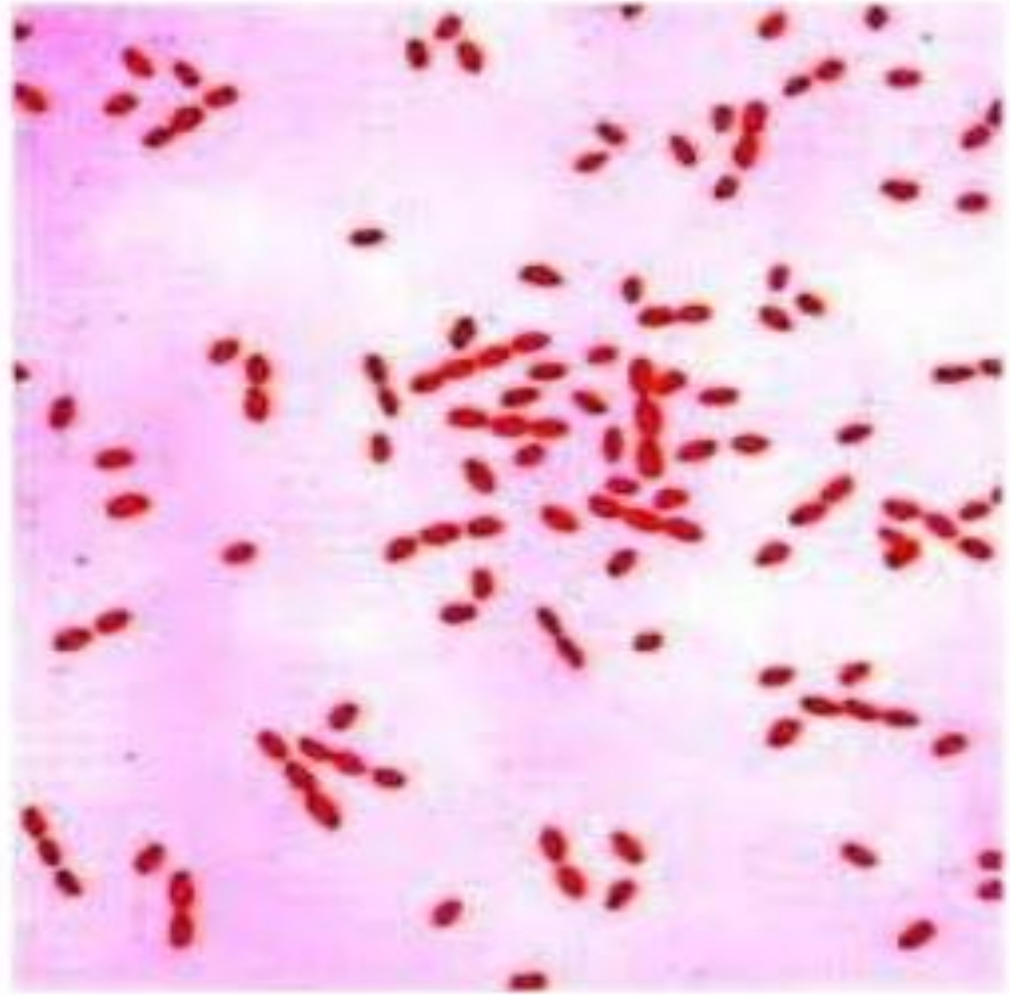
Species	Natural Host	Human Pathogen
B. abortus	cattle	yes
B.melitensis	goats, sheep	yes
B. suis	swine	yes
	hares	yes
	reindeer	yes
	rodents	yes
B. canis	dogs, other canids	yes
B. ovis	sheep	no
B. neotomae	desert wood rat	no
B. pinnipediae	otter , seal	?
B. cetaceae	dolphin , porpoise	?

INTRODUCTION

- The genus *Brucella* consists of Gram-negative coccobacilli, They are strict **intracellular** parasites of animals and may also infect humans.
- Brucellosis is a zoonotic disease, primarily affecting goats, sheep, cattle, buffaloes, pigs and other animals and transmitted to humans by contact with infected animals or through ingestion of their products.
- The human diseases with various names: **Mediterranean fever, Malta fever, undulant fever/remittent fever, Gibraltar fever, Cyprus fever**.
- The diseases caused by members of this genus are characterized by a number of names based on the original microbiologists who isolated and described the organisms

MORPHOLOGY

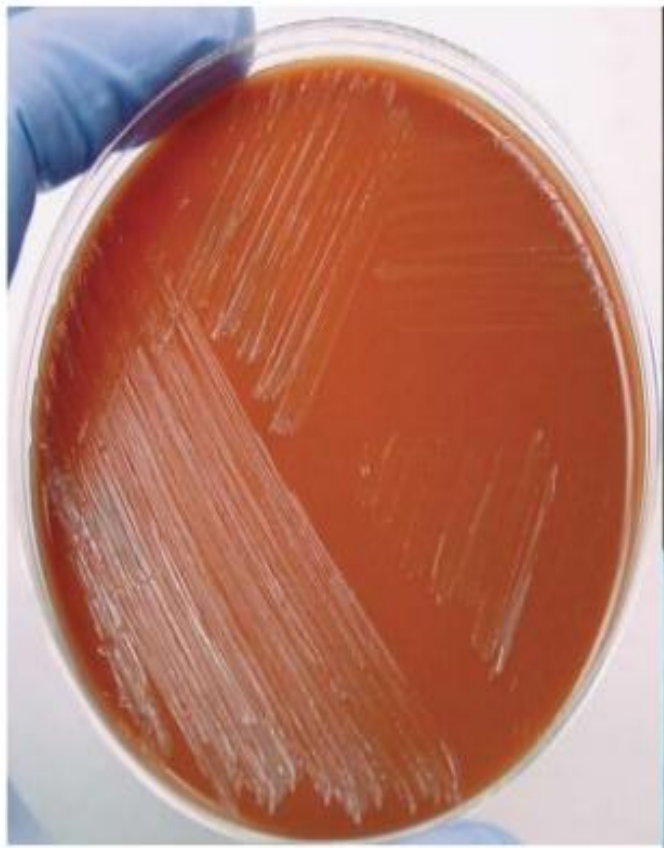
- Brucellae species are small, gram-negative aerobic coccobacilli, 0.5-0.7 µm x 0.6-1.5 µm in size.
- They are nonmotile, noncapsulated, nonsporing and non-acid fast.



CULTURAL CHARACTERISTICS

- Brucellae are strict aerobes.
- *Br. Abortus* is capnophilic, many strains requiring 5-10% C02 for growth.
- Optimum temperature is 37°C (range 20-40 °C) and pH 6.6-7.4.
- Grow on simple media, though growth is slow and scanty.
- Growth is improved by the addition of serum or liver extract.
- The media employed currently are serum dextrose agar, serum potato infusion agar, trypticase soy agar, or tryptose agar.

CULTURE CHARACTERISTICS



RESISTANCE

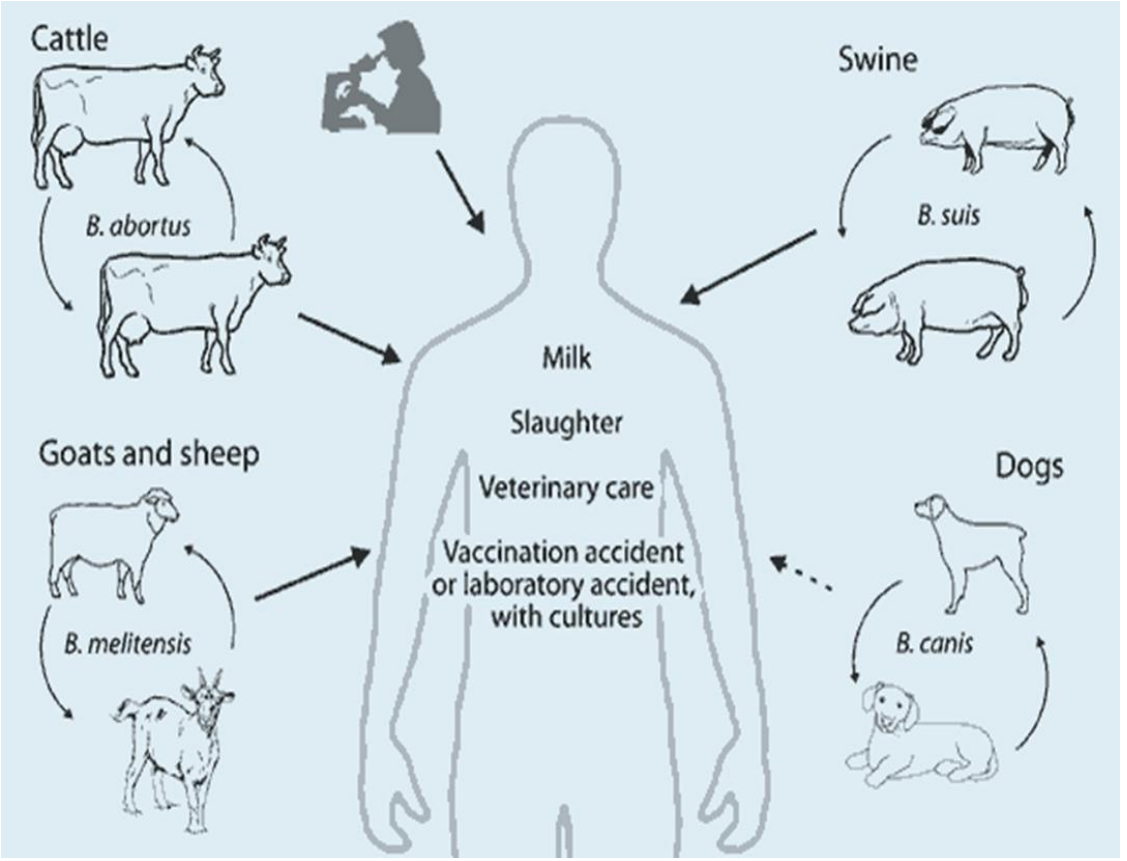
- Brucellae are destroyed
- by heat at 60 °C in 10 minutes
- by 1% phenol in 15 minutes.
- are killed by pasteurization.
- They may survive in soil and manure for several weeks.
- The organism survives for 10 days in refrigerated milk, for months in butter, one month in ice cream.
- They are sensitive to direct sunlight and acid.
- **They are resistant to penicillin but are susceptible to streptomycin, tetracycline, chloramphenicol and ampicillin.**

PORTALS OF ENTRY

1. Oral entry :
   - Ingestion of contaminated animal products (often raw milk or its derivatives).
   - contact with contaminated fingers.
2. Aerosols:
   - Inhalation of bacteria.
   - Contamination of the conjunctivae.
3. Percutaneous infection: through skin abrasions or by accidental inoculation.

EPIDEMIOLOGY

- Exposure to infectious aerosols during manipulation of cultures is one of the most common source of laboratory infection.
- Mainly Farmers, abattoir workers, butchers, veterinarians are at risk.
- Infection can occur through **contamination of conjunctiva and skin** with discharges
- Main source of infection to general population is by **dairy products** prepared **from infected milk.**
- **Neonatal infection** can be acquired by the **transplacental route,** during **delivery** or via the ingestion of **contaminated breast milk**.



PATHOGENESIS

- Intracellular location & survival of the organism contribute to its virulence & pathogenesis.
- All three major species of Brucella are pathogenic to human beings.
- *Br. melitensis* is the most pathogenic, *Br. abortus and Br. suis of* intermediate pathogenic.
- Incubation period is 1-4 weeks.

- **Human infection may be of three types:**
- 1. Latent infection: with only serological but no clinical evidence;
- 2. Acute or sub-acute brucellosis; and
- 3. Chronic brucellosis.

Brucellae

↓

Phagocytized

↓

Resistant to intracellular killing

↓

Evades immune control

↓

Growth in phagocytes

↓

Spreads → spleen, liver, lymph nodes, bone marrow

↓

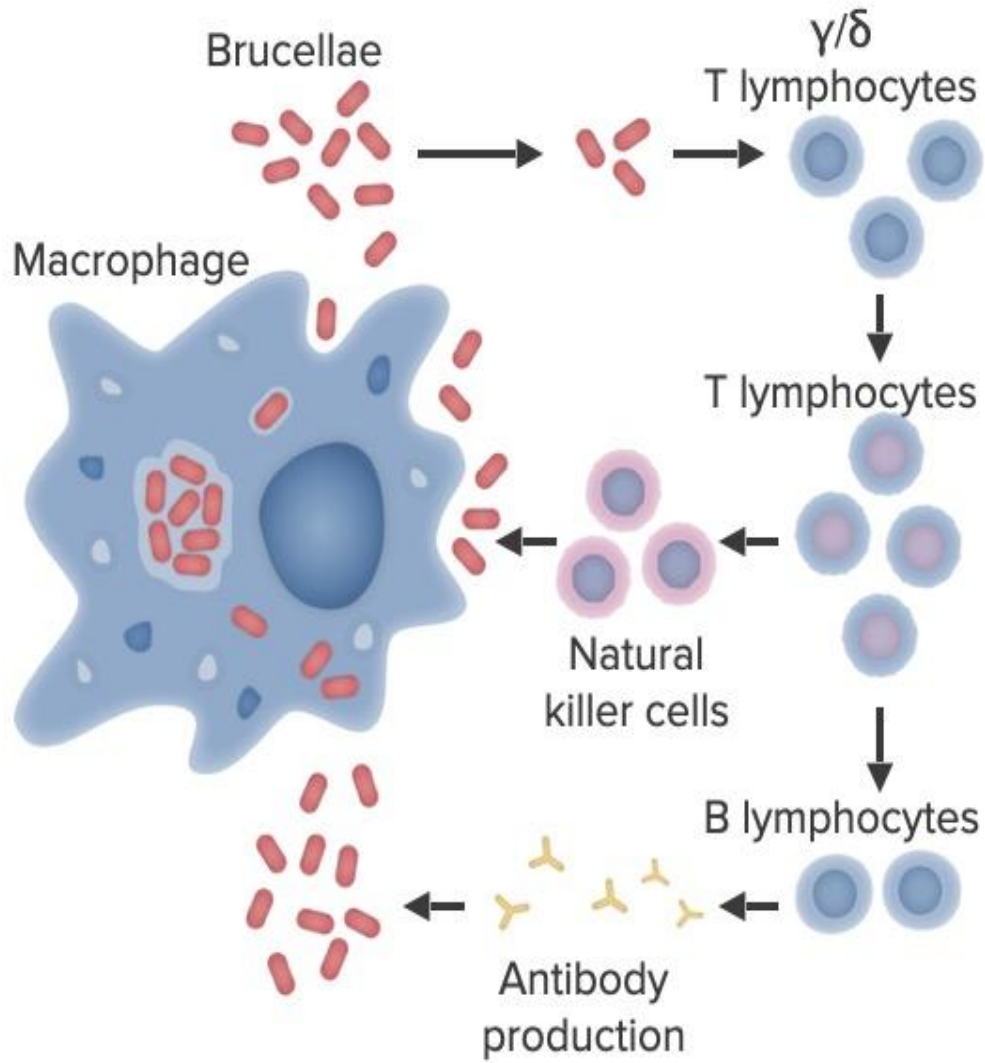
Infection

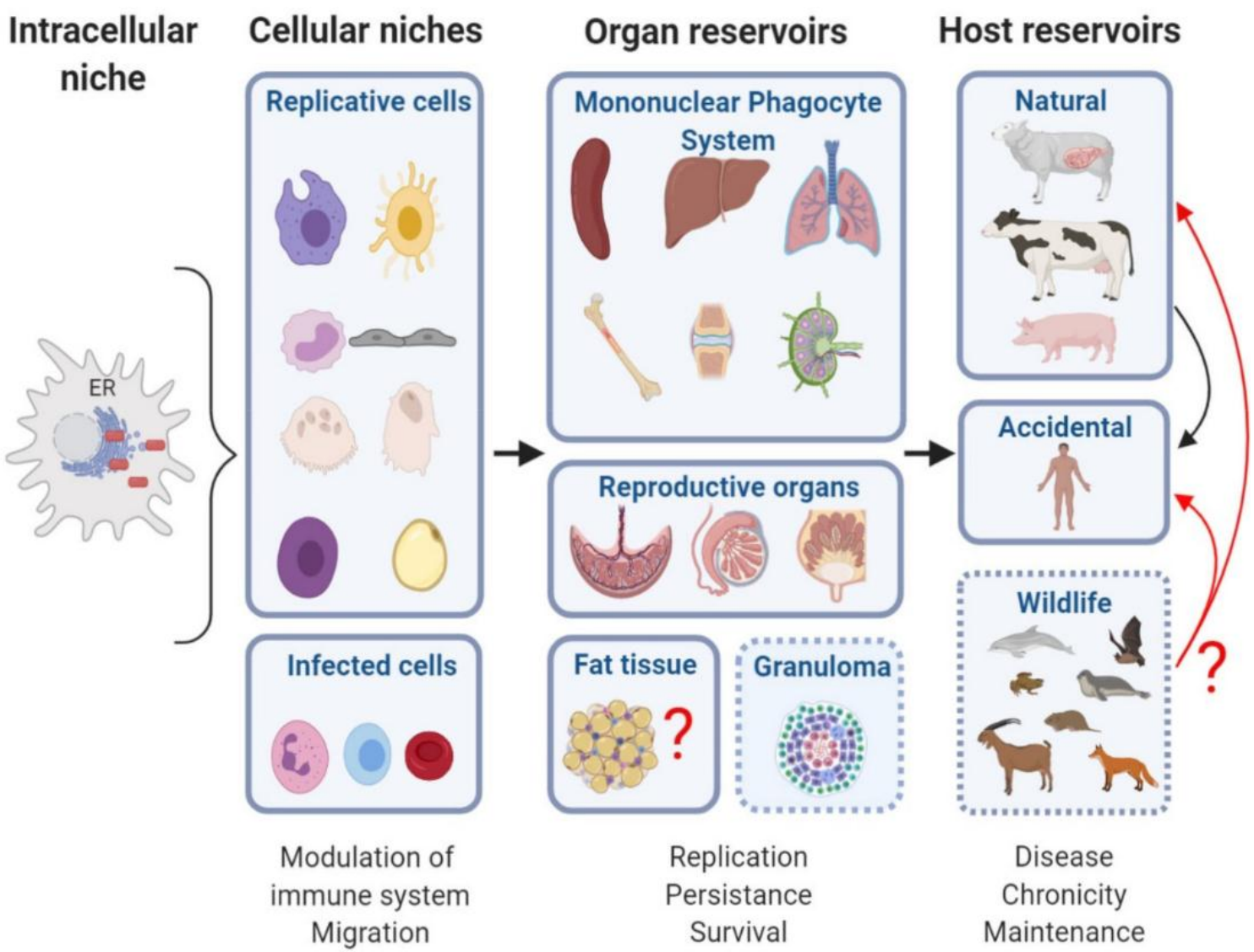
↓

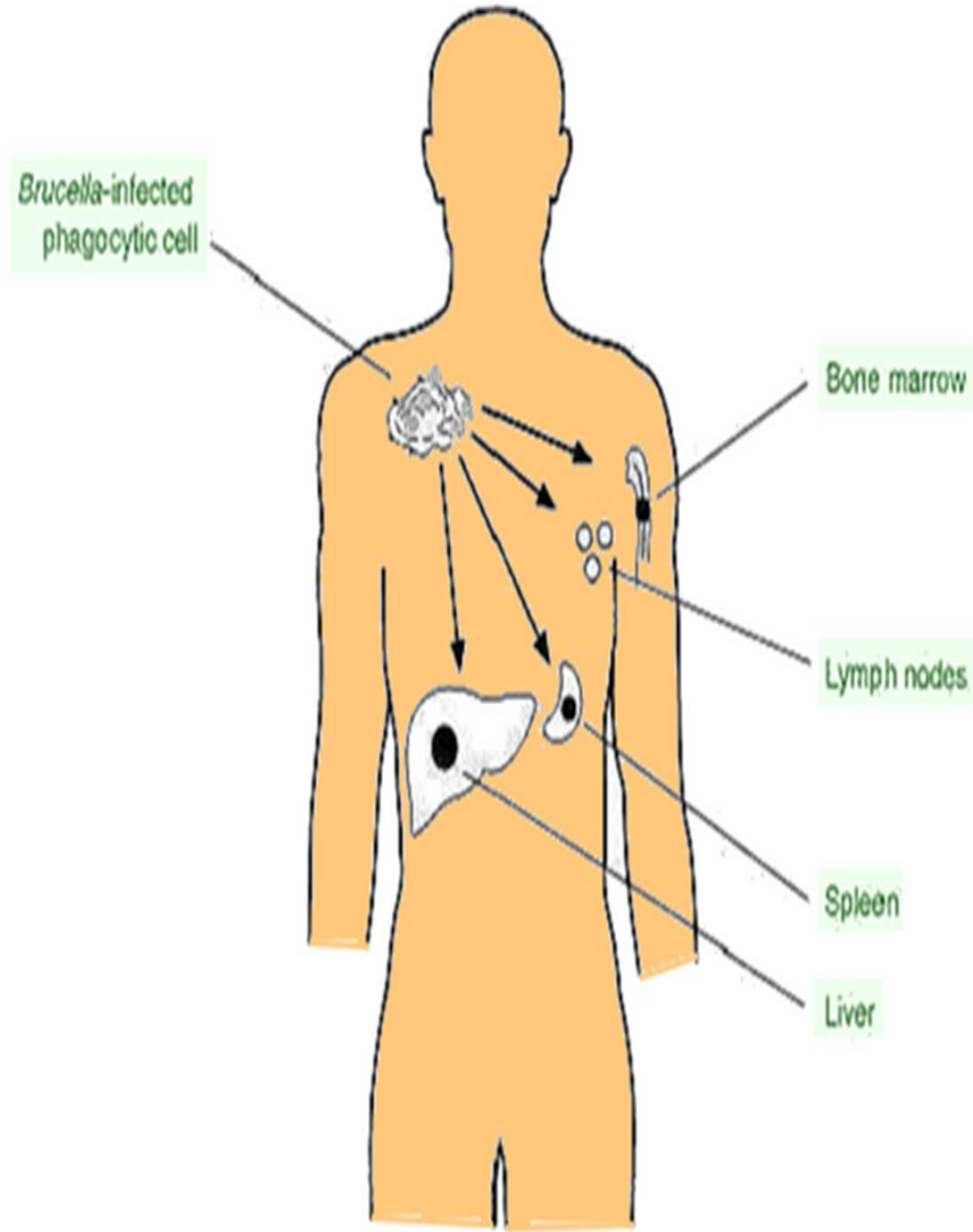
Host TH1 response

↓

Granulomas around infection foci




Intracellular niche
Cellular niches
Organ reservoirs
Host reservoirs
ER
Replicative cells
Infected cells
Mononuclear Phagocyte System
Reproductive organs
Fat tissue
?
Granuloma
Natural
Accidental
Wildlife
?
Modulation of immune system Migration
Replication Persistance Survival
Disease Chronicity Maintenance


