AZERBAIJAN MEDICAL UNIVERSITY
DEPARTMENT OF MEDICAL MICROBIOLOGY and IMMUNOLOGY

Lesson 6.

Microbiology diagnosis of zoonotic infections (brucellosis, anthrax, listeriosis, plague, tularemia)

FACULTY: General Medicine
SUBJECT: Medical microbiology - 2

Discussed questions:

1. Zoonotic infections and their characteristics
2. Understanding of particularly dangerous infections, rules for working with their agents.
3. Morpho-biological characteristics of *Brucella*. Pathogenicity factors of Brucella, pathogenesis and clinical manifestations of brucellosis. Microbiological diagnosis of brucellosis. Diagnostic value of tests used in the serological method (approximate agglutination, Wright's reaction, Coombs' reaction, etc.). Specific treatment and prevention of brucellosis.
4. Understanding of bacteria from the genus *Bacillus*. Morpho-biological characteristics of antrax pathogens. Pathogenicity factors of *B.anthrachis*. Pathogenesis and clinical manifestations of antrax, microbiological diagnosis, specific treatment and prevention.
5. The causative agent of listeriosis - *Listeria monocytogenes*, its morpho-biological characteristics, ecology. Pathogenesis and clinical manifestations of listeriosis, the role of listeria in neonatal pathology. Microbiological diagnosis of listeriosis.
6. Yersinia genus. Plague - *Yersinia pestis*, its morpho-biological characteristics, pathogenicity factors. Pathogenesis and clinical manifestations of plague. Microbiological diagnosis, specific treatment and prevention of plague. The causative agents of intestinal yersiniosis - *Y.enterocolitica and Y.pseudotuberculosis*, their morpho-biological characteristics, pathogenesis and clinical manifestations, microbiological diagnosis
7. Morpho-biological characteristics of *tularemia* agents, pathogenicity factors. Pathogenesis and clinical manifestations of tularemia, microbiological diagnosis, specific treatment and prevention by serological, biological, skin-allergic and emergency methods.

Purpose of the lesson:

- To inform the students with the morpho-biological characteristics of the causative agents of brucellosis, black sore, listeriosis, plague and tularemia, the characteristics of the diseases they cause, microbiological diagnostic methods, specific treatment and prevention principles.

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.

BIOSAFETY LEVELs (BSL)