Brucella-infected phagocytic cell
Bone marrow
Lymph nodes
Spleen
Liver

ACUTE BRUCELLOSIS

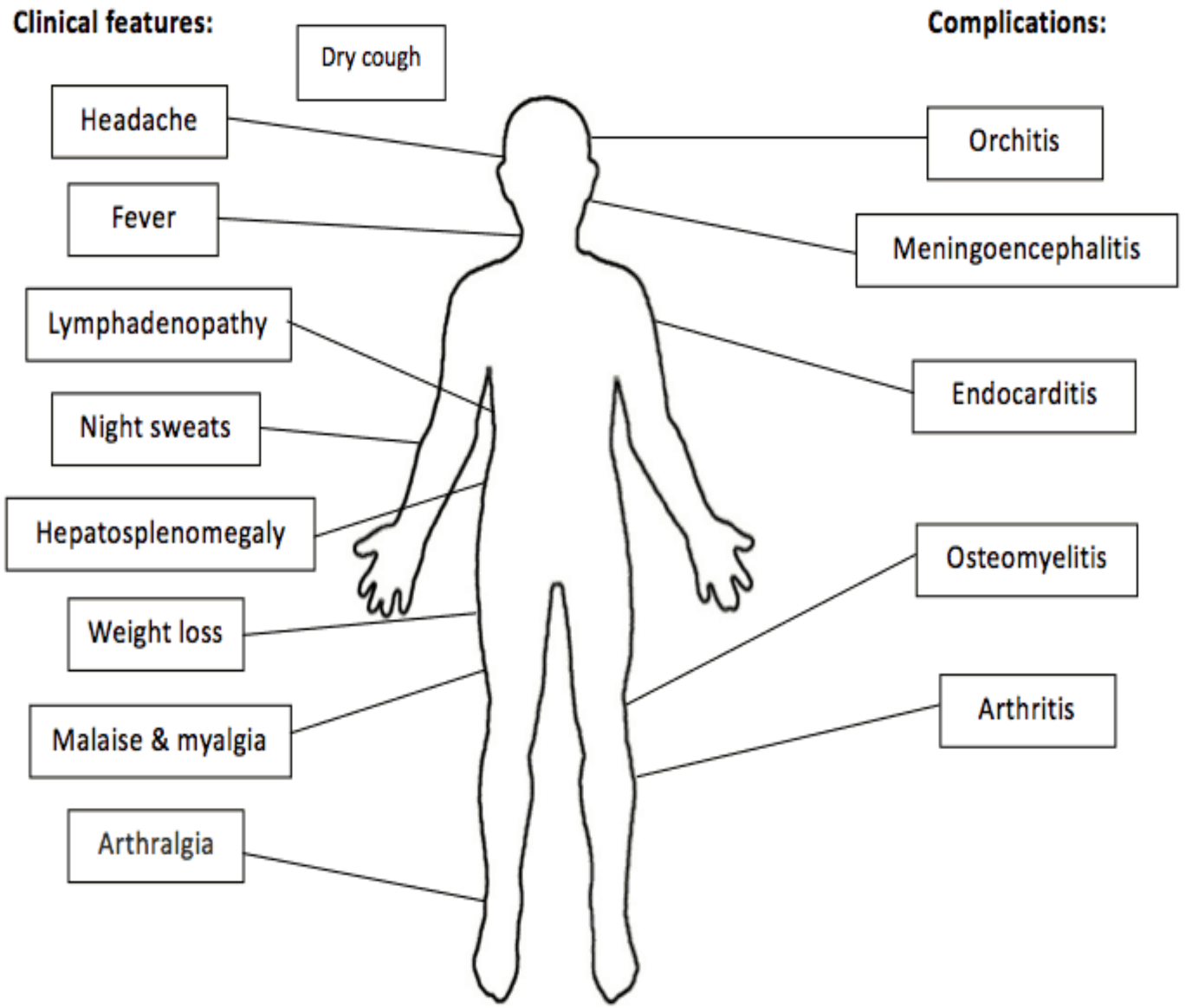
- Acute brucellosis is mostly due to Br melitensis.
- It is usually known as undulant fever, but this is misleading as only some cases show the undulant pattern
- It is associated with prolonged bacteraemia and irregular fever.
- The symptomatology is varied, consisting of muscular and articular pains, asthmatic attacks, nocturnal drenching sweats, exhaustion, anorexia, constipation, nervous irritability and chills.
- The usual complications are articular, osseous, visceral or neurological.
- Sub-acute brucellosis: It may follow acute brucellosis. Blood culture is less frequently positive.

CHRONIC BRUCELLOSIS

- Chronic brucellosis, which may be nonbacteremic, is a low-grade infection with periodic exacerbations.
- The symptoms are generally related to a state of hypersensitivity in the patient.
- Common clinical manifestations are sweating, lassitude and joint pains, with minimal or no pyrexia.
- The illness lasts for years.

CLINICAL MANIFESTATIONS

- Fever
- Night sweats
- Malaise
- Anorexia
- Arthralgia
- Fatigue
- Weight loss
- Depression.



CLINICAL MANIFESTATIONS

- Gastrointastinal tract: anorexia, abd. pain, vomiting, diarrhea,constipation, hepatosplenomegaly.
- LIVER: Involved in most cases but LFTs normal or mildly abnormal.
  - granulomas (B. abortus).
  - hepatitis (B.melitensis).
  - abscesses (B.suis).
- Skeletal:
- Arthritis, spondylitis, osteomyelitis.
- Sacroiliitis.
- Arthritis - Hip, Knee & Ankles.

CLINICAL MANIFESTATIONS

- Neurologic
  - Meningitis, encephalitis, radiculopathy & peripheral neuropathy, intracerebral abscesses
- Cardiovascular
  - Endocarditis 2% (major cause of mortality)
  - Rx: valve replacement and antibiotics
  - Pericarditis & myocarditis
- Pulmonary
  - Inhalation or hematogenous
  - Cause any chest syndrome
  - Rarely *Brucella* isolated from sputum
- Genitourinary
  - Epidydemoorchitis
  - Pyonephrosis (rare)
- Cutaneous
  - Nonspecific
- Hematologic
  - Anemia
  - Leukopenia
  - Thrombocytopenia

LABORATORY DIAGNOSIS

- Specimen: Blood, Urine, sputum, breast milk
  Lymph node biopsy and Bone marrow aspirate.
- Laboratory methods for diagnosis include
- Culture,
- Serology.
- Hypersensitivity tests.
- Molecular testing.

SPECIMENS

- Blood is the specimen of choice and is collected for culture and for serological test.
- Bone marrow and sometimes synovial fluid, and pleural fluid are also collected for culture.
- Specimens such as liver, and lymph nodes can also be cultured for isolation of Brucella organisms.
- Rarely, the bacteria can be isolated from cerebrospinal fluid (CSF), urine, sputum, breast milk, vaginal discharge, and seminal fluid.

Laboratory diagnosis of Brucellosis:

No.	Methods	Time Consuming
Serology level		
1	Rose Bengal test by Rapid Slide agglutination (screening) test	2 min
2	Rose Bengal test by Tube Agglutination test	2-4 hours
3	Brucella IgG/IgM by Immunochromatographic assay	5 minutes
4	2 Mercaptoethanol Test	15 minutes
5	ELISA (enzyme-linked immunosorbent assay) (IgG/ IgM)	45 minutes – 2 hours
Molecular methods level		
6	PCR (Polymerase Chain Reaction)	7-10 days

*The rapid slide test is brucella used in private laboratory and hospitals in Hawler.
*PCR an excellent tool for the early diagnosis of Brucellosis, due to its very high sensitivity and specificity.

SEROLOGY

- Most serological studies for diagnosis of Brucellosis are based on antibody detection, These include:
- Serum agglutination test –SAT (standard tube agglutination)
- Rose Bengal test- Slide agglutination
- ELISA
- Complement fixation
- Indirect Coombs
- Immunecapture-agglutination
- Whole cell preparations of Brucella antigens are used in IFA, Agglutination.
- Purified LPS/ Protein extracts are used for ELISA.

SEROLOGY

- Specific brucella antibodies, both IgG and IgM antibodies appear in the serum 7-10 days after infection.
- IgM antibodies persist for up to 3 months after which these antibodies decline.
- Then IgG and IgA antibodies appear after 3 weeks of infection and persist for longer time.
- In acute stage or subclinical brucellosis both IgG and IgM can be demonstrated.
- In chronic brucellosis only IgG can be demonstrated, as IgM are absent.
- As IgG antibodies persist for many months or years, demonstration of significant rise in the antibody titer is the definitive serological evidence of brucellosis.
- **Antibody titer of 1: 160 is the presumptive evidence of Brucella infection.**

TREATMENT

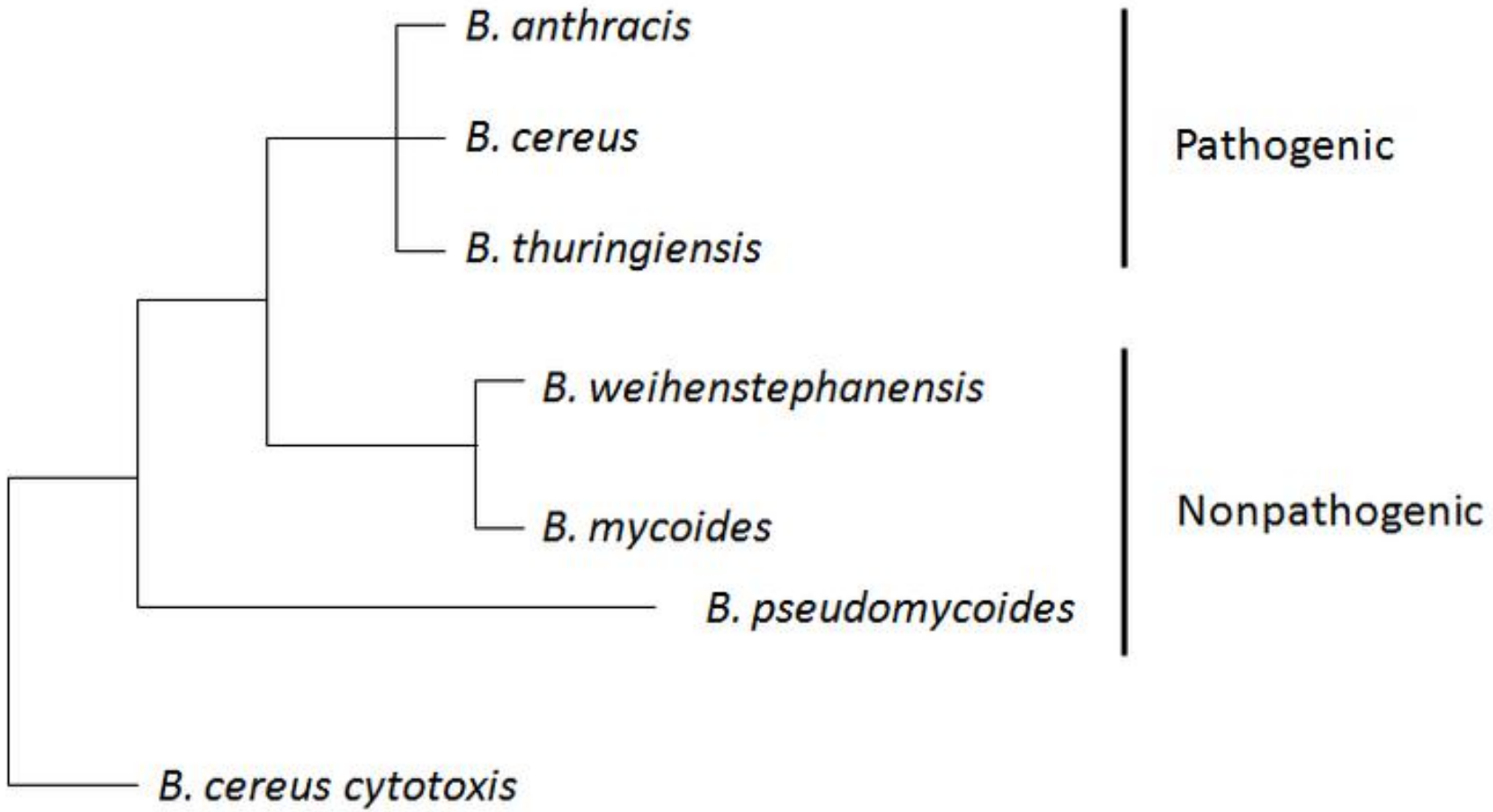
- Brucellae are sensitive to a number of oral antibiotics and aminoglycosides.
- The combination of tetracycline and doxycycline is effective against most species of Brucella.

PREVENTION & CONTRPOL

- 1. Persons handling the animals should use protective clothing and gloves.
- 2. Pasteurisation or boiling of milk should be done.
- 3. Vaccination: Cattle should be vaccinated with live attenuated Br. abortus strain 19, RB 51 for cows.
- 4. Unimmunized infected animals should be slaughtered.
- 5. Br. abortus strain 19-BA, a more attenuated variant of strain 19, has been widely employed for human immunisation in USSR(Union of Soviet Socialist Republics) for protection of population exposed to infection.
- **Vaccine is given intradermally.**

Bacillaceae – Taxonomy

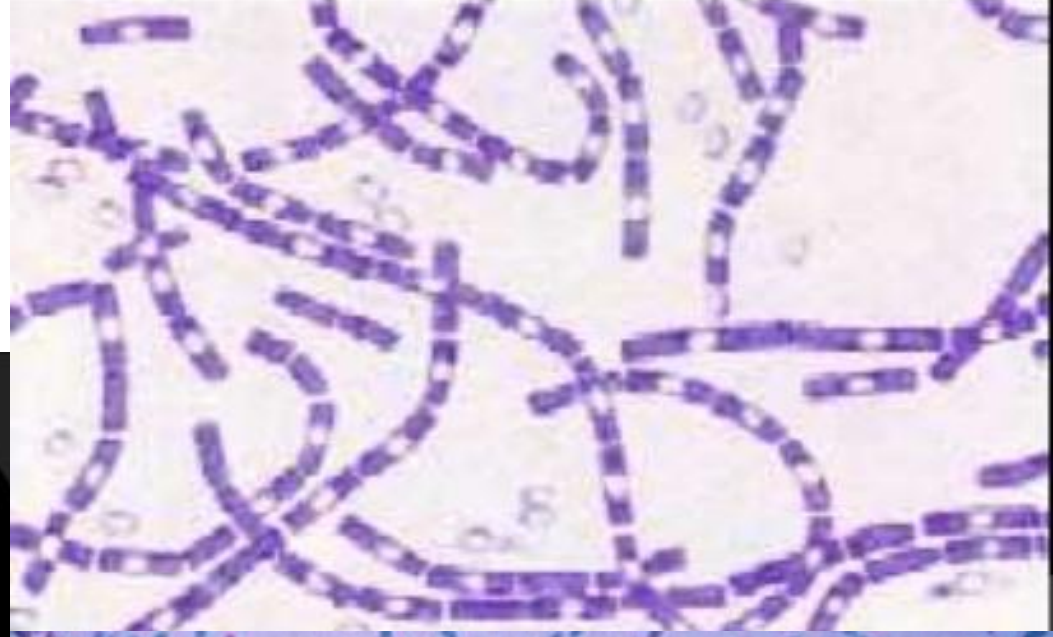
- (Domain): Bacteria
- (Kingdom): Bacillota
- (Class): Bacilli
- (Order): Bacillales
- (Family): Bacillaceae
- (Genus): Bacillus

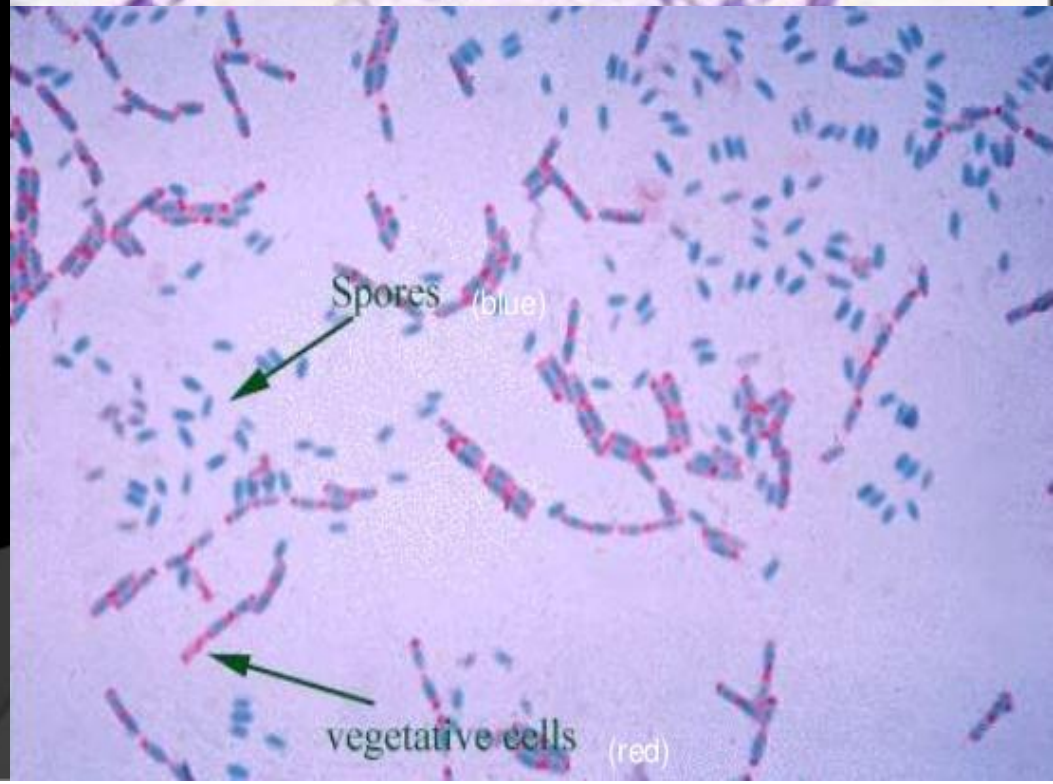


Antrax

Causative organism

- - Etiologic agent: *Bacillus anthracis* Cohn 1875.
- - Large (8 x 1.2 mm) Gram positive, nonmotile, weakly hæmolytic; central spores, straight ends, encapsulated in vivo, produces long chains.
- - Pathogenic to herbivores, man, lab animals.





CULTURAL CHARECTERISTICS

-Aerobic ,Facultative anaerobic

-On NA : Colonies are round, grayish white,irregular,raised with "frosted glass" appearance,2-3 mm in diameter.