BSL-1

Lowest safety level

Not known to cause disease in adult human

Non-pathogenic microbe

BSL-2

Moderate danger if inhale, swallow or expose to skin

Influenza

BSL-3

Severe or potentially lethal disease

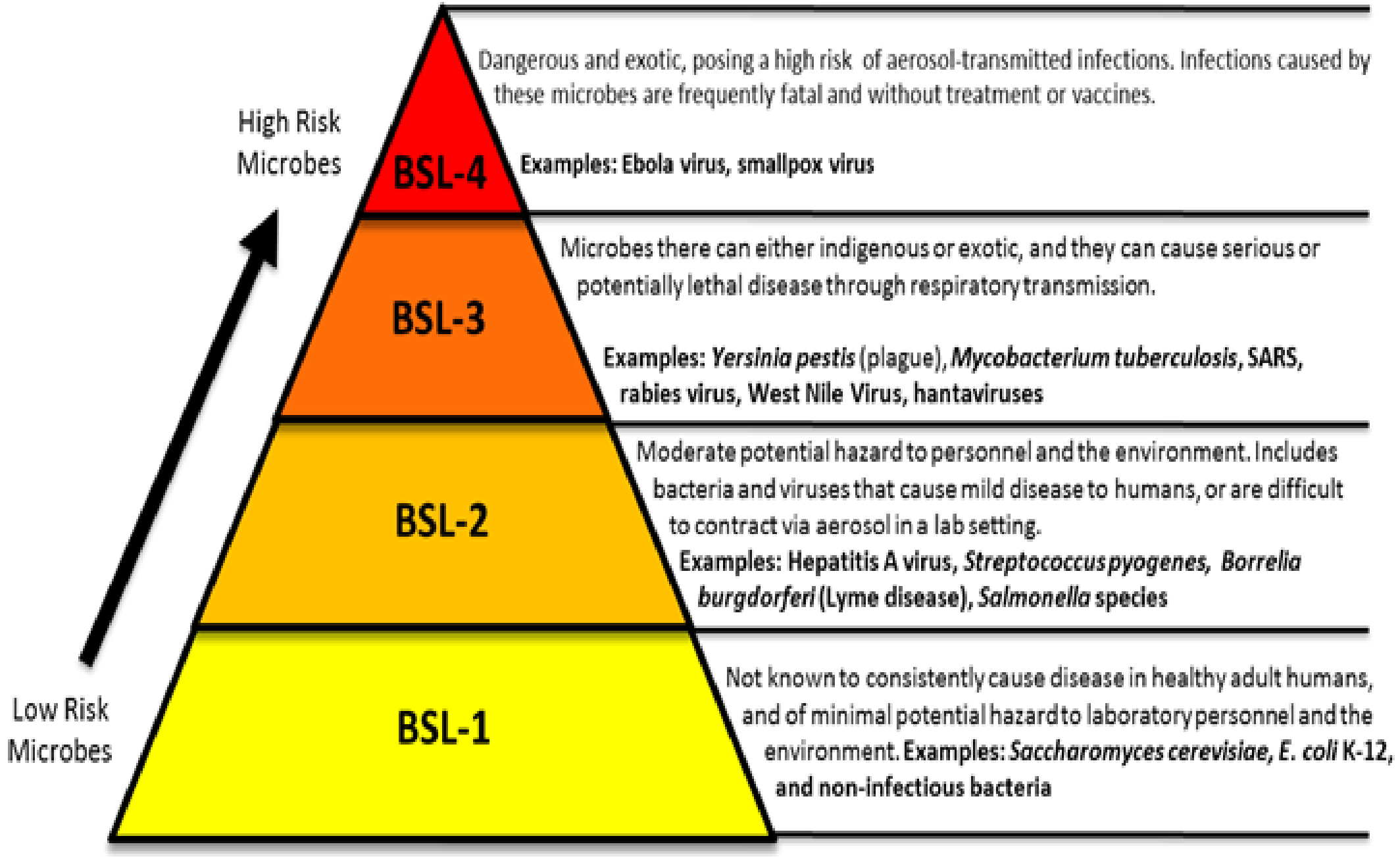
HIV, H5N1 flu