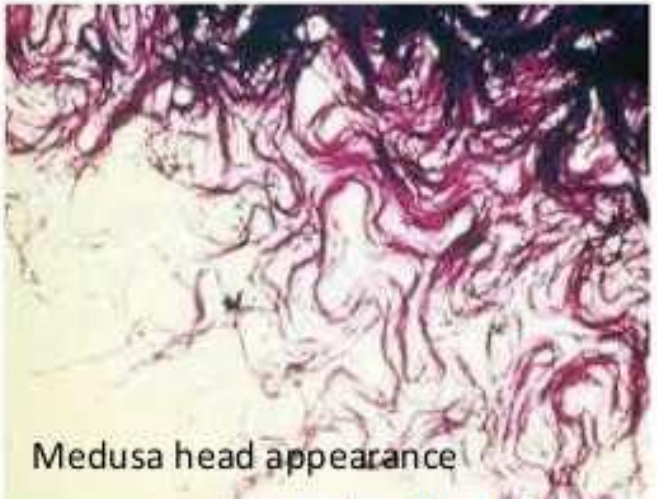
•Medusa head /Barrister's wig appearance of colonies under low magnification

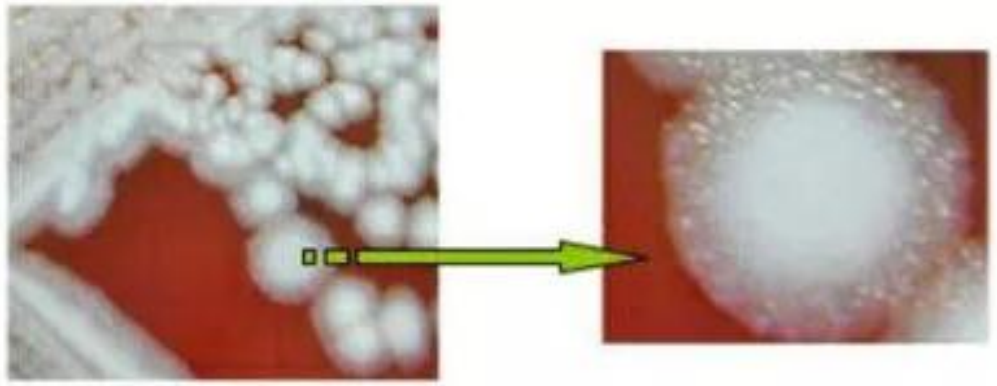
-On BA: Non-hemolytic colonies

-Gelatin stab culture: "Inverted fir tree" appearance

-PLET medium: for selective isolation

CULTURAL CHARECTERISTICS





Colonies of *Bacillus anthracis on blood agar.*

On blood agar, nonhemolytic colonies characterized by a rough, uneven surface with multiple curled extensions at the edge resembling a "Medusahead"

Differentiation of bacteria from the Bacillus genus

Characteristic	*B. cereus*	*B. thuringiensis*	*B. mycoides*	*B. weihenstephanensis*	*B. anthracis*
Gram reaction	+[b]	+	+	+	+
Catalase	+	+	+	+	+
Motility	+/–[c]	+/–	–[d]	+	–
Reduction of nitrate	+	+	+	+	+
Tyrosine decomposed	+	+	+/–	+	–[e]
Lysozyme-resistant	+	+	+	+	+
Egg yolk reaction	+	+	+	+	+
Anaerobic utilization of glucose	+	+	+	+	+
VP reaction	+	+	+	+	+
Acid produced from mannitol	–	–	–	–	–
Hemolysis (Sheep RBC)	+	+	+	ND	–[e]
Observation	Produces enterotoxins	Produces endotoxin crystals, pathogenic to insects	Rhizoidal growth	Growth at 6°C; no growth at 43°C	Pathogenic to animals and humans

TABLE 28-2 Virulence factors of *Bacillus anthracis*

Virulence factors	Biological functions
Bacterial capsule	Protects anthrax bacilli against leukocytic phagocytosis and lysis; if engulfed, resist killing and digestion
Anthrax toxin complex	Anthrax toxin complex is plasmid-encoded and comprises of the following three proteins
Protective antigen (PA)	Entry of the bacilli into the host cell. Binds the complex to receptors on macrophage surface
Edema factor (EF)	Causes cellular edema within the target tissue and also inhibits neutrophil function. Blocks adenyl cyclase pathway within cells
Lethal factor (LF)	Release of tumor necrosis factor-α and interleukin-1 by macrophages

Transmission:

- **Contact with tissues** of animals (cattle, sheep, goats, horses, pigs and others) dying of the disease.
- **Biting flies** that have partially fed on such animals.
- **Contact with contaminated hair, wool, hides or products** made from them (e.g. drums, brushes, rugs).
- **Contact with soil** associated with infected animals or with contaminated bone meal used in gardening.
- Inhalation anthrax results from **inhalation of spores** in risky industrial processes—such as tanning hides and processing wool or bone—with aerosols of *B. anthracis* spores in an enclosed, poorly-ventilated area.
- Intestinal and oropharyngeal anthrax may arise from **ingestion of contaminated undercooked meat**; there is no evidence that milk from infected animals transmits anthrax.

Transmission cont.

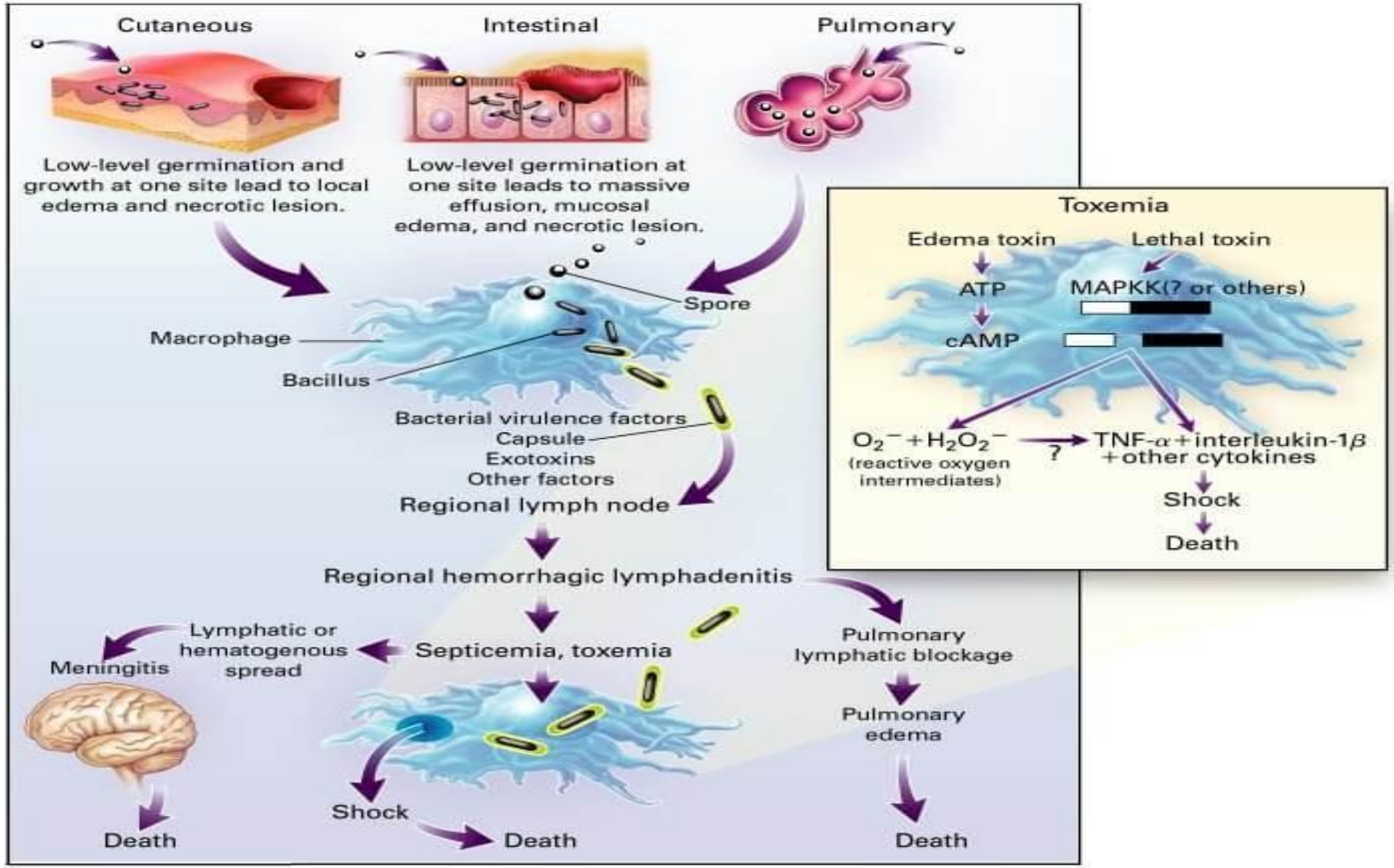
- The disease spreads among grazing animals through **contaminated soil and feed**; and among omnivorous and carnivorous animals through contaminated meat, bone meal or other feeds derived from infected carcases.

- **Accidental** infections may occur among laboratory workers.

- Anthrax is not transmitted person to person.

- Articles and soil contaminated with spores in endemic areas may remain infective for many years.

Pathogenesis



Clinical manifestations:

- Anthrax is an illness with acute onset.
- characterised by several distinct clinical forms including:

1. a skin lesion
2. a respiratory illness
3. abdominal distress

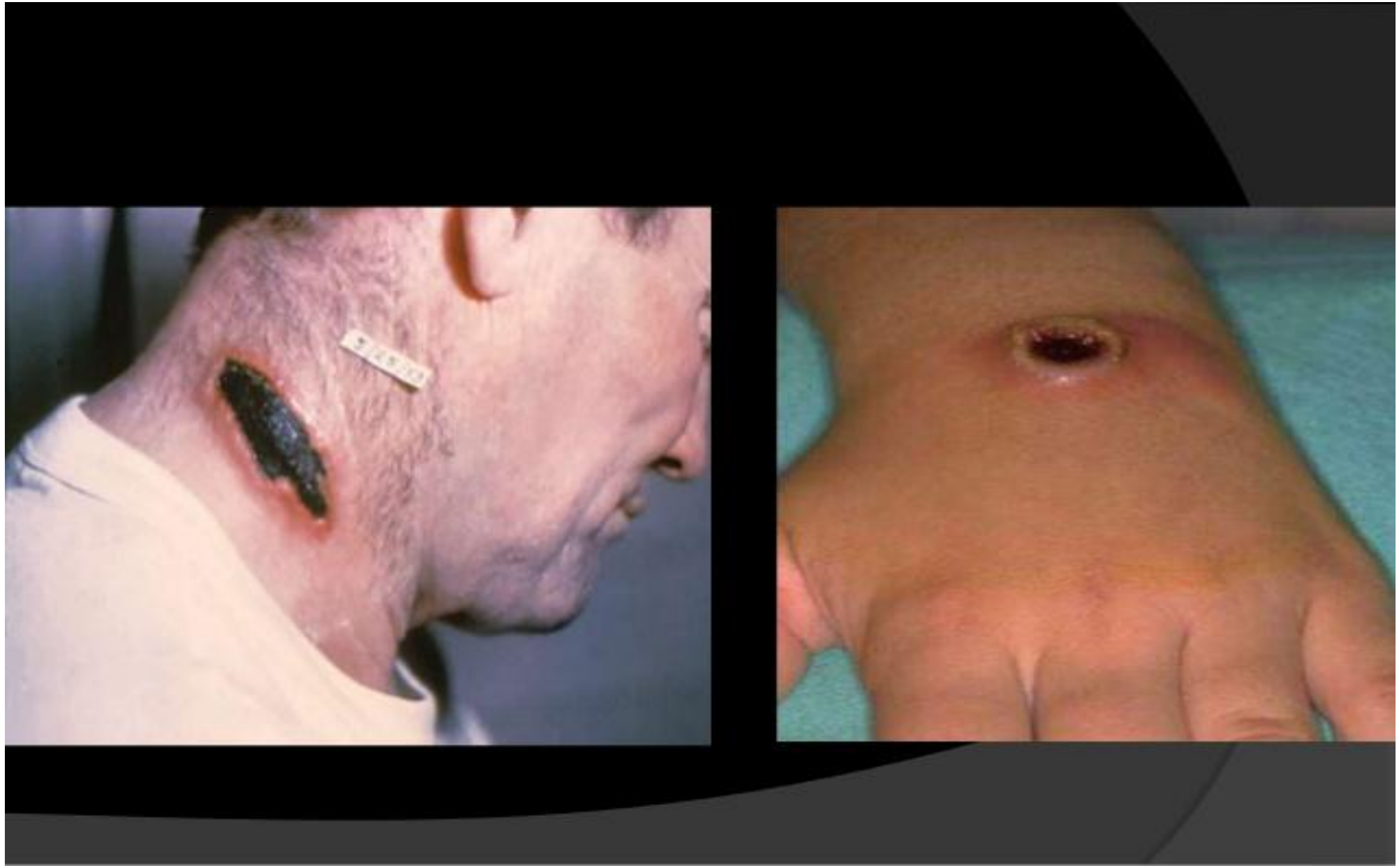
- Ninety percent of cases are cutaneous anthrax

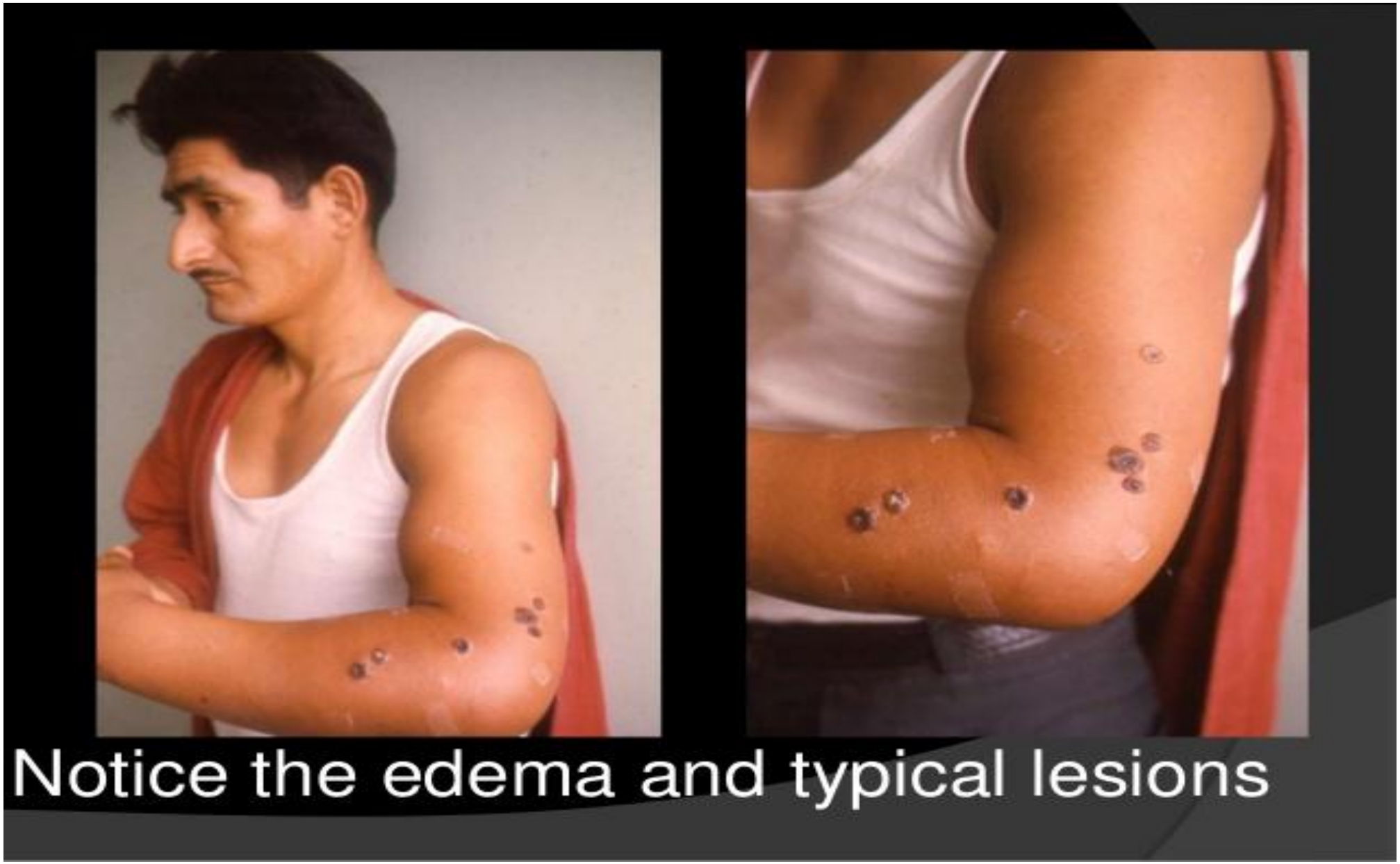
Cutaneous Anthrax

- Mainly in professionals(Veterinarian, butcher, Zoo keeper
- Spores infect skin- a characteristic gelatinous edema develops at the site (Papule- Vesicle-Malignant Pustule-Necrotic ulcer)
- 80-90% heal spontaneously (2-6wks)
- 0-20% progressive disease – develop septicemia
- 95-99% of all human anthrax occur as cutaneous anthrax

Site of Malignant pustule

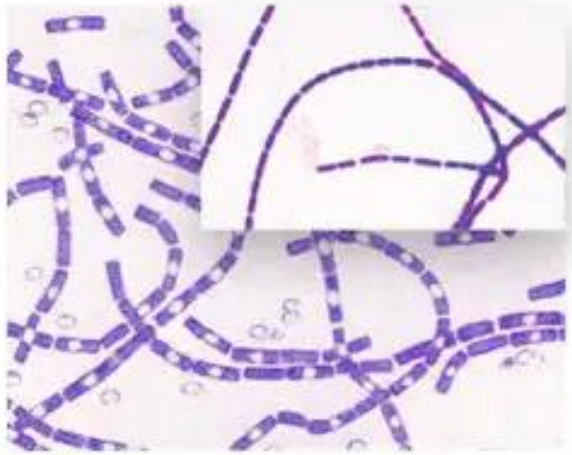
- Head: usually no complication
- Face: severe, superinfection; gangrene near eye
- Neck, breast or chest wall: massive edema, over thorax and sometimes involving scrotum
- Shoulders, arms: may be multiple, small lesions
- Forearms, fingers: atypical on palms
- General symptoms, fever, chills, depend on site.
- Weakness, hypotension are danger signs.




Notice the edema and typical lesions

- ***Laboratory confirmation*** requires at least one of the following:

1. isolation of Bacillus anthracis from a clinical specimen
2. demonstration of B. anthracis in a clinical specimen by immunofluorescence
3. significant antibody titres developing in an appropriate clinical case.



GPR in chains with elliptical and central spores

Non-hemolytic colonies with "frosted glass" and/ or "medusa head" appearance

Bacillus anthracis

Inverted "fir tree" appearance

Used to identify culture isolate

Treatment

- The case should be under the care of an infectious diseases physician.
- Penicillin is the drug of choice for cutaneous anthrax and is given for 5–7 days.
- Tetracyclines, erythromycin and chloramphenicol are also effective.
- The U.S. military recommends parenteral ciprofloxacin or doxycycline for inhalation anthrax though the duration of treatment is not well defined.

Vaccination

- Cell-free filtrate
- At risk groups
  - Veterinarians
  - Lab workers
  - Livestock handlers
  - Military personnel
- Immunization series
  - Five IM injections over 18-week period
  - Annual booster

Listeriaceae - TAXONOMY

- (Domain): Bakteriyalar
- (Kingdom): Bacillota
- (Class): Bacilli
- (Order): Bacillales
- (Family): Listeriaceae
- (Genus): Listeria
- (Species):

LISTERIA MONOCYTOGENES

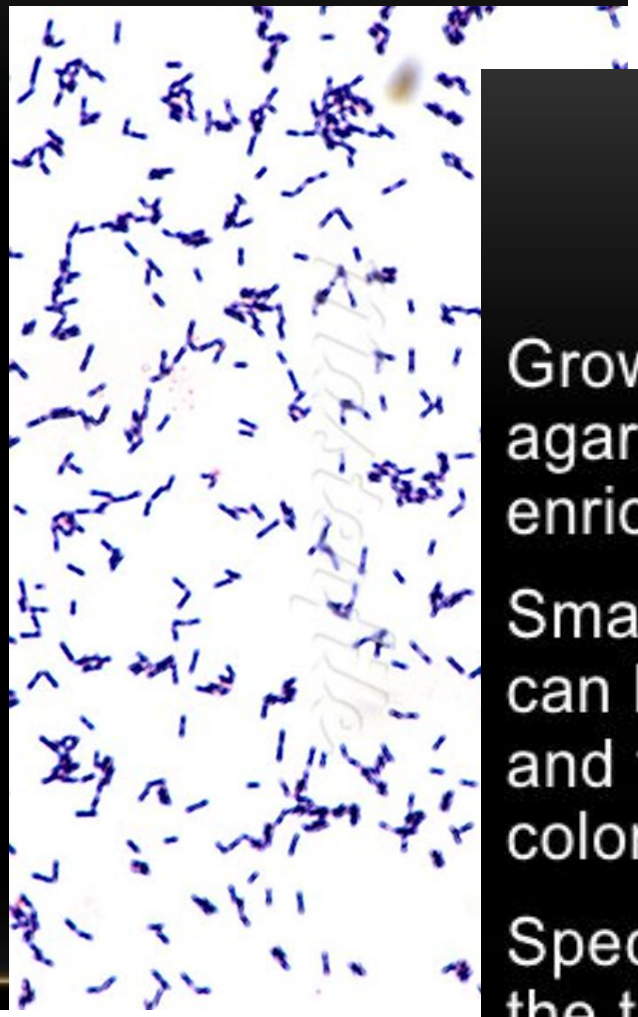
L.monocytogenes is a Gram + ve non spore forming.

A specific character of the organism manifest with tumbling end or over end motility at

22^0c – 28^0c but not at

37^0c

But makes the Microbiologists to identify from Diptheriods, which are mistaken and specimens are discarded.



CULTURE AND GROWTH CHARACTERISTICS

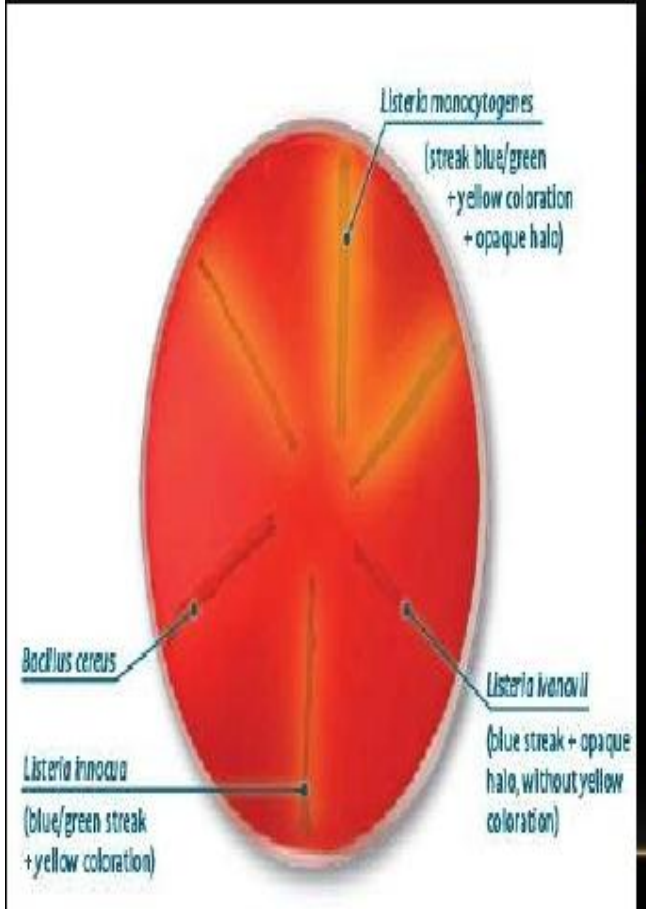
Grows on Muller Hinton agar with sheep blood as enrichment.

Small zone of Hemolysis can be observed around and the underneath of the colony.

Specimens are enriched if the tissues are kept at 4^0c and plated on the media

(Cold enrichment)

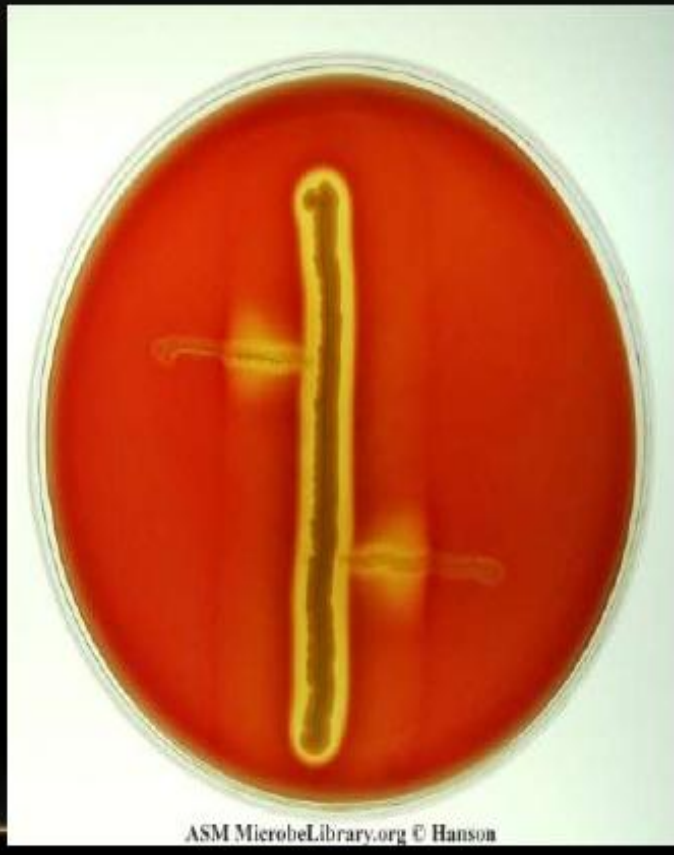
BIOCHEMICAL REACTIONS



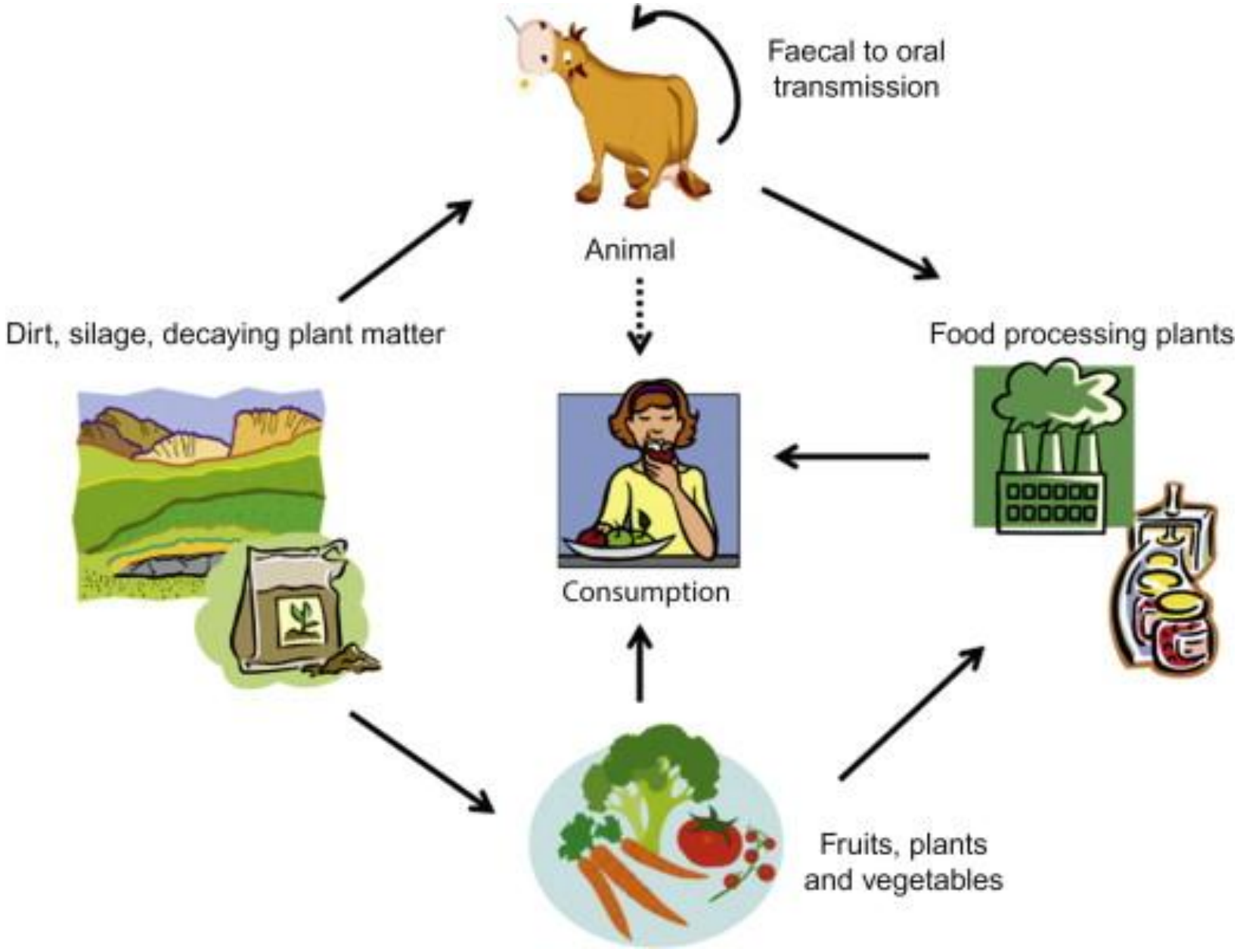
- Bacteria are facultative anaerobic microbes
- Catalase + motile
- Listeria produce acid and not gas in various sugar fermentation tests

CAMP POSITIVE LISTERIA

CAMP-positive Listeria monocytogenes inoculated at right angles to b-hemolytic Staphylococcus aureus. Note the arrow-shaped zone of weak enhanced hemolysis indicating a positive CAMP test. Gloves are worn when working with L. Monocytogenes. (Anne Hanson, University of Maine)

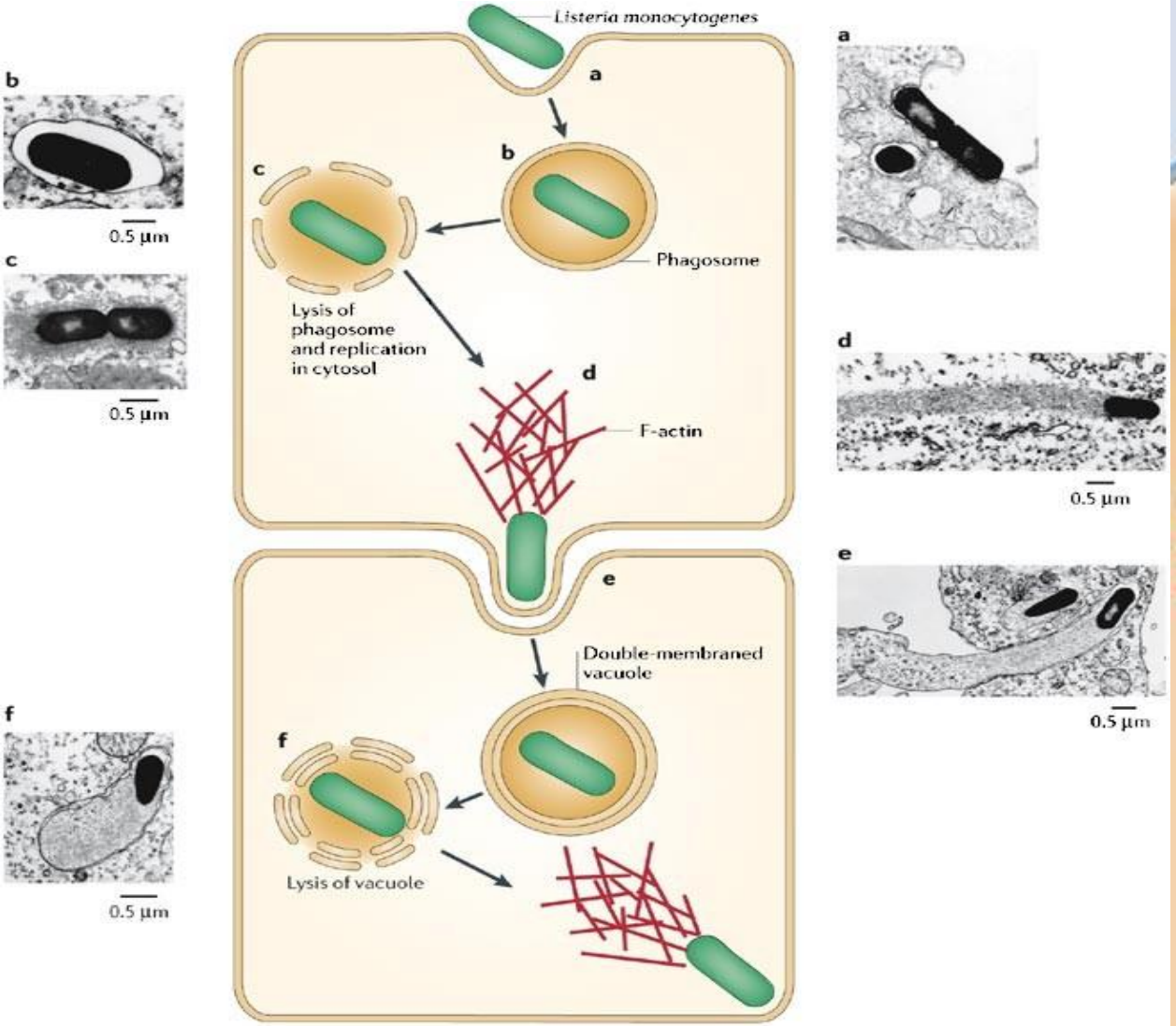


Mode of transmission

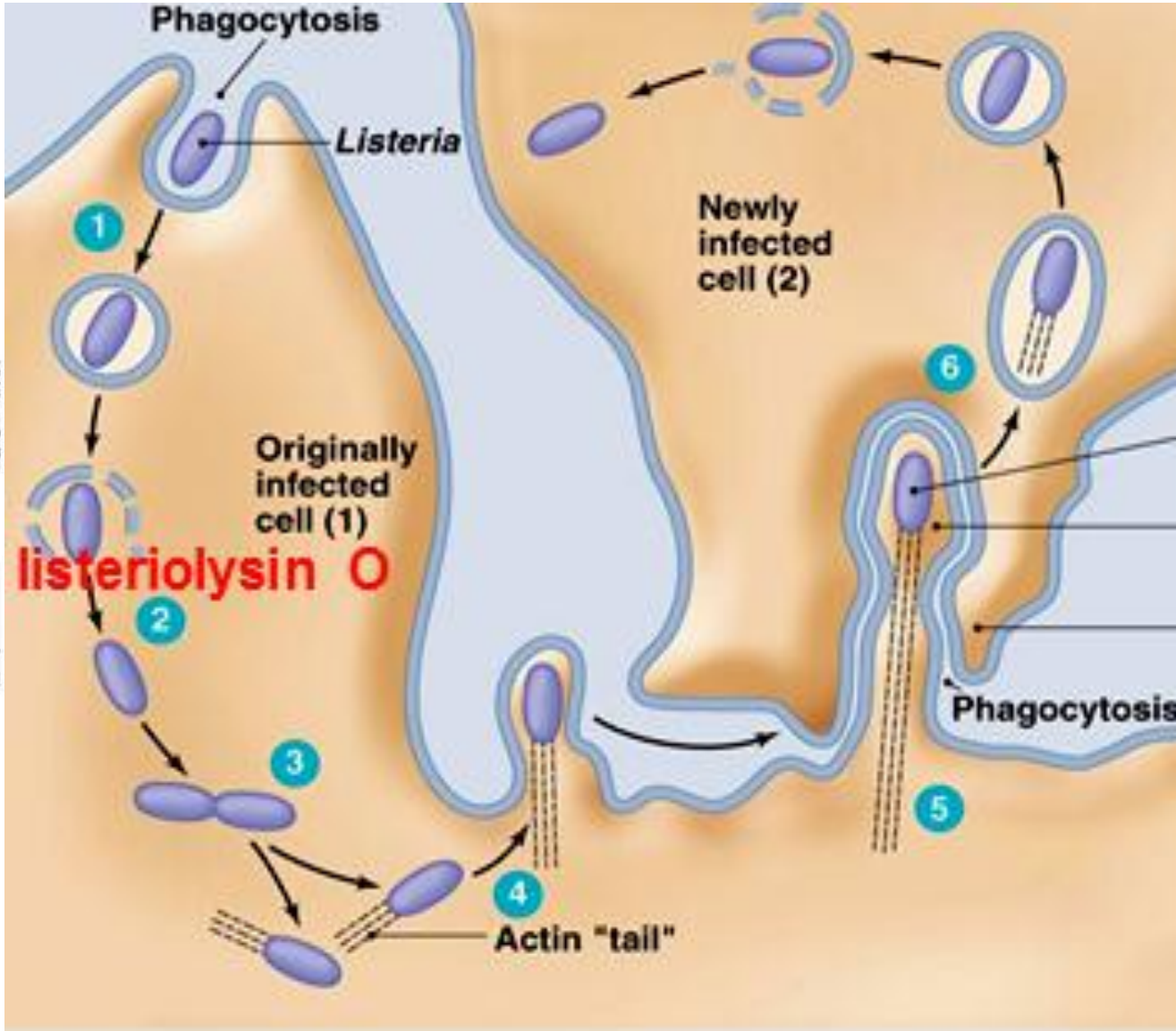


Listeria monocytogenes

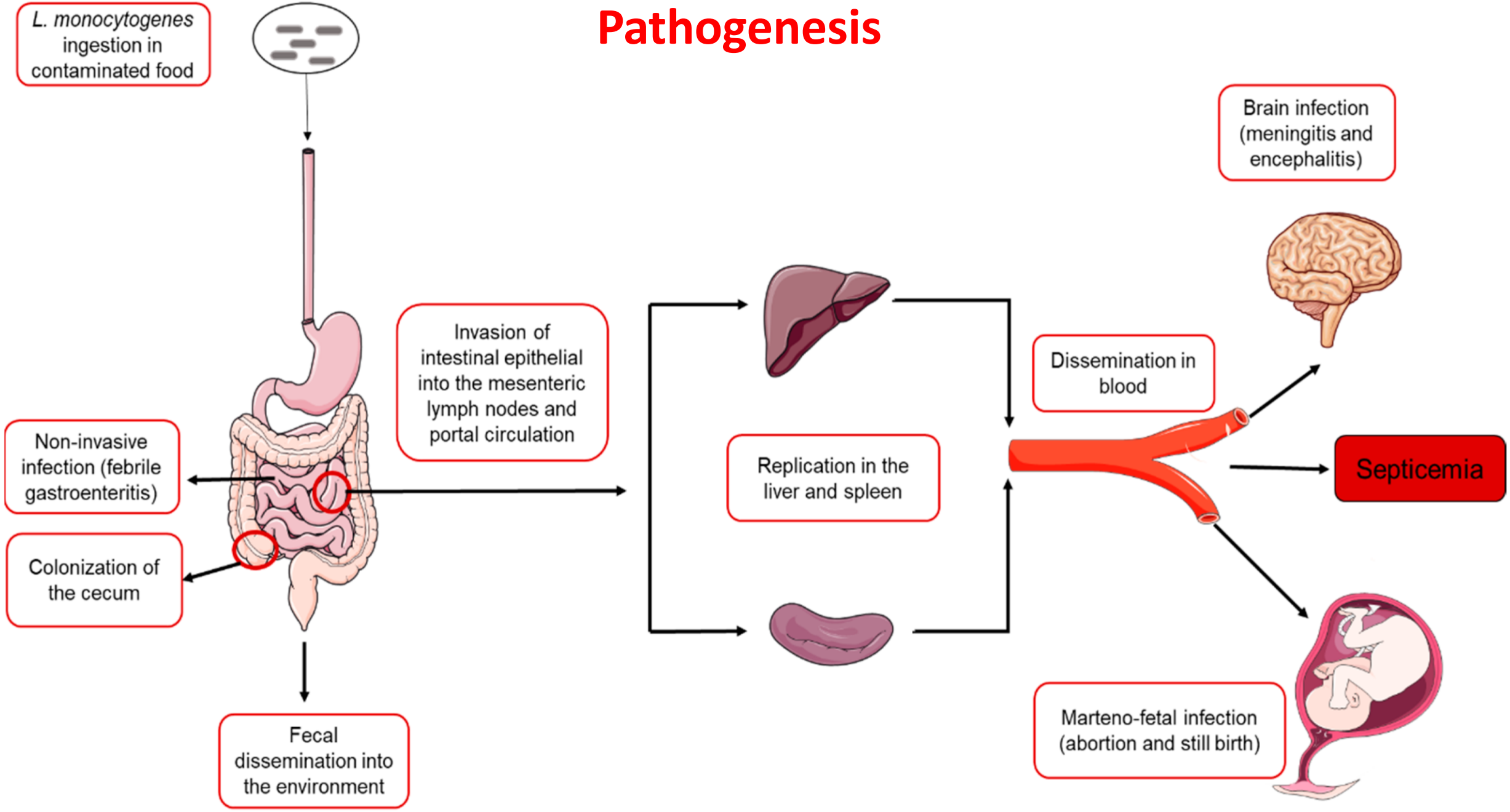
Schematic illustration and electron micrographs of the life cycle



Mechanism of transfer from one cell to another



Pathogenesis



PATHOGENESIS AND PATHOLOGY

- Listeria Monocytogenes enters through the Gastro – intestinal tract after infections of contaminated foods such as cheese or vegetables,
- The cell wall surface protein called Interanalin interacts with E –CADHERIN and enters into epithelial cells
- Bacteria produce Listeriolysin
- L.monocygenes can move from cell to with out being exposed to Antibodies,Complement,Polymorphonuclear cells

WHAT IS LISTERIOSIS

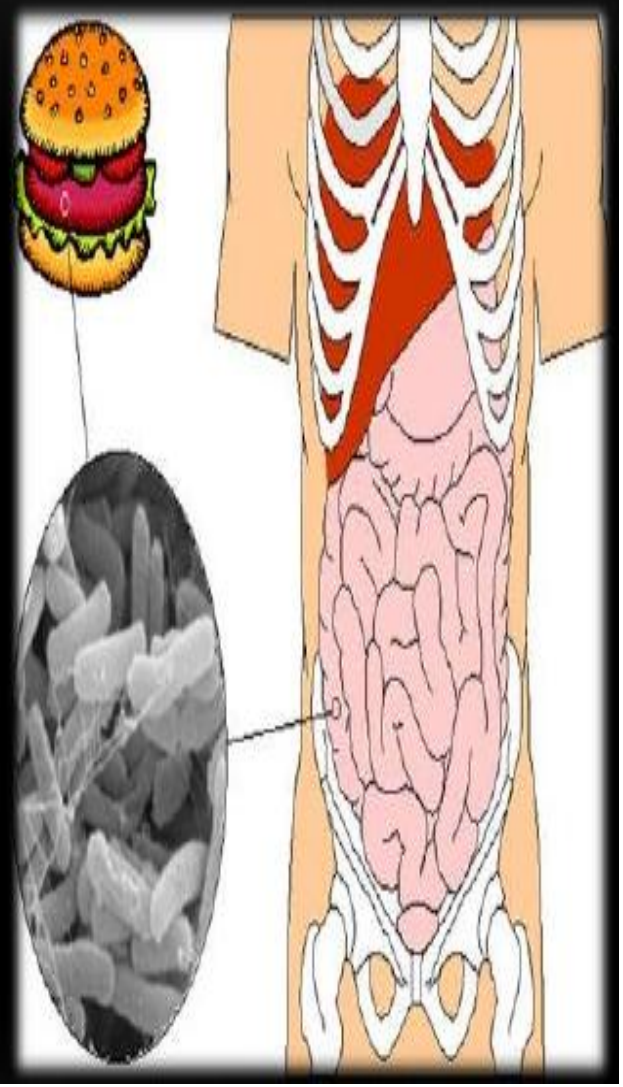
- **Listeriosis,** a serious infection caused by eating food contaminated with the bacterium ***Listeria Monocytogenes***, has recently been recognized as an important public health problem in the United States. The disease affects primarily persons of advanced age, pregnant women, new-borns, and adults with weakened immune systems.
  However, persons without these risk factors can also rarely be affected.