BSL-4

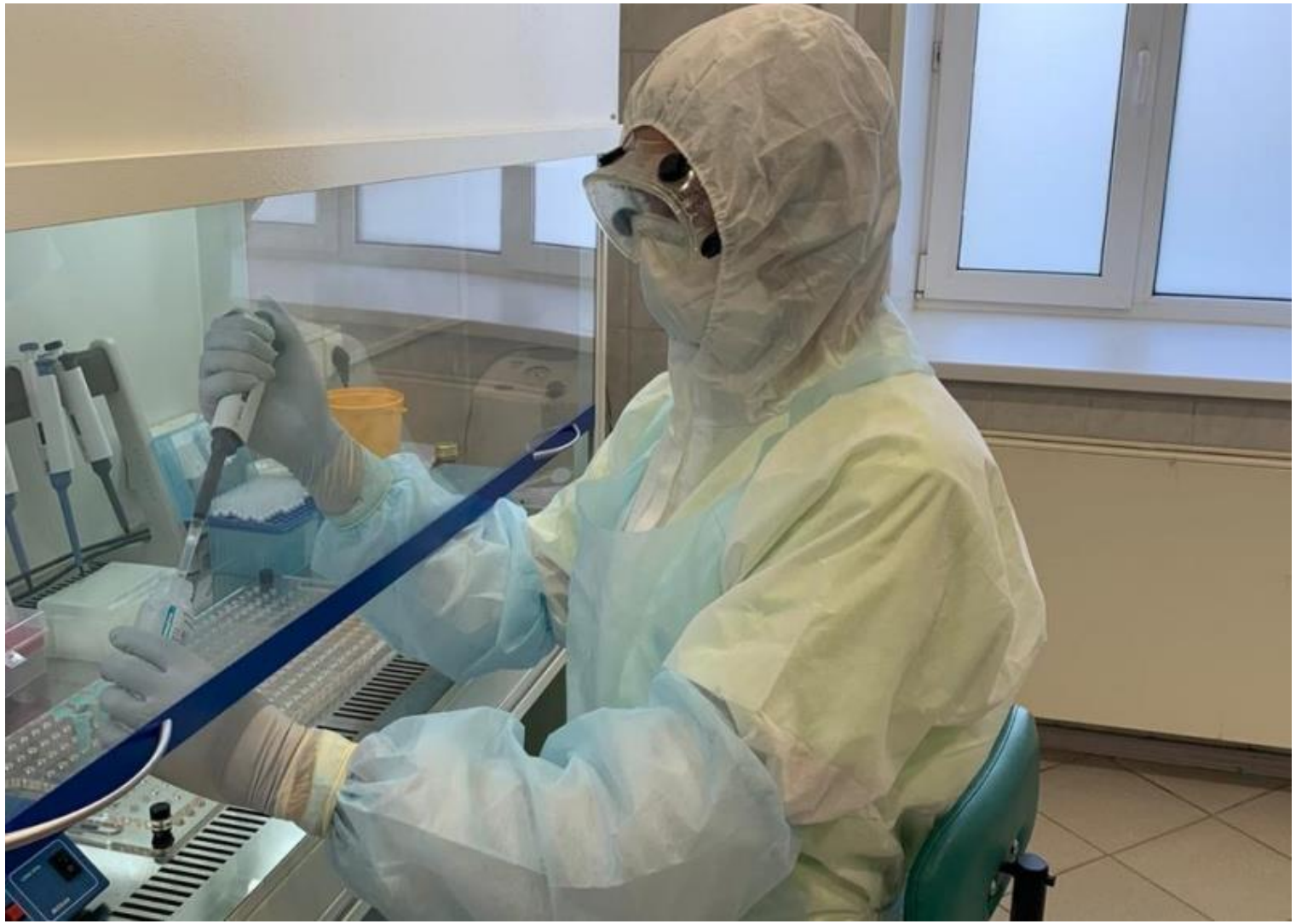
Highest safety level

Life threatening disease

Ebola, SARs - CoV2


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

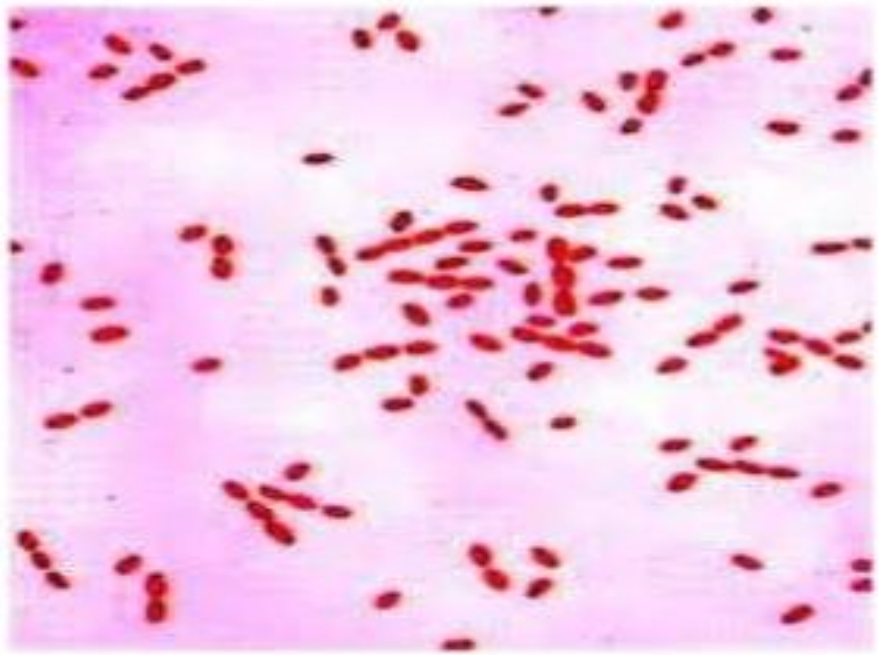
The procedure for working in a special regime laboratory