WHO ARE AT RISK WITH LISTERIOSIS

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

COMMON PRESENTING MANIFESTATION OF LISTERIOSIS

- Vomiting;
- Nausea;
- Cramps;
- Diarrhea;
- Severe Headache;
- Constipation; or
- Persistent fever.



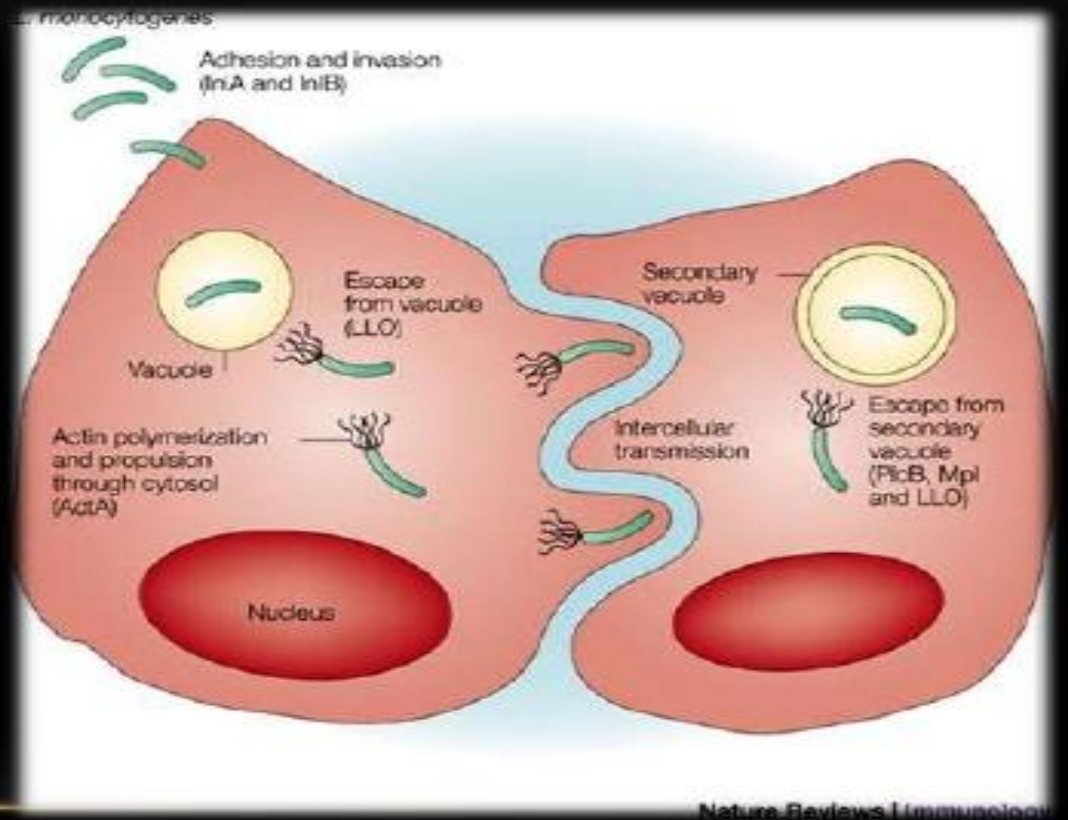
SYMPTOMS

- Fever
- Muscle ache
- GI Sx: Nausea, diarrhea
- Pregnant women: mild flu-like Sx, miscarriage, still birth, premature delivery, or infected newborn.
- Lethargy
- irritability

- If infection spreads to the nervous system: headache, stiff neck, confusion, loss of balance, or convulsions.
- Listeria can cause Pneumonia, Meningitis, a nd Sepsis.

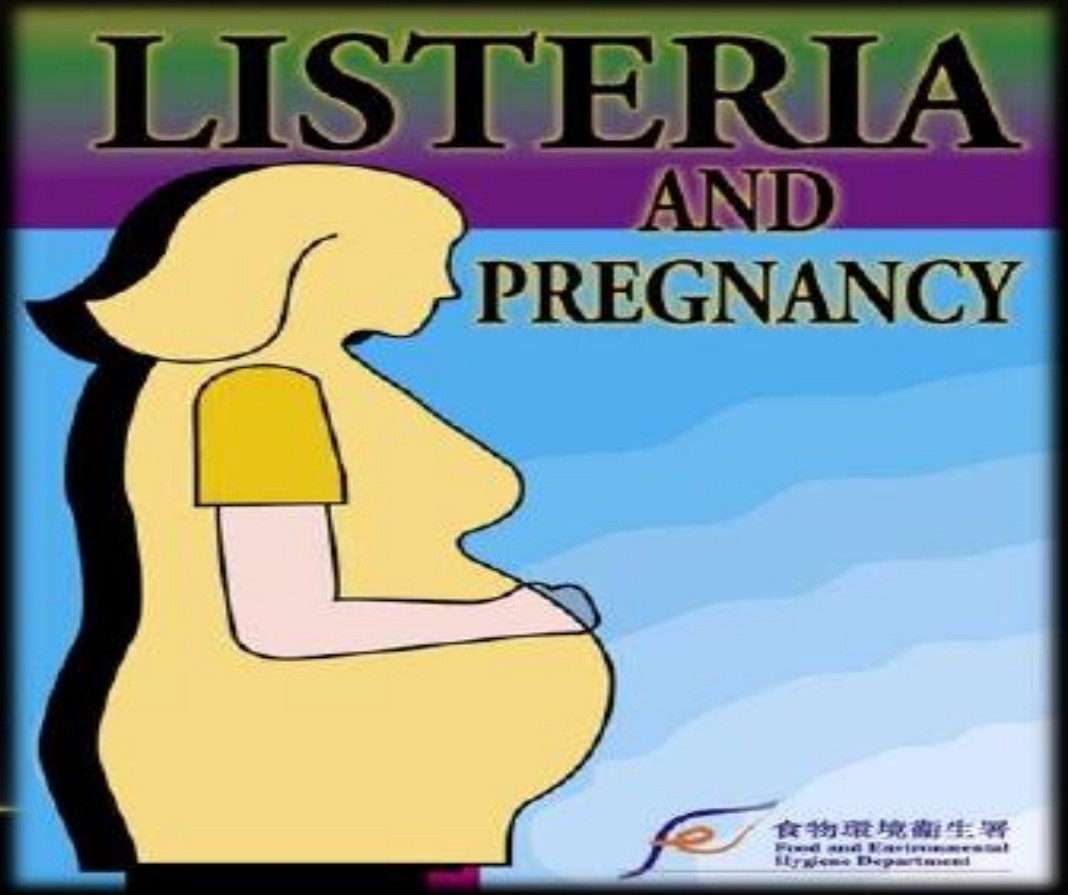
LISTERIOSIS IN ADULTS

- Adults may present with bacterimia . Meingoencephalitis and occur most commonly in Immunosupressed patients in whom Listeria is one of the more common cause of Meningitis
- Disease can be insidious to fulminant



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.



LISTERIOSIS IN NEW BORNS

- New-borns - New-borns rather than the pregnant women themselves suffer the serious effects of infection in pregnancy.

DIAGNOSIS

- There is no routine screening test for susceptibility.
- Patient may present with fever, or stiff nec, .
- A blood or spinal fluid examination (to cultivate the bacteria) will confirm the clinical diagnosis. .
- During pregnancy, patients need detailed investigations to rule our Listeriosis.

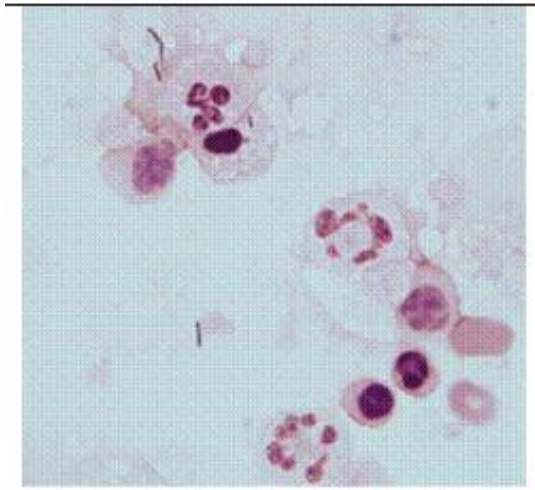
Diagnosis dependent on isolation of Organisms in cultures obtained on CSF, Blood, and other fluids

Listeria monocytogenes

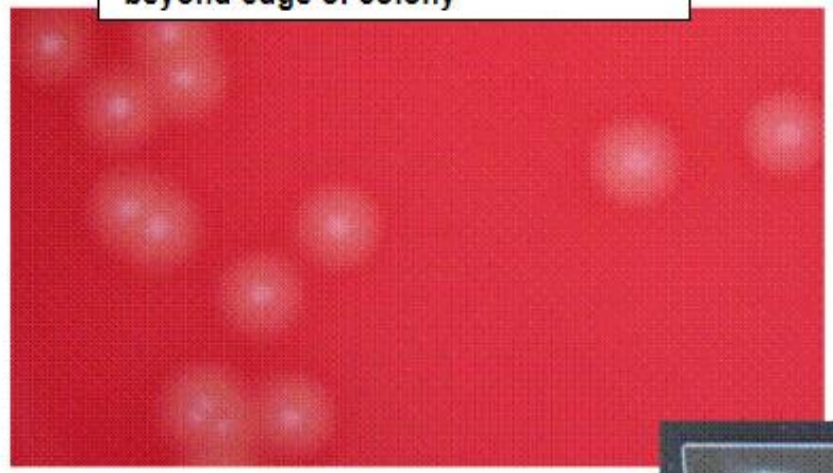
Major pathogen in CSF and Blood only

@ Ellen Jo Baron 2007

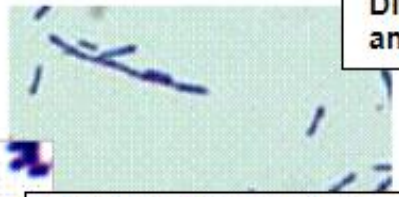
CSF showing PMNs, Monocytes, & Gram positive rods (may be intracellular)

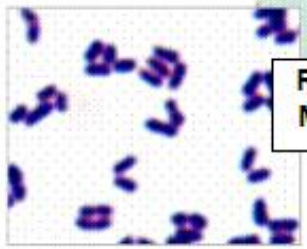


Beta hemolysis does NOT extend beyond edge of colony

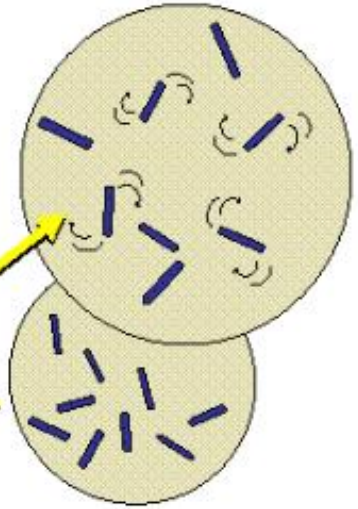


Colony looks like Group B streptococci. Differentiate from Group B strep by Gram stain and positive catalase reaction

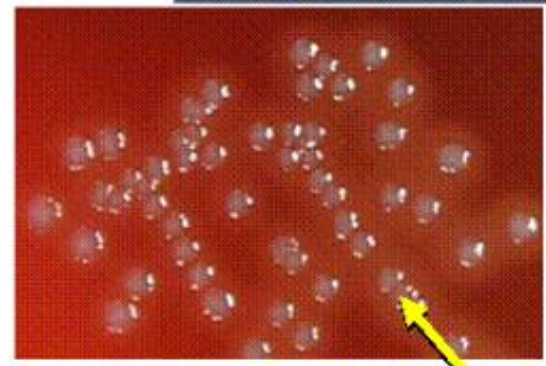




Regular Gram + rods
May be short or longer



Motility:
Tumbling motility at 26°C
Non-motile at 35° C



This organism is NOT Listeria because beta hemolysis extends beyond edge of colony

TREATMENT

- ***Listeriosis* is a serious disease** requiring hospitalization.
- A combination of antibiotics is given intravenously through a small straw-like catheter.
- When infection occurs during pregnancy, antibiotics must be given promptly to the mother to prevent infection of the fetus or newborn.
- The duration of antibiotic treatment is at least 2 weeks.
- Even with prompt treatment, some infections result in death.

ANTIBIOTIC TREATMENT

- Ampicillin
- Erythromycin
- Intravenous **Trimethoprim – Sulphmethoxazole**
- Cephalosporins and Fluroquinoles are not active against l.monocytogens
- A commination of Gentamycin and Ampicillin on clinical basis

Yersiniaceae - TAXONOMY

KINGDOM : Eubacteria

PHYLUM : Proteobacteria

CLASS : Gammaproteobacteria

ORDER : Enterobacteriales

FAMILY : Enterobacteriaceae

GENUS : *Yersinia*

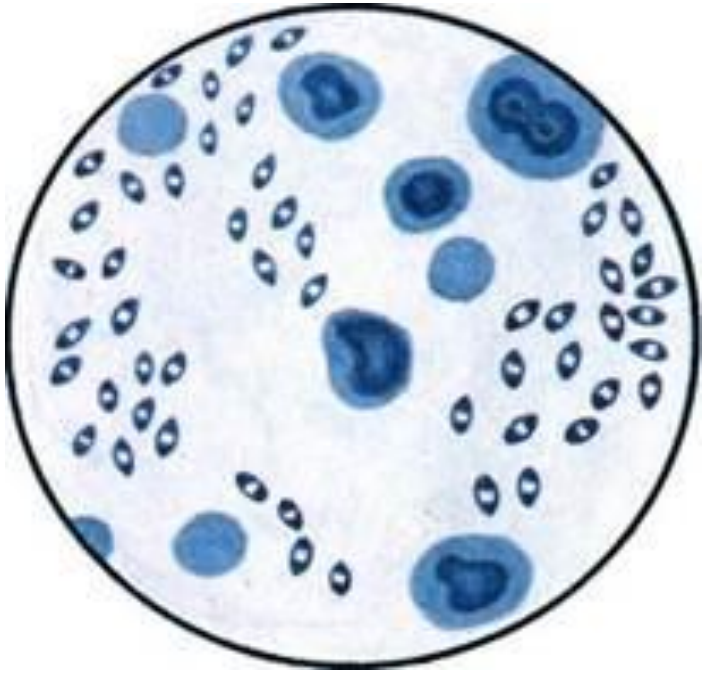
SPECIES : *pestis, enterocolitica, pseudotuberclosis*

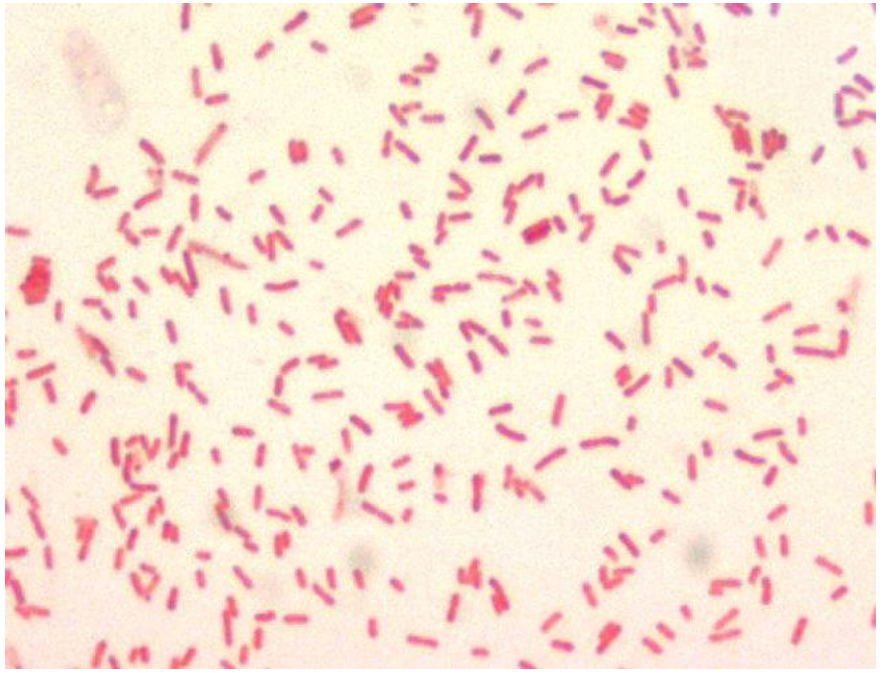
Yersinia Pestis

- *Yersinia Pestis* is a gram negative and noncapsulated, facultative anaerobic microorganism (family enterobacteriaceae)
- It can infect humans and animals via the oriental rat flea which called (Xenopsylla Cheopis).
- It can reproduce inside cells, so even if phagocytosed they can still survive, because it produces an anti-phagocytic slime layer.
- *Yersinia pestis* was discovered in 1894 by Alexandre yersin .

Yersinia pestis
morpho-biological characteristics

1-2x0.4-0.7 μm in size, non-motile, non-spore-forming, ovoid Gram-negative rod-shaped bacteria. Forms a delicate capsule. They are polymorphic. Since the cytoplasm is unevenly distributed, it is stained more intensively in the ends. This is called bipolar staining.





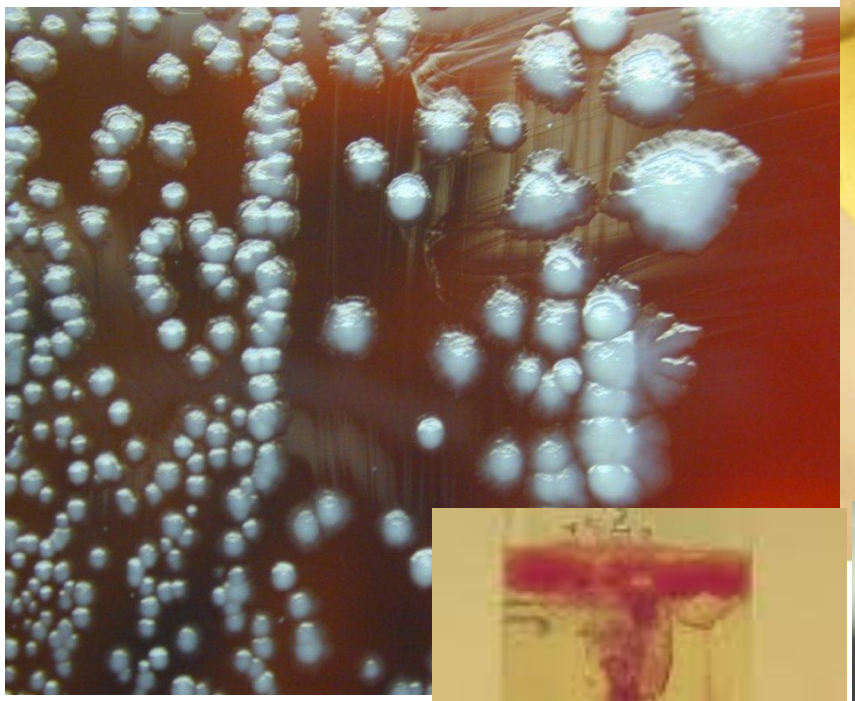
Yersinia pestis

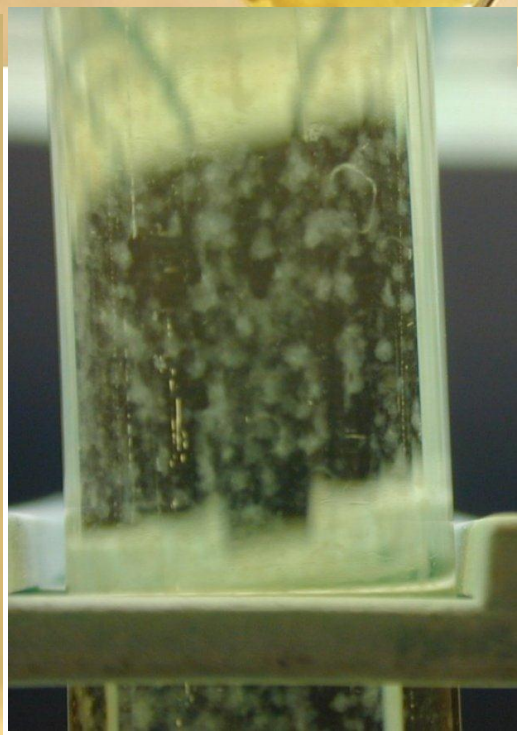
Yersinia pestis cultural characteristics

- It is a facultative anaerobe.
- They develop in normal nutrient environment. Casein medium and blood clot hydrolyzate are selective media for them.
- In a solid nutrient environment, it forms an uneven colony. Virulent bacteria form R-colonies resembling a "handkerchief with bordered edges", and weakly virulent ones form smooth S-colonies.

stalaktit

- When growing in a **liquid nutrient** medium, it forms crusts on the surface of the broth, and flakes inside. Later, threads reminiscent of stalactites are observed from the surface of the broth to the inside.