Brucellaceae - Taxonomy

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

BRUCELLA



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

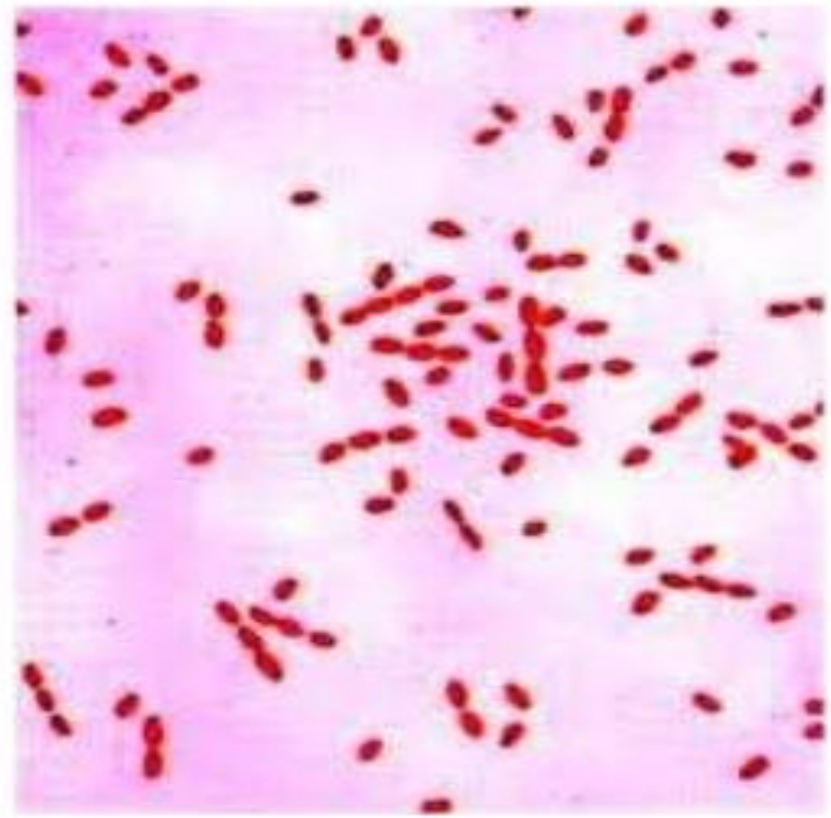
TAXONOMY

- 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	?

MORPHOLOGY

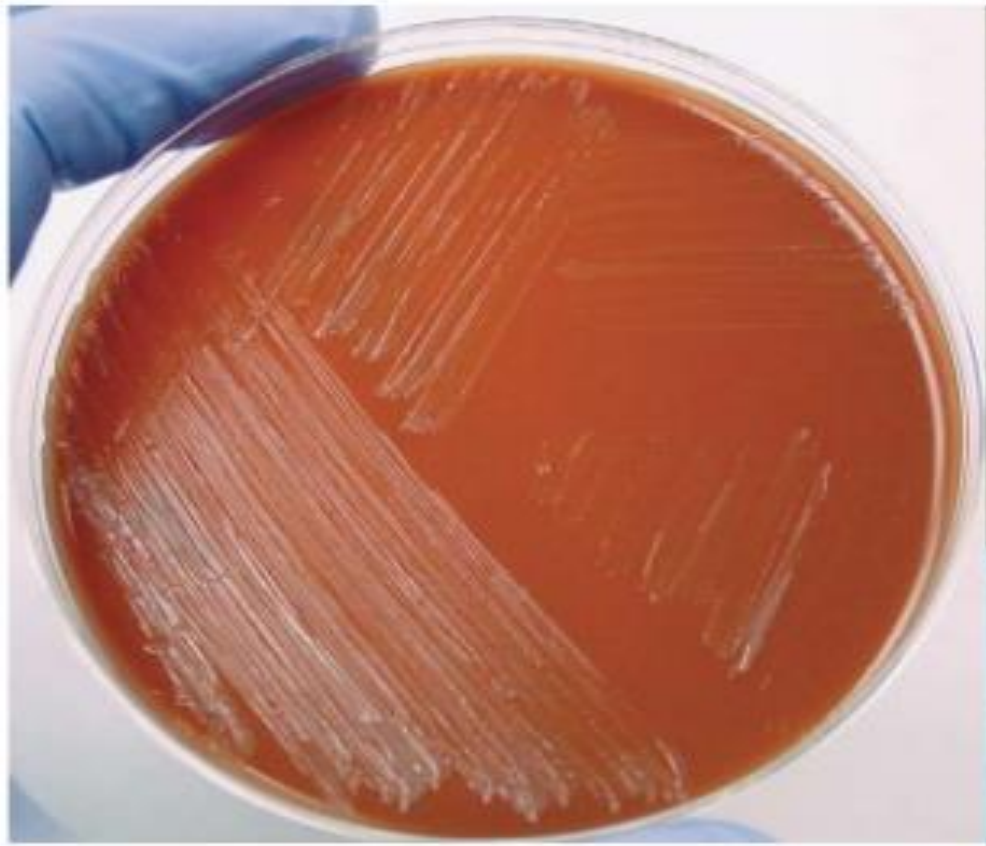
- Brucellae species are small, gram-negative aerobic coccobacilli, 0.5-0.7 µm x 0.6-1.5 µm in size.
- They are nonmotile, noncapsulated, nonsporing and non-acid fast.



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



BIOCHEMICAL REACTIONS

- No carbohydrates are fermented.
- Catalase and oxidase positive (except for Br. Neotomae and Br. ovis which are negative).
- Nitrite reduction positive,
- IMViC- All negative & Urease positive.

Species	CO_2 test	H_2S test	Urea test	Bacteriostatic dyes				
				Thionin			Fuchsin	
				A	B	C	A	B
B. melitensis	-	-	+	-	+	+	+	+
B. abortus	+	+	+	-	+	+	+	+
B. canis	-	-	+	+	+	+	+	+
B. suis	-	-	+	+	+	+	-	-
B. ovis	+	-	+	+	+	+	+	+

RESISTANCE

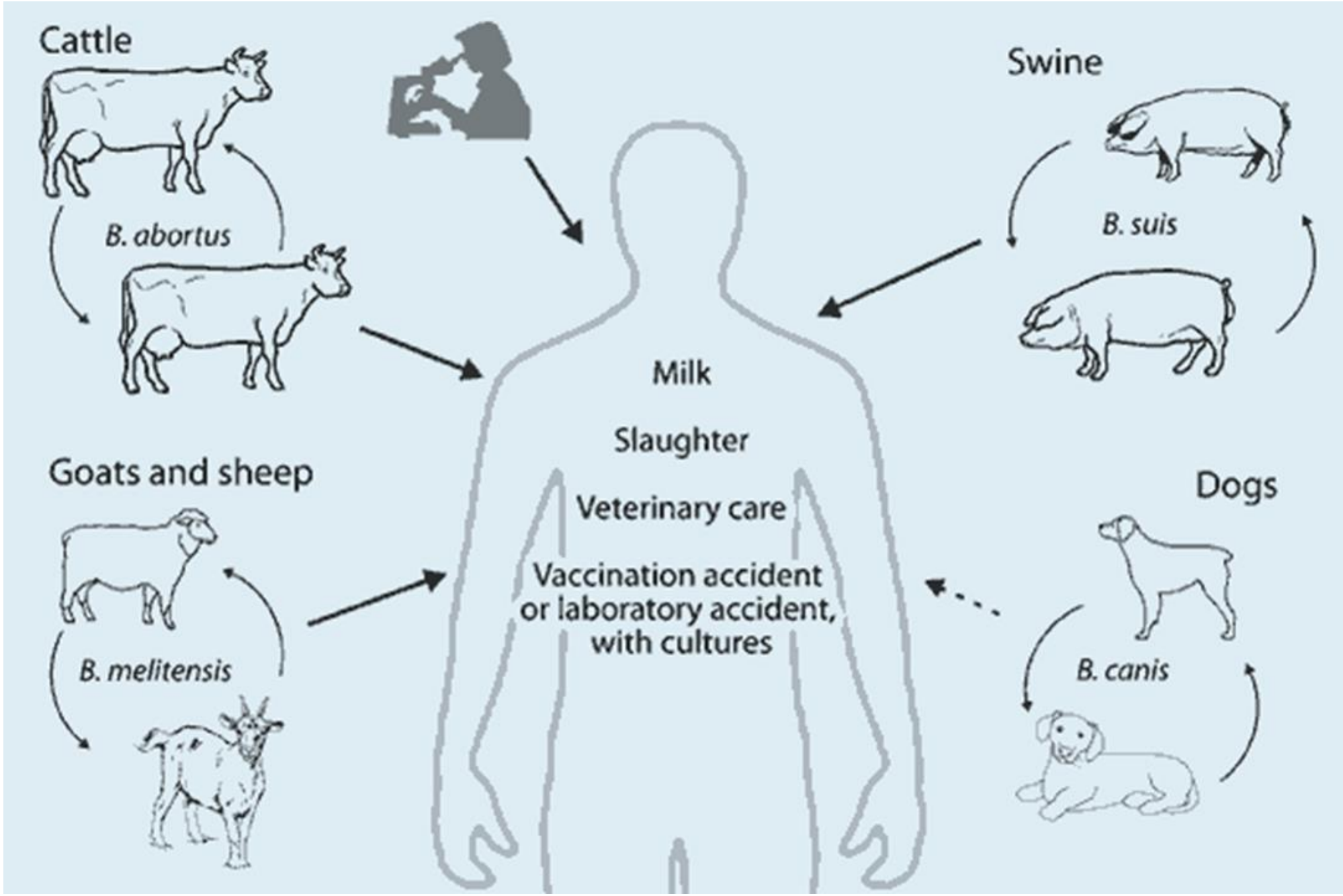
- Brucellae are destroyed
- by heat at 60 °C in 10 minutes
- by 1% phenol in 15 minutes.
- are killed by pasteurization.
- They may survive in soil and manure for several weeks.
- The organism survives for 10 days in refrigerated milk, for months in butter, one month in ice cream.
- They are sensitive 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**.