Biochemical Characteristics of *Yersinia* species			
Reaction	***Yersinia* species**		
	Y. pestis	***Y. pseudo-tuberculosis***	***Y. enterocolitica***
Lysine	-	-	-
Ornithine	-	-	+
Motility at RT (22-26°C)	-	+	+
Urea	-	+	+
Mannitol	+	+	+
Sorbitol	+/-	-	+
Voges- Proskauer	-	-	+/-
Indole	-	+/-	+/-

Pathogenicity

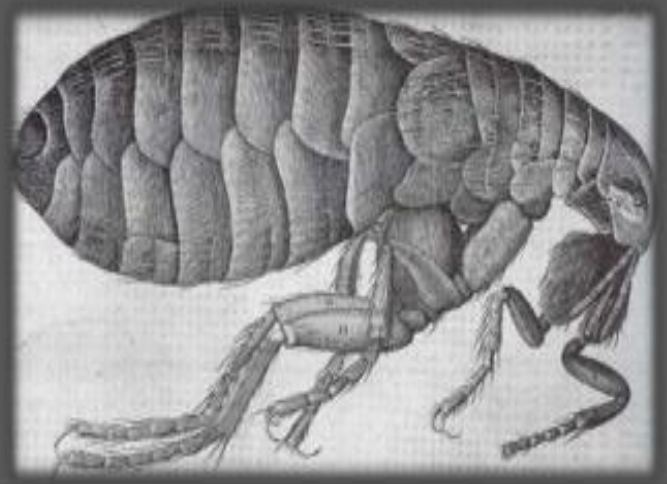
- Two important anti-phagocytic antigens, named F1 (Fraction 1) and V antigen, which both are important for virulence. Furthermore , Y. pestis survives and produces F1 and V antigens while it is residing within WBC such as monocytes, but not in neutrophils .
- In addition, the Type-III Secretion System (T3SS) allows Y. pestis to inject proteins into macrophages and other immune cells.

TABLE 34-2 Virulent factors of *Yersinia pestis*

Virulence factors	Biological functions
Plague toxin	Causes systemic manifestations of plague
F1 envelope antigen	Inhibits phagocytosis
V and W antigens	Inhibit phagocytosis and intracellular killing of the plague bacillus inside macrophages
Type III secretion systems	Facilitate secretion of virulence factors of *Y. pestis* into host cells Prevent phagocytic killing of the pathogenic *Yersinia* species
Plasminogen activator (pla) protease	Degrades C3b and C5a components of the complement Also degrades fibrin clots
Yersinia Outer Membrane Proteins(YOPs)	Cell surface adhesion,iron acquisition,inhibition of phagocytosis and intracellular killing.

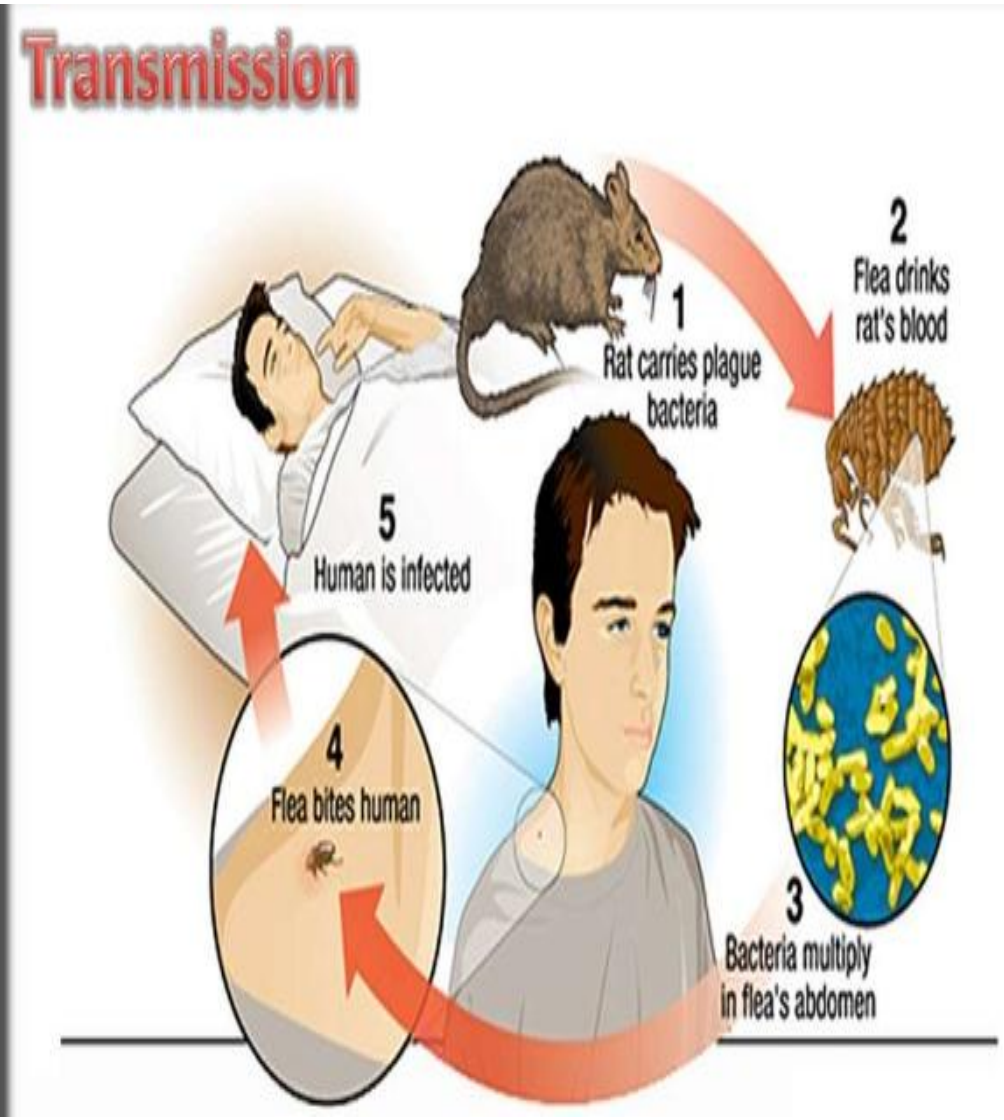
Transmission ...

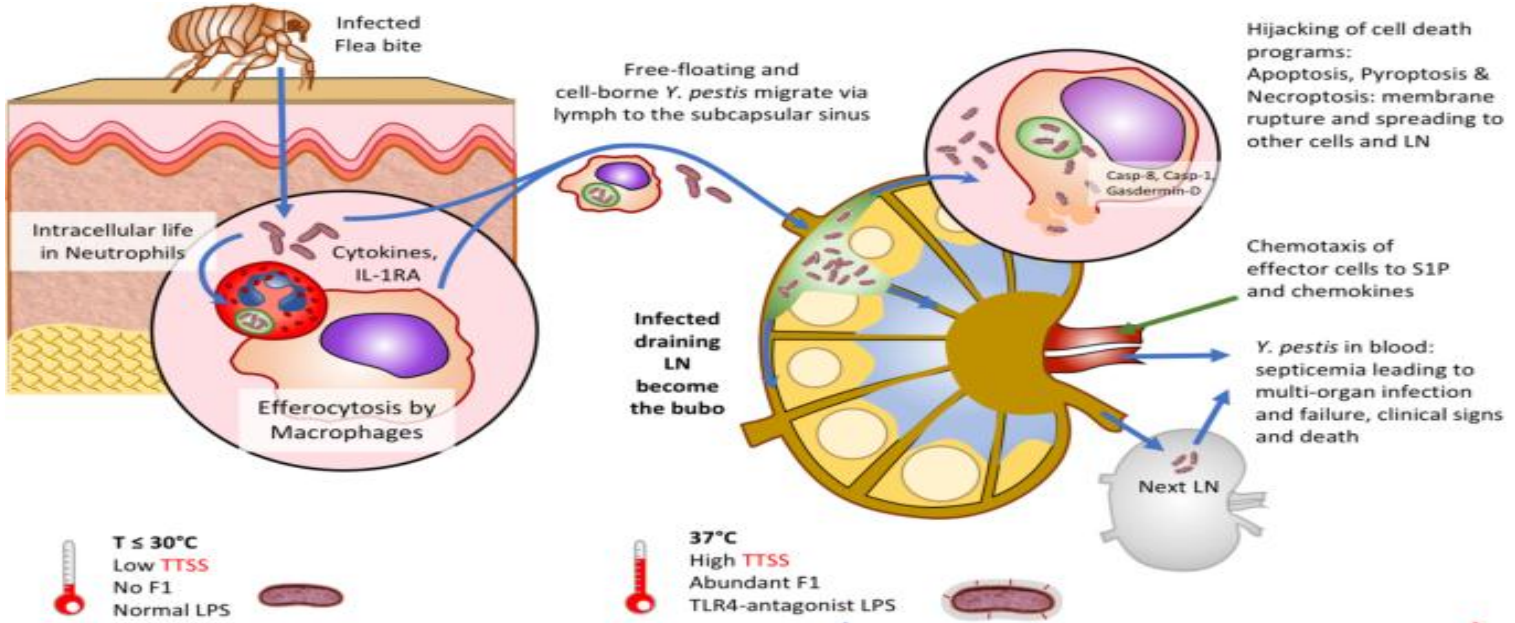
- Air droplet : coughing or sneezing on another person
- Direct physical contact : touching an infected person, including sexual contact .
- Indirect contact : usually by touching contaminated soil or a contaminated surface
- Fecal-oral transmission : usually from contaminated food or water sources
- Vector borne transmission : carried by insects or other animals.

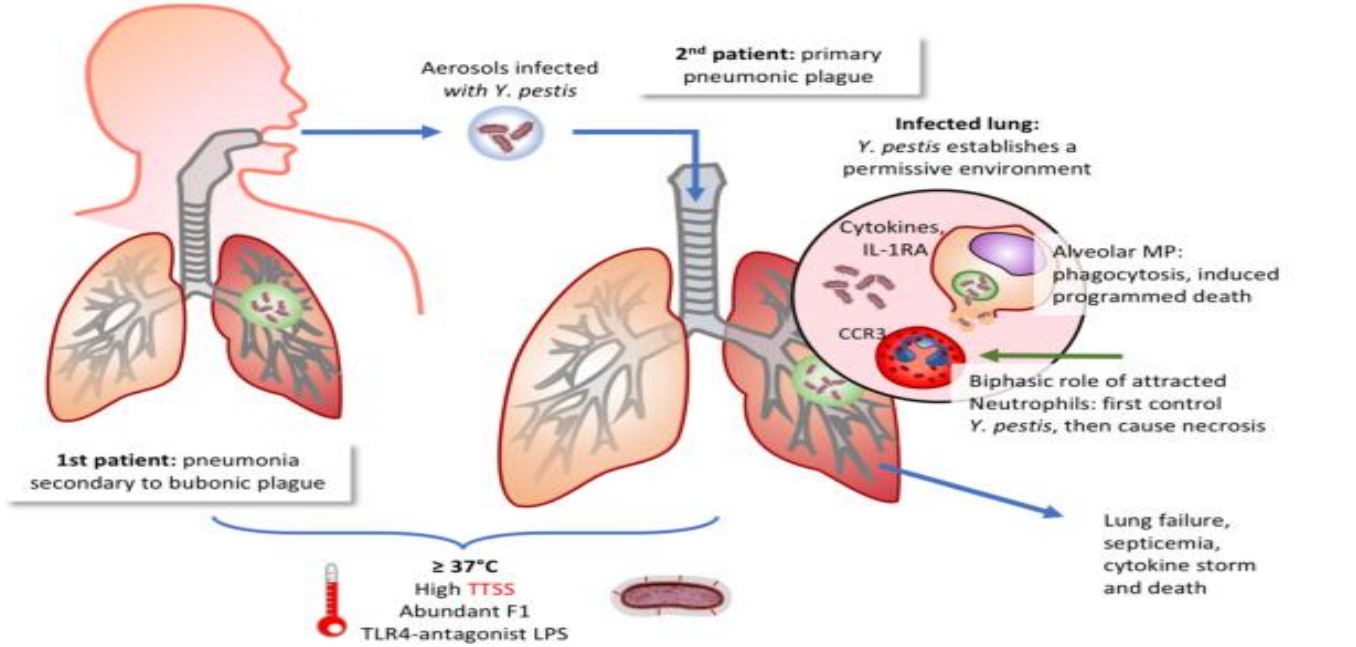


How the flea can infect us ?

When a flea bites a human and contaminates the wound with regurgitated blood, the plague carrying bacteria are passed into the tissue. Once in the body, the bacteria can enter the lymphatic system.


Transmission
1
Rat carries plague bacteria
2
Flea drinks rat's blood
3
Bacteria multiply in flea's abdomen
4
Flea bites human
5
Human is infected


Infected Flea bite
Intracellular life in Neutrophils
Cytokines, IL-1RA
Efferocytosis by Macrophages
Free-floating and cell-borne Y. pestis migrate via lymph to the subcapsular sinus
Hijacking of cell death programs: Apoptosis, Pyroptosis & Necroptosis: membrane rupture and spreading to other cells and LN
Casp-8, Casp-1, Gasdermin-D
Chemotaxis of effector cells to S1P and chemokines
Infected draining LN become the bubo
Y. pestis in blood: septicemia leading to multi-organ infection and failure, clinical signs and death
Next LN
T ≤ 30°C
Low TTSS
No F1
Normal LPS
37°C
High TTSS
Abundant F1
TLR4-antagonist LPS
Pre-inflammatory phase
Migration, multiplication and immune evasion
Inflammatory phase
Systemic dissemination, clinical signs and tissue destruction


Aerosols infected with Y. pestis
2nd patient: primary pneumonic plague
Infected lung: Y. pestis establishes a permissive environment
Cytokines, IL-1RA
Alveolar MP: phagocytosis, induced programmed death
CCR3
Biphasic role of attracted Neutrophils: first control Y. pestis, then cause necrosis
1st patient: pneumonia secondary to bubonic plague
Lung failure, septicemia, cytokine storm and death
≥ 37°C
High TTSS
Abundant F1
TLR4-antagonist LPS

What is the Plague ?? ....

- Plague is a bacterial infection, which Yersinia pestis is the etiological agent of this disease, and mostly affects (lungs and lymph nodes and blood vessels). Plague is a fatal disease , which approximately more than 200 million people have been dead by this disease.
- The word of plague is believed to come from the Latin word plāga ("blow, wound")

Types Of The Plague Disease

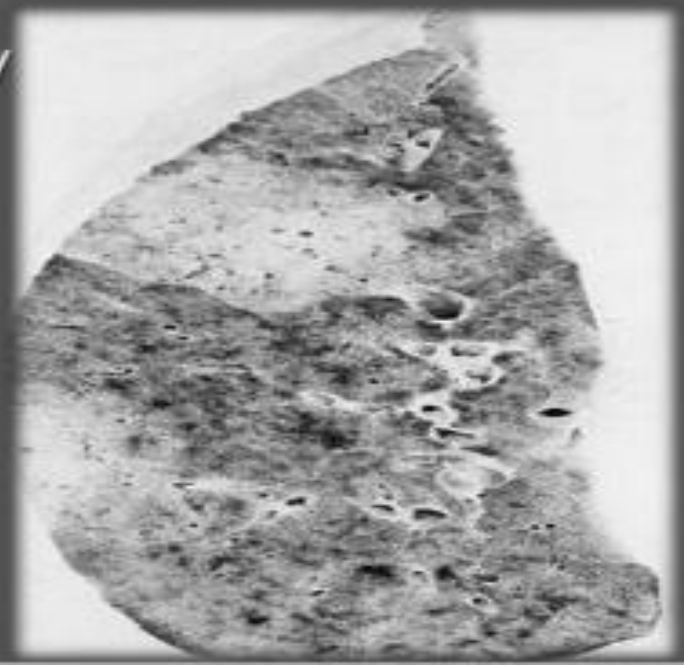
- **Bubonic Plague**
- **Pneumonic Plague**
- **Septicemic plague**
  - *Meningeal plague*
  - *Cellulocutaneous plague*

Bubonic plague ...

- Bubo is a Greek word which is termed for swollen lymph glandes. It is the acute inflammation and painful swellings of the lymph nodes , it also is the most common type of Plague.
- It will occur when an infected rodent or flea bites you.
- The Bacteria will spread through the lymphatic vessels of the infected human until it reaches a lymph node, where it stimulates the inflammation that causes the lymph nodes to expand. The expansion of lymph nodes is the cause of the characteristic lymphadenopathy "bubo"

Pneumonic Plague

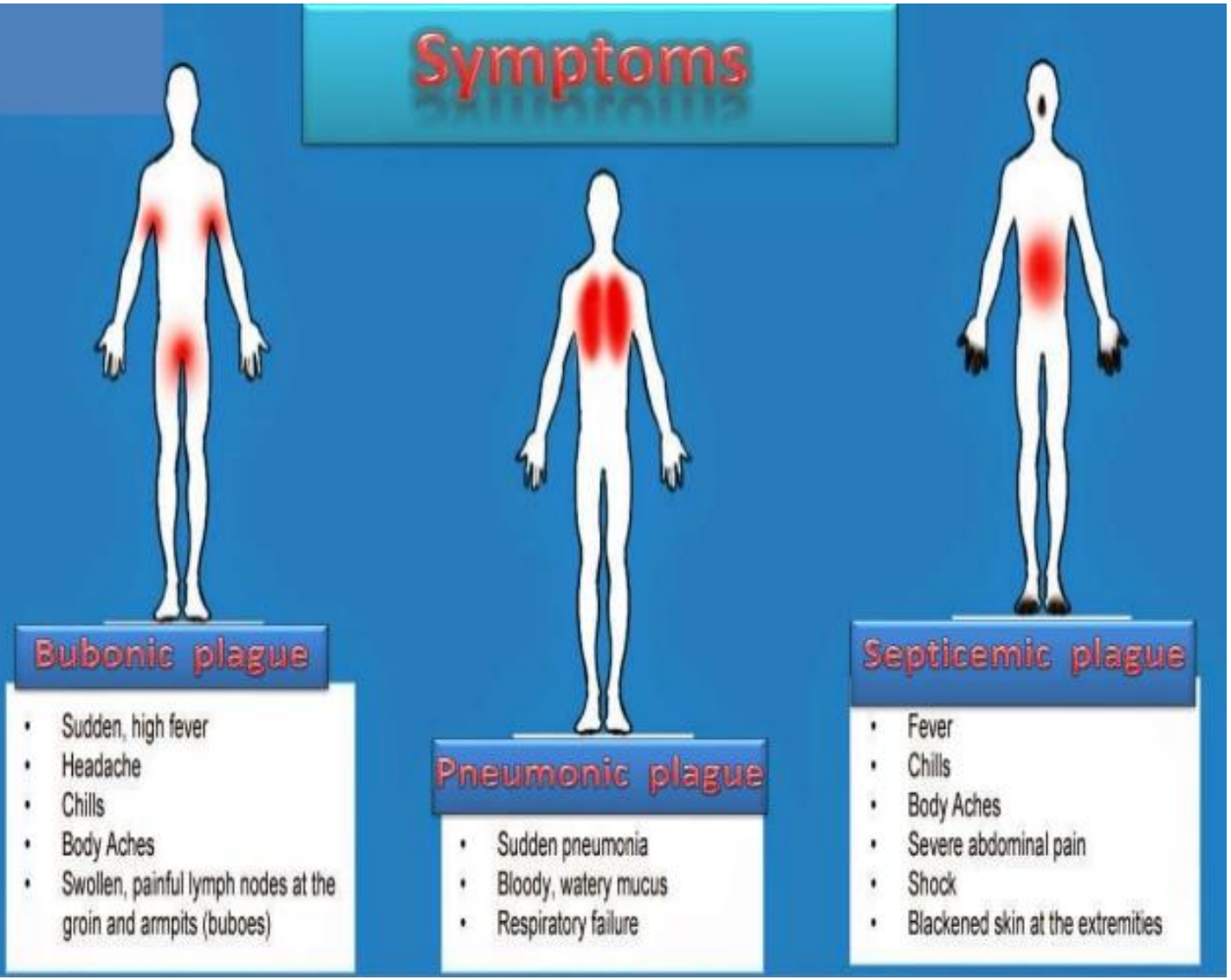
- Is the another type of plague , which arises from infection of the lungs. It causes coughing and sneezing.
- Pneumonic plague is the only form of plague that can be transmitted from person to person.
- The course of the disease is rapid, unless diagnosed and treated soon enough, typically within a few hours . Death may follow in one to six days.



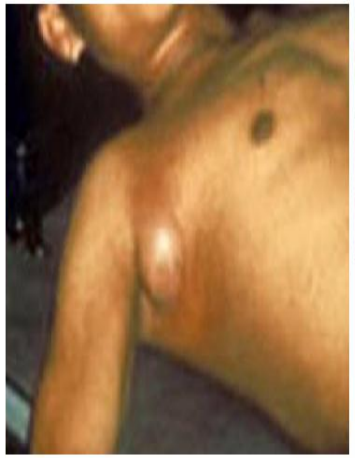
Septicemic plague

- When the bacteria enter the bloodstream directly and multiply there, it's known as septicemic plague. When they're left untreated, both bubonic and pneumonic plague can lead to septicemic plague.

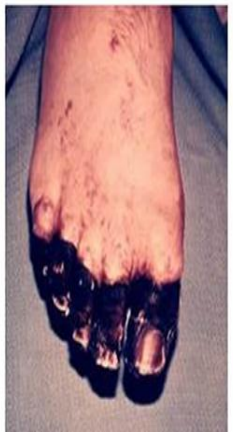
- Septicemic plague is the least common of there form with a mortality rate close to one hundred percent .



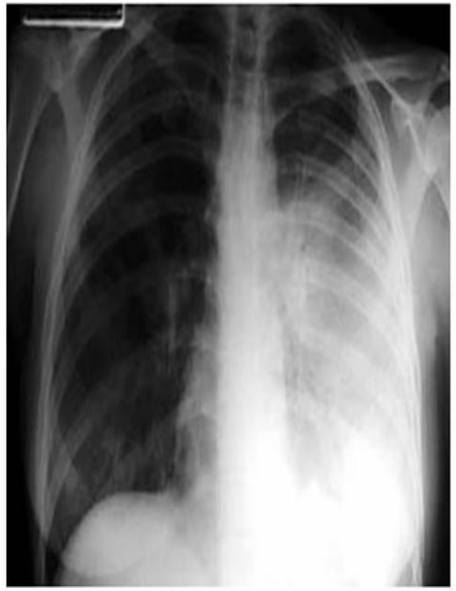
Bubonic



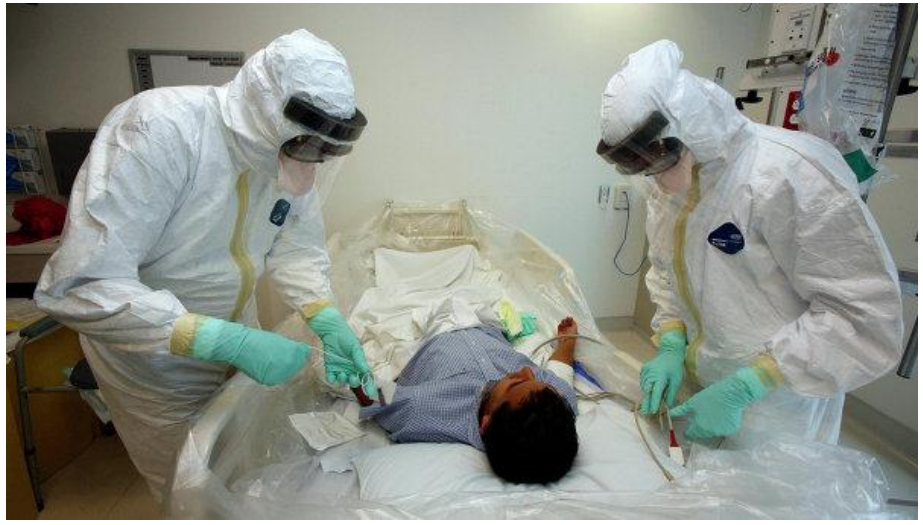
Septicaemic



Pneumonic



Diagnosis of plague is carried out in a special laboratory.



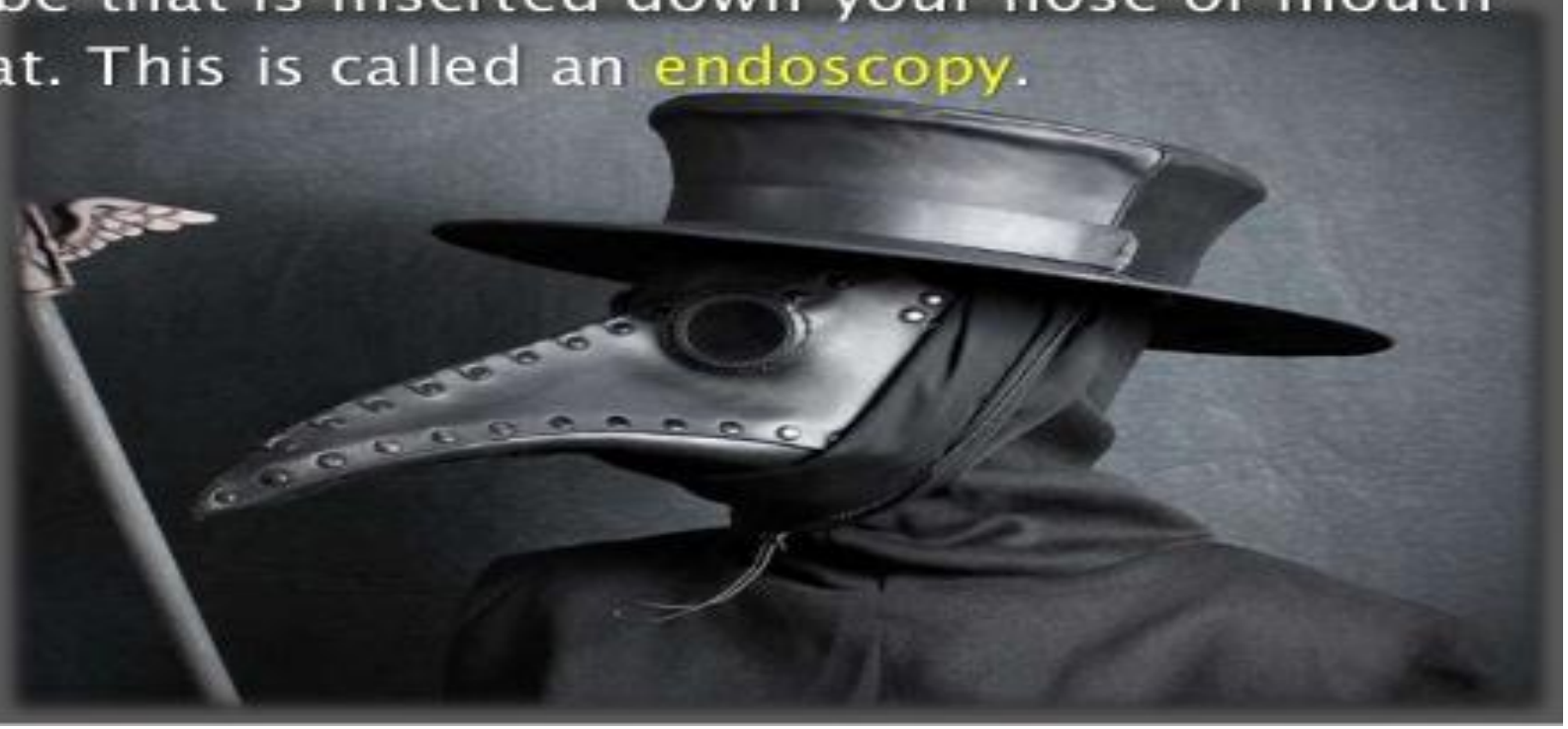
Diagnostic techniques

1. A blood test can reveal if you have septicemic plague.

2. To check for bubonic plague, use a needle to take a sample of the fluid in swollen lymph nodes.

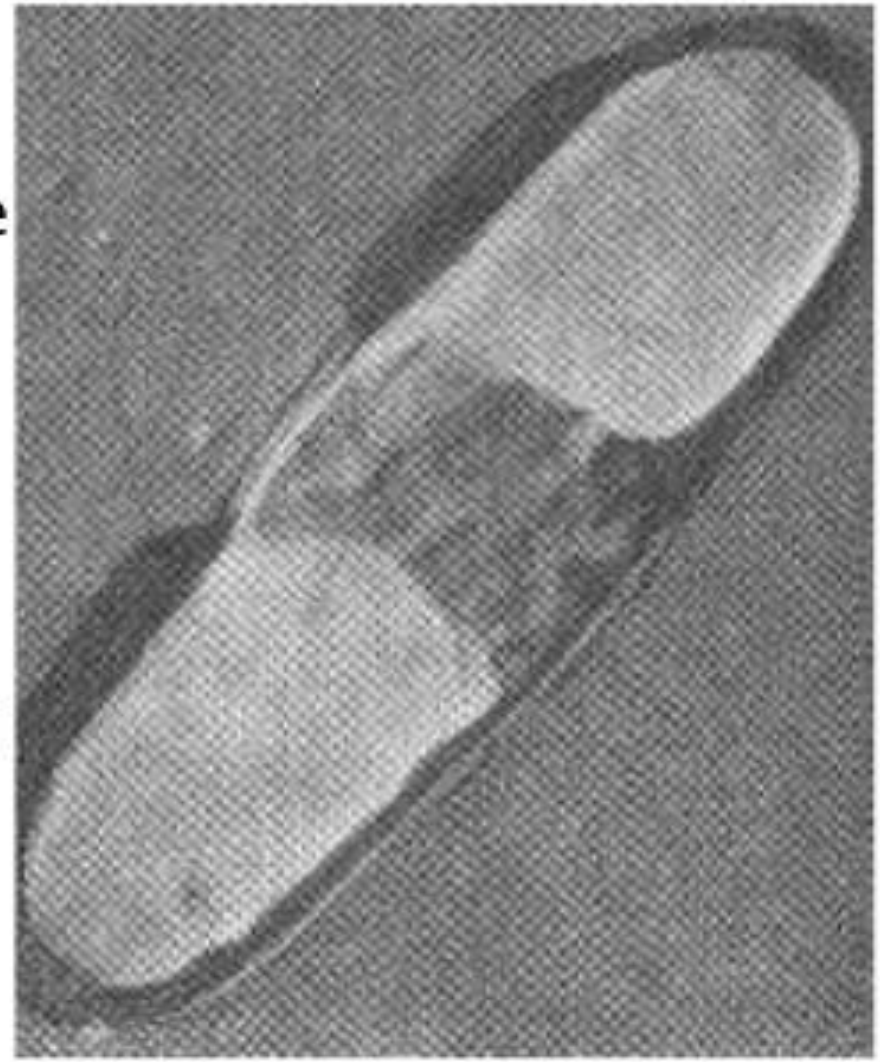
3. To check for pneumonic plague, fluid will be extracted from your airways by a tube that is inserted down your nose or mouth and down your throat. This is called an endoscopy.

4. By using X-ray .



LABORATORY DIAGNOSIS

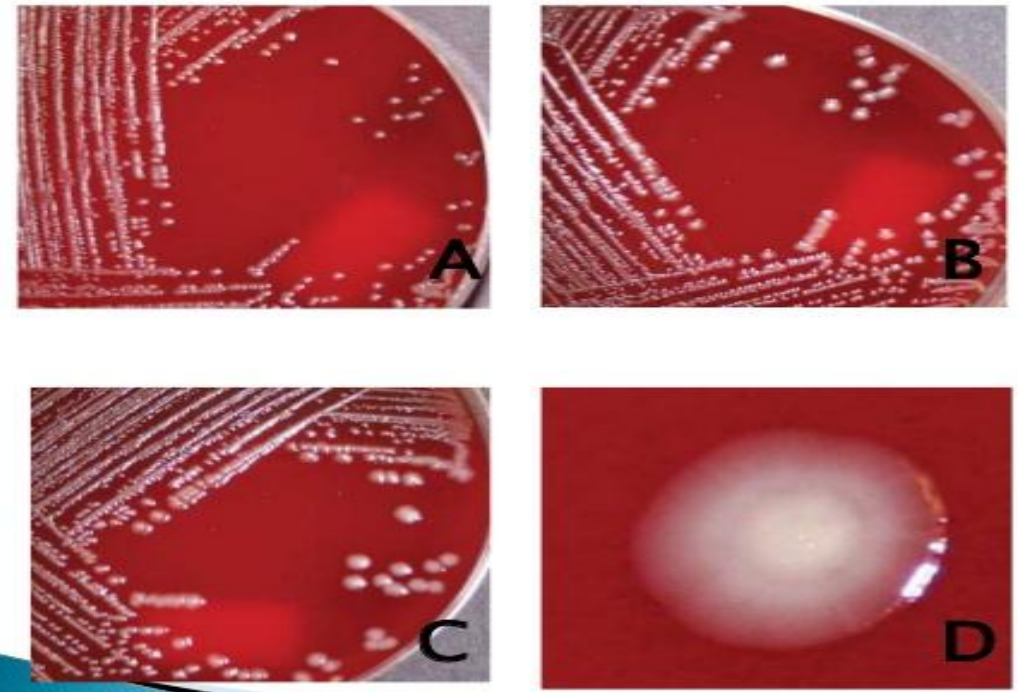
- The organism gives a bipolar "closed-safety pin" appearance on Giemsa, Wright, or Wayson stains (but not on Gram stain) (Guarner *et al.*, 2002).
- The organism also may be identified via immunohistochemical staining using a monoclonal anti-F1 *Y. pestis* antibody on formalin-fixed tissue samples
- Plate 1 (Guarner *et al.*, 2002).



• Colonies are smooth, opaque, and round but may have irregular edges. Under magnification, colonies can be smooth or finely granular and might have a raised center with a flat periphery (“fried egg” appearance) or a “hammered copper” appearance (Brubaker, 1991).

• Colonies are visible on plates after 48 hours, and it is recommended that plates be incubated for a total of 7 days before being discarded(Smego *et al.*, 1999).

Yersinia pestis



Yersinia pestis growth on BA at

(A) 48 h,

(B) 72 h,

(C) 96 h,

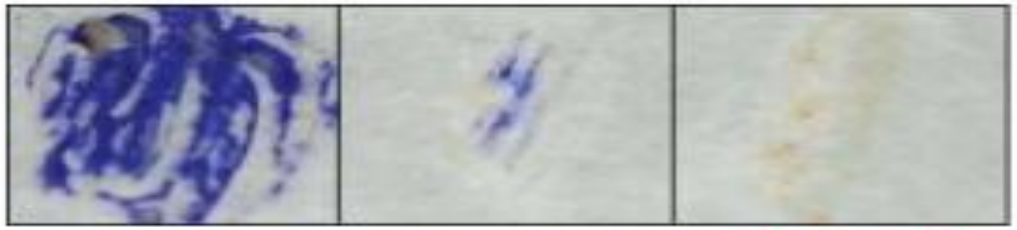
(D) 96 h “Fried egg” Plate 2. wadsworth center , 2007.

BIOCHEMICAL TESTS

CATALASE TEST

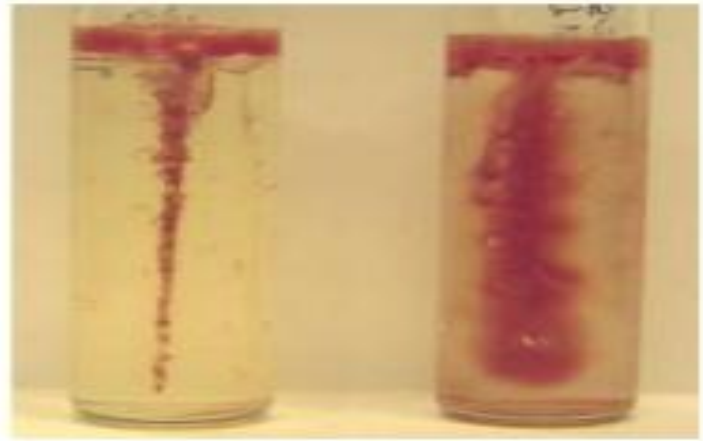
POSITIVE WEAKLY POSITIVE NEGATIVE

OXIDASE TEST



POSITIVE WEAKLY POSITIVE NEGATIVE

MOTILITY TEST



NON MOTILE MOTILE

UREASE TEST

NEGATIVE POSITIVE

- Catalase positive
- Urease, indole and oxidase negative
- Non- motile at 35 –37^{0}C (Wadsworth Centre, 2007)

Treatment

If diagnosed in time ,the various forms of plague are usually highly responsive to antibiotic therapy. The antibiotics often used are Streptomycin, Chloramphenicol and Tetracycline. the newer generation of antibiotics are Gentamicin and Doxycycline have proven to use against this bacteria.

Vaccination

- Natural or induced immunity is achieved by the production of specific antibodies against F1 and V antigens; antibodies against F1 and V induce phagocytosis by neutrophils.
- The (USAMRIID) have found that an experimental F1/V antigen-based vaccine protects crab-eating macaques but fails to protect African green monkey species , but then it was solved by changing more genes.

In a new study , researchers tested three vaccines that were designed to protect people against infection from the bacteria that cause plague , to create this vaccine researchers modified several genes of bacteria so that they couldn't cause disease , specifically the vaccines were designed to protect people against pneumonic plague.

Francisellaceae - TAXONOMY

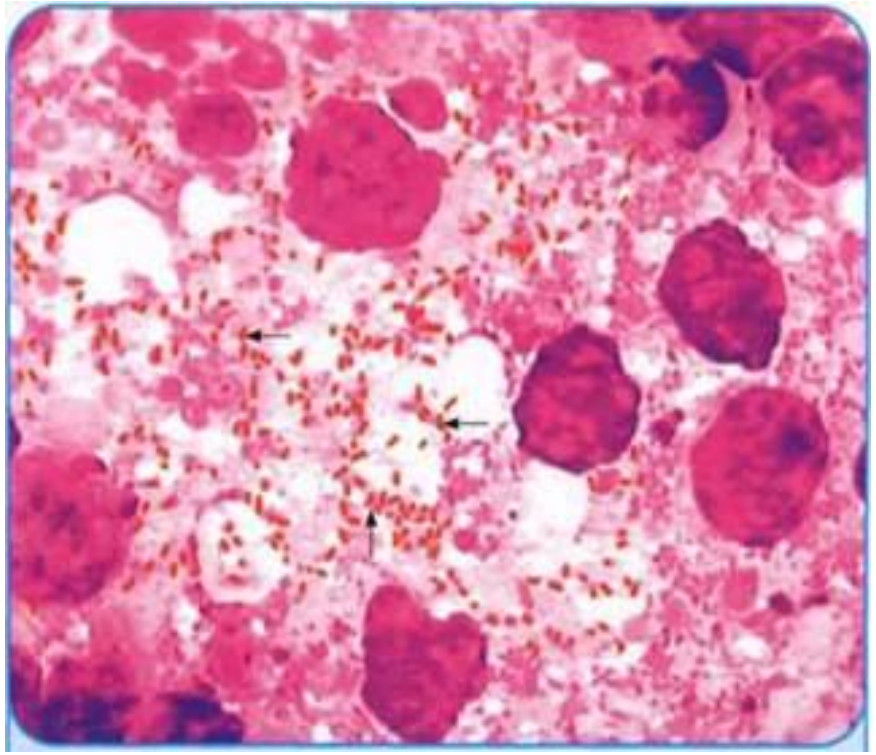
- (Domain): Bacteria
- (Kingdom): Pseudomanadota
- (Class): Gammaproteobacteria
- (Order): Thiotrichales
- (Family): Francisellaceae
- (Genus): Francisella
- (Species): *F.tularensis*

Biovars : type A – tularensis (non-arctic or American type; high virulent)

type B – palearctika (European and Asian type; low virulent)

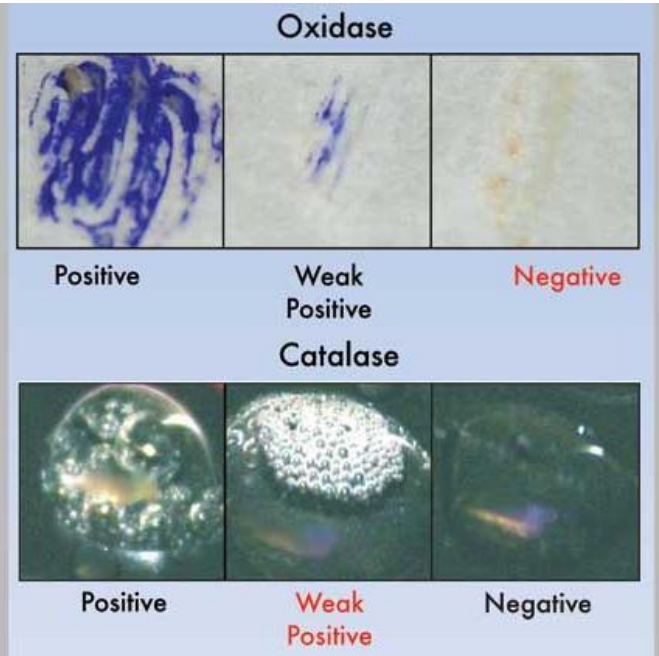
Francisella tularensis
Morphology and Physiology I

- Small, weakly staining gram-negative coccobacillus 0.2 to 0.2 – 0.7 um in size.
- Nonmotile, displays bipolar staining with Giemsa stain, obligate anaerobe, and is weakly catalase positive.
- Young cultures are relatively uniform in appearance while older cultures display extreme pleomorphism.
- Carbohydrates are dissimilated slowly with the production of acid but no gas.
- Displays a thick capsule whose loss is accompanied by loss of virulence.