Cattle
B. abortus
Swine
B. suis
Milk
Slaughter
Veterinary care
Vaccination accident
or laboratory accident,
with cultures
Goats and sheep
B. melitensis
Dogs
B. canis

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.

PATHOGENESIS

- **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

↓

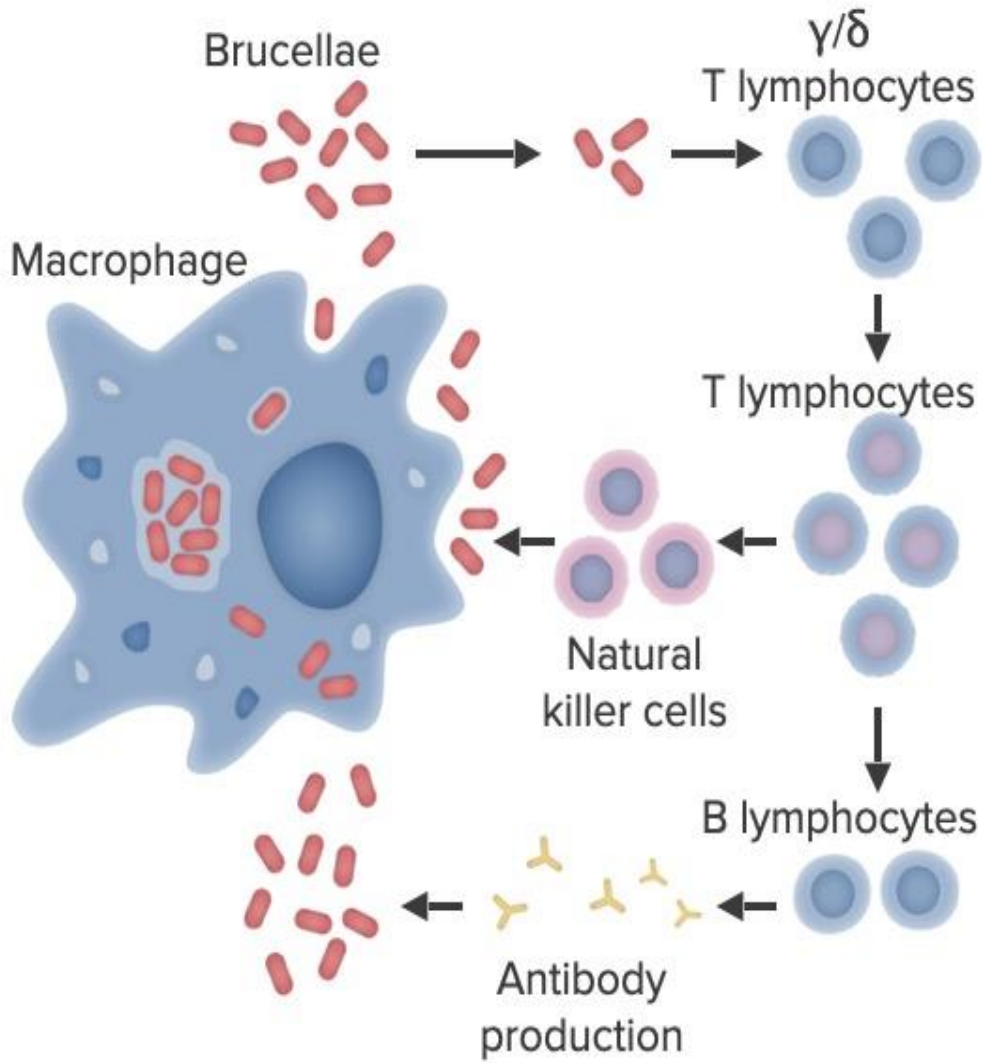