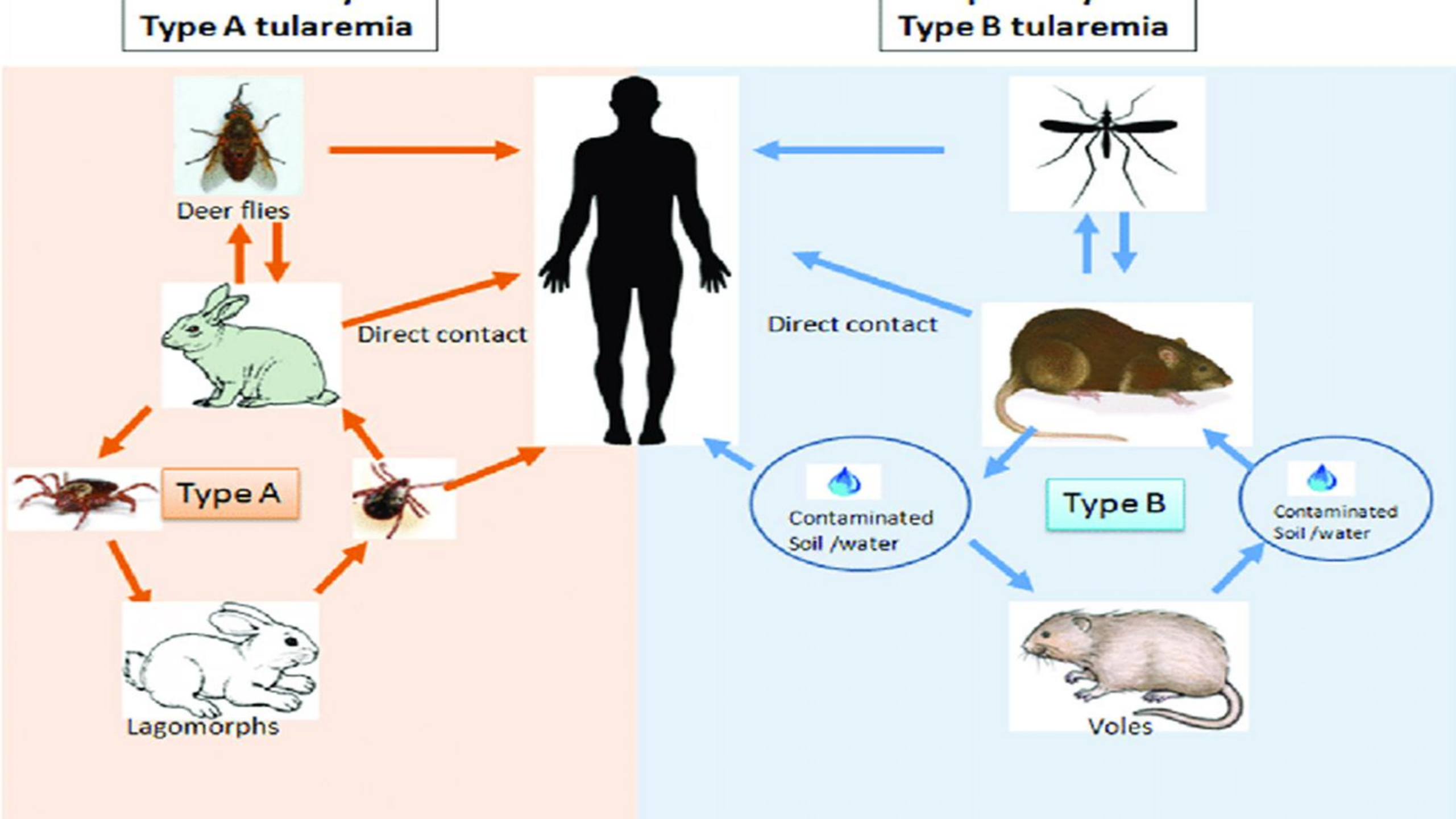
Francisella tularensis
Culture Characteristics

- Optimal growth at 37^0 C, growth range 24^0 to 39^0 C. Survival rate is best at lower temperatures.
- Slow growing with a requirement for iron and cysteine or cystine.
- No growth on routine culture media but small colony growth after 2 - 4 days on glucose-cysteine-blood agar or peptone-cysteine agar.
- No true hemolysis on blood containing media only a greenish discoloration.

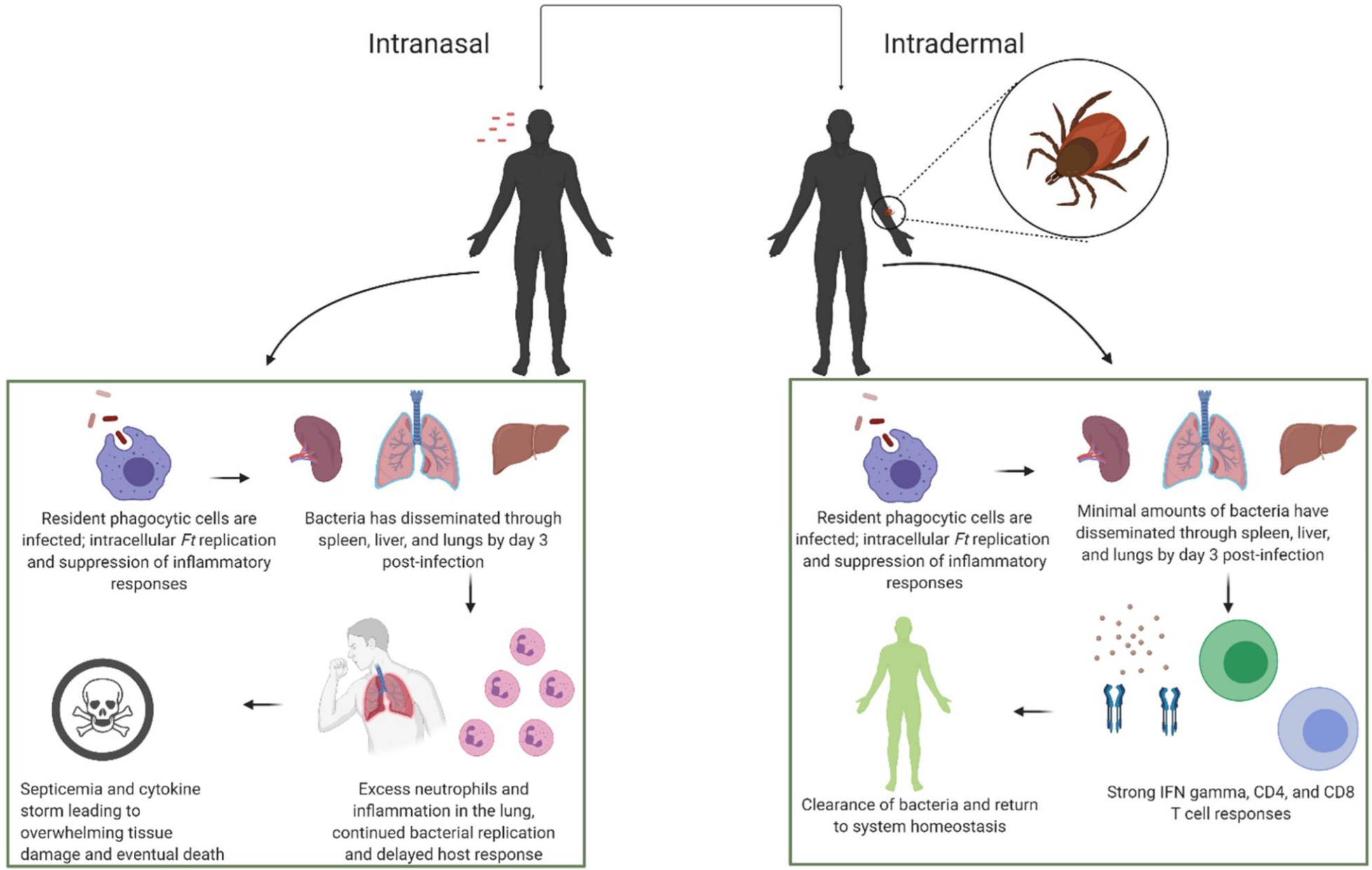


Etiology

- People can become sick with tularemia, but it's not a disease that naturally occurs in humans. It often affects rabbits and other animals including rodents, sheep, and birds. House pets like dogs and cats can get tularemia too.
- These are some of the ways people can get it:
- Insect bites, especially from a deer fly or tick
- Coming into contact with the skin, hair, or meat of an animal that's infected
- Consuming contaminated water or food, such as undercooked meat
- Breathing in bacteria that comes up from the soil during an activity like construction or gardening
- It's also possible to become infected if you're exposed to the bacteria in a laboratory setting, or potentially, in an act of bioterrorism.


Type A tularemia
Type B tularemia
Deer flies
Direct contact
Type A
Lagomorphs
Direct contact
Contaminated
Soil /water
Type B
Contaminated
Soil /water
Voles

Francisella tularensis Infection



Pathogenesis

- Infection with *F. tularensis* can occur by several routes. Portals of entry are through blood and the respiratory system. The most common occurs via skin contact, yielding an ulceroglandular form of the disease. Inhalation of bacteria - particularly biovar *F. t. tularensis*, leads to the potentially lethal pneumonic tularemia. While the pulmonary and ulceroglandular forms of tularemia are more common, other routes of inoculation have been described and include oropharyngeal infection due to consumption of contaminated food and conjunctival infection due to inoculation at the eye.
- *F. tularensis* is capable of surviving outside of a mammalian host for weeks at a time and has been found in water, grassland, and haystacks. Aerosols containing the bacteria may be generated by disturbing carcasses due to brush cutting or lawn mowing; as a result, tularemia has been referred to as "lawnmower disease". Recent epidemiological studies have shown a positive correlation between occupations involving the above activities and infection with *F. tularensis*.

Lifecycle

- *F. tularensis* is a facultative intracellular bacterium that is capable of infecting most cell types, but primarily infects macrophages in the host organism. Entry into the macrophage occurs by phagocytosis and the bacterium is sequestered from the interior of the infected cell by a phagosome. *F. tularensis* then breaks out of this phagosome into the cytosol and rapidly proliferates. Eventually, the infected cell undergoes apoptosis, and the progeny bacteria are released to initiate new rounds of infection.

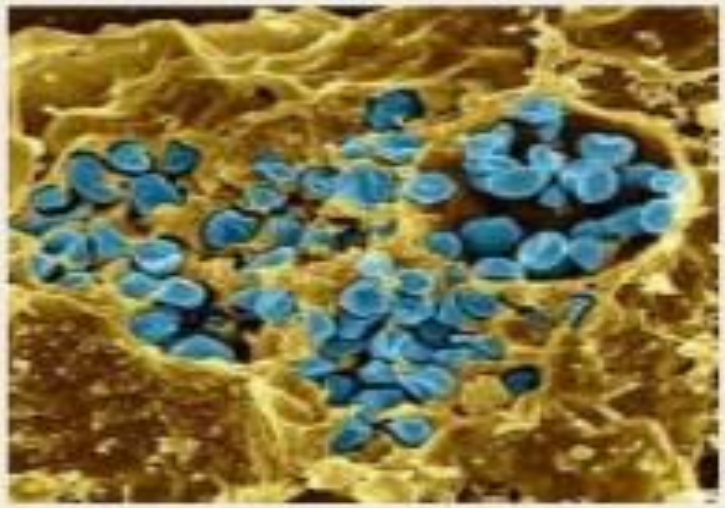
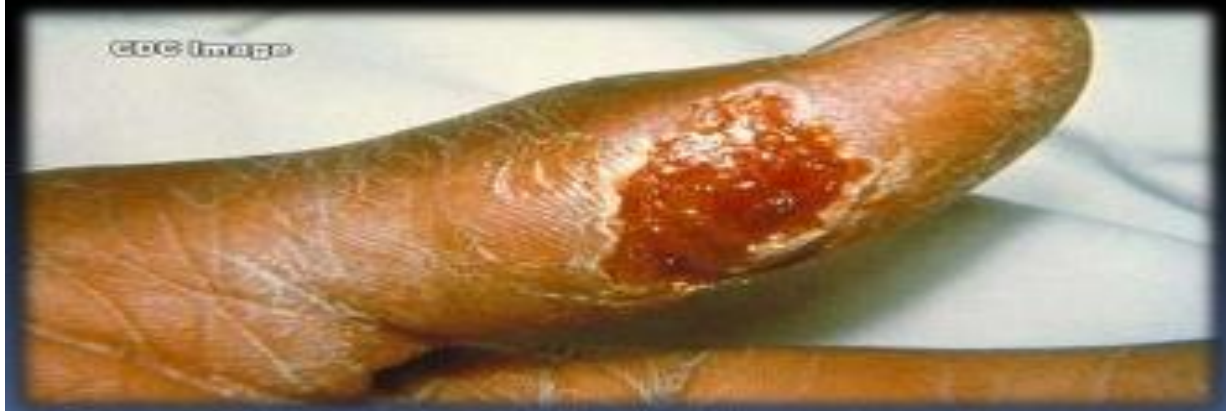


Fig : Francisella tularensis bacteria (blue) infecting a macrophage (yellow)

What is Tularemia?

Tularemia is a rare infectious disease that can attack your skin, lungs, eyes, and lymph nodes. Sometimes it's called rabbit fever or deer fly fever. It's caused by a bacteria called *Francisella tularensis*.

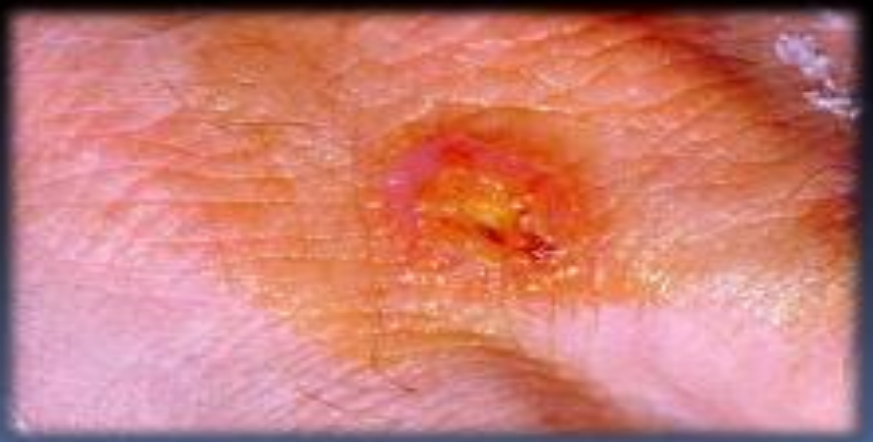


Epidemiology

- The majority of infections in humans and animals are caused by *F. tularensis* subspecies *tularensis* (the more virulent species) and *F. tularensis* subspecies *holarctica*. Human disease is rarely associated with the subspecies *novicida*, *Francisella philomiragia*, and *Francisella hispaniensis* .

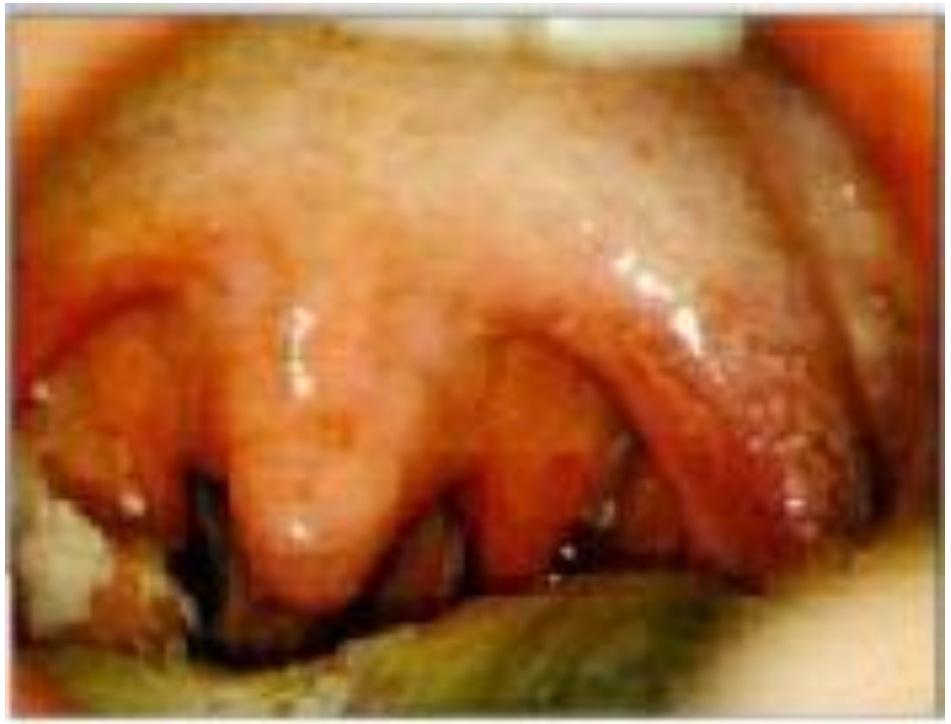
Symptoms

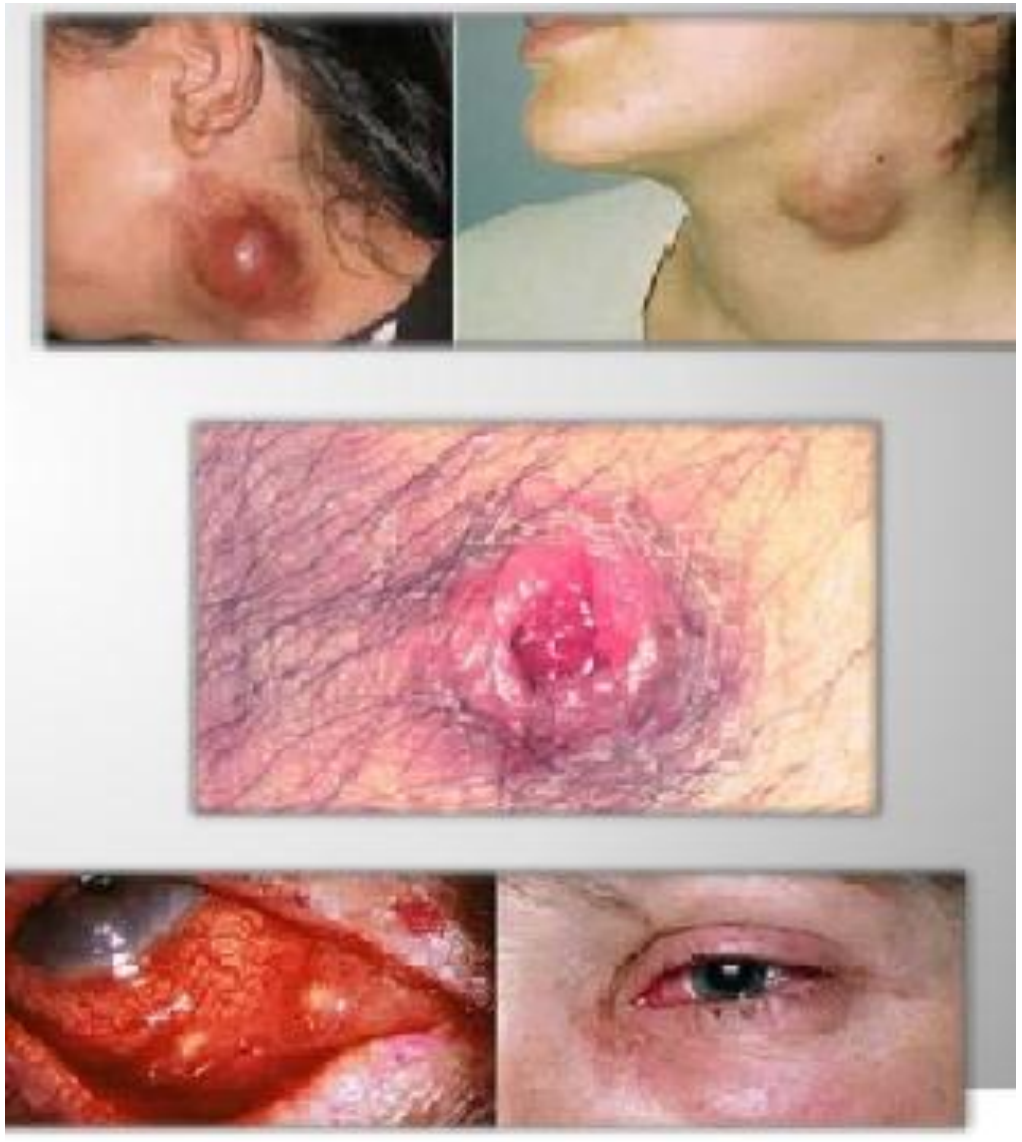
- **Ulceroglandular tularemia** is the most common variety of the disease. Symptoms can include:
- Lymph glands that are painful
- swollen
- Fever
- Chills
- Headache
- Fatigue
- **Oculoglandular tularemia** affects the eyes. Symptoms can include:
- Pain, swelling, or discharge in the eye
- Redness in the eye
- Light sensitivity
- An ulcer that forms inside the eyelid
- Tender lymph glands around the ear, neck, and jaw



Clinical manifestations of tularemia

(bubonic, oropharyngeal, ulceroglandular, oculoglandular forms)





Diagnosis & Treatment

- Tularemia can be difficult to diagnose. It is a rare disease, and the symptoms can be mistaken for other, more common, illnesses. For this reason, it is important to share with your health care provider any likely exposures, such as tick and deer fly bites, or contact with sick or dead animals.

- Blood tests and cultures can help confirm the diagnosis. Antibiotics used to treat tularemia include streptomycin, gentamicin, doxycycline, and ciprofloxacin. Treatment usually lasts 10 to 21 days depending on the stage of illness and the medication used. Although symptoms may last for several weeks, most patients completely recover.

Prevention

- When hiking, camping or working outdoors:
  - *Use insect repellents containing 20% to 30% DEET (N,N-diethyl-meta-toluamide), picaridin or IR3535. EPA provides information on the proper use of repellents.*
  - *Wear long pants, long sleeves, and long socks to keep ticks and deer flies off your skin.*
  - *Remove attached ticks promptly with fine-tipped tweezers.*
  - *Don't drink untreated surface water.*

- When mowing or landscaping:
  - *Don't mow over sick or dead animals. When possible, check the area for carcasses prior to mowing.*
  - *Use of masks during mowing and other landscaping activities may reduce your risk of inhaling the bacteria, but this has not been studied.*