Infection

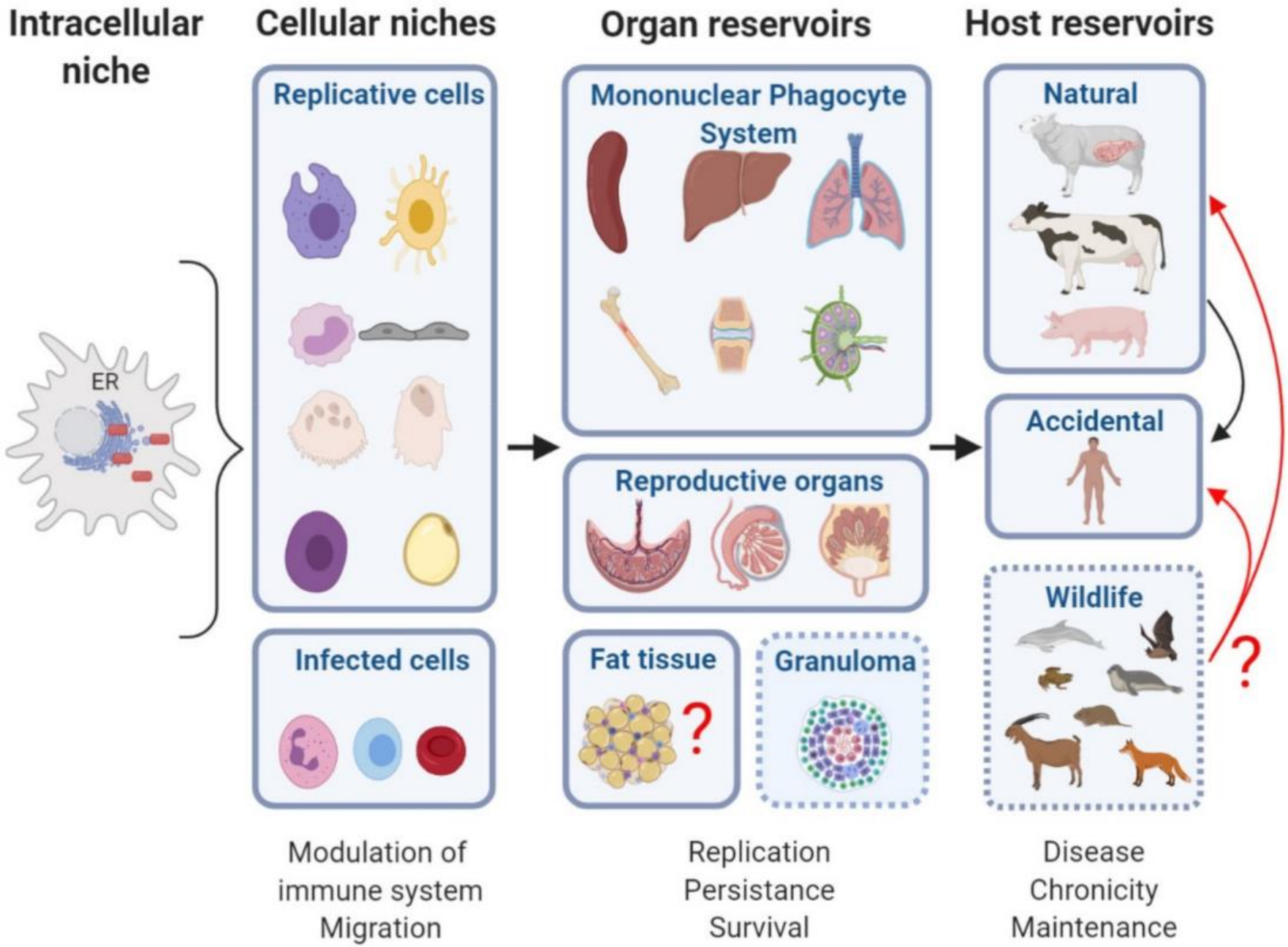
↓

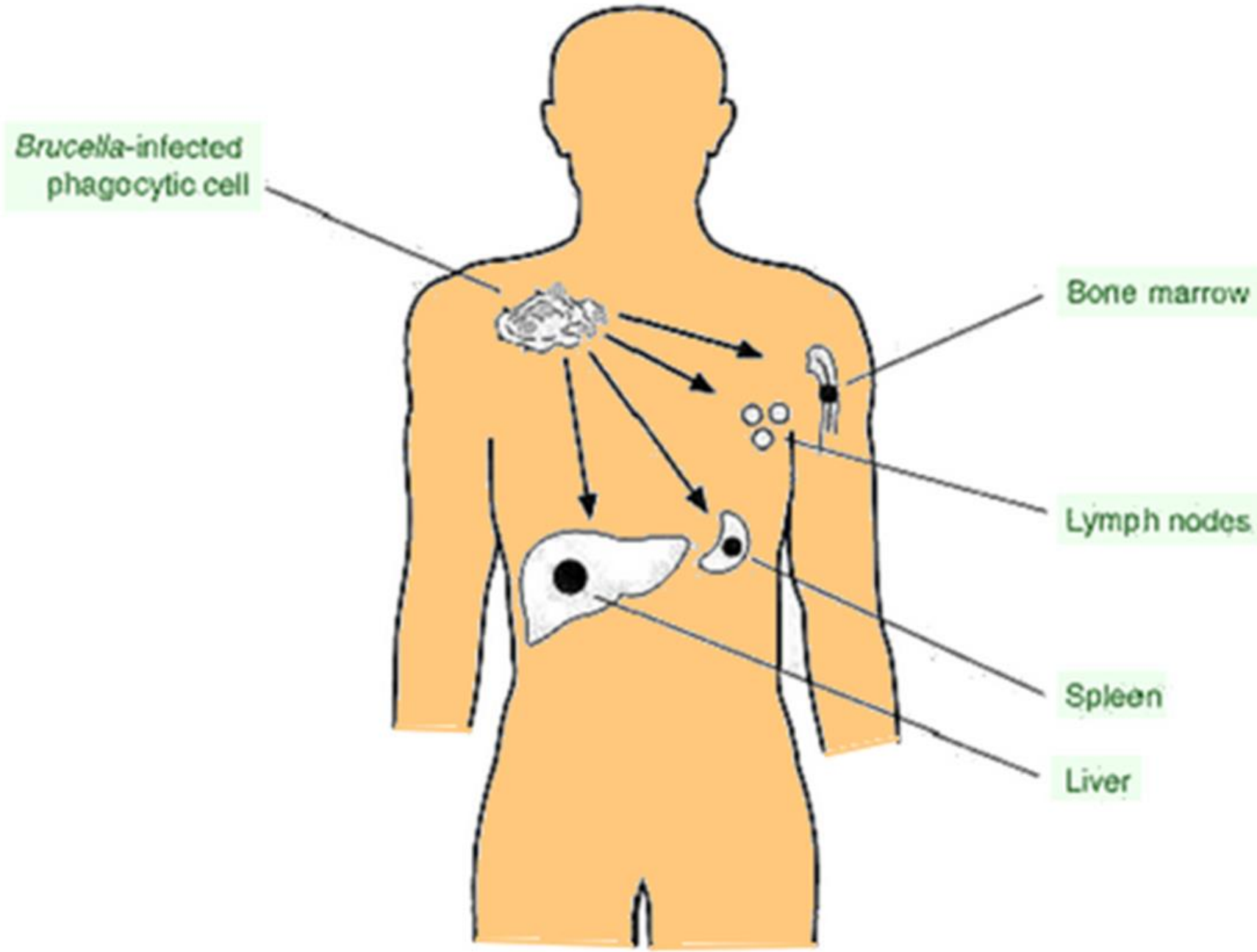
Host TH1 response

↓

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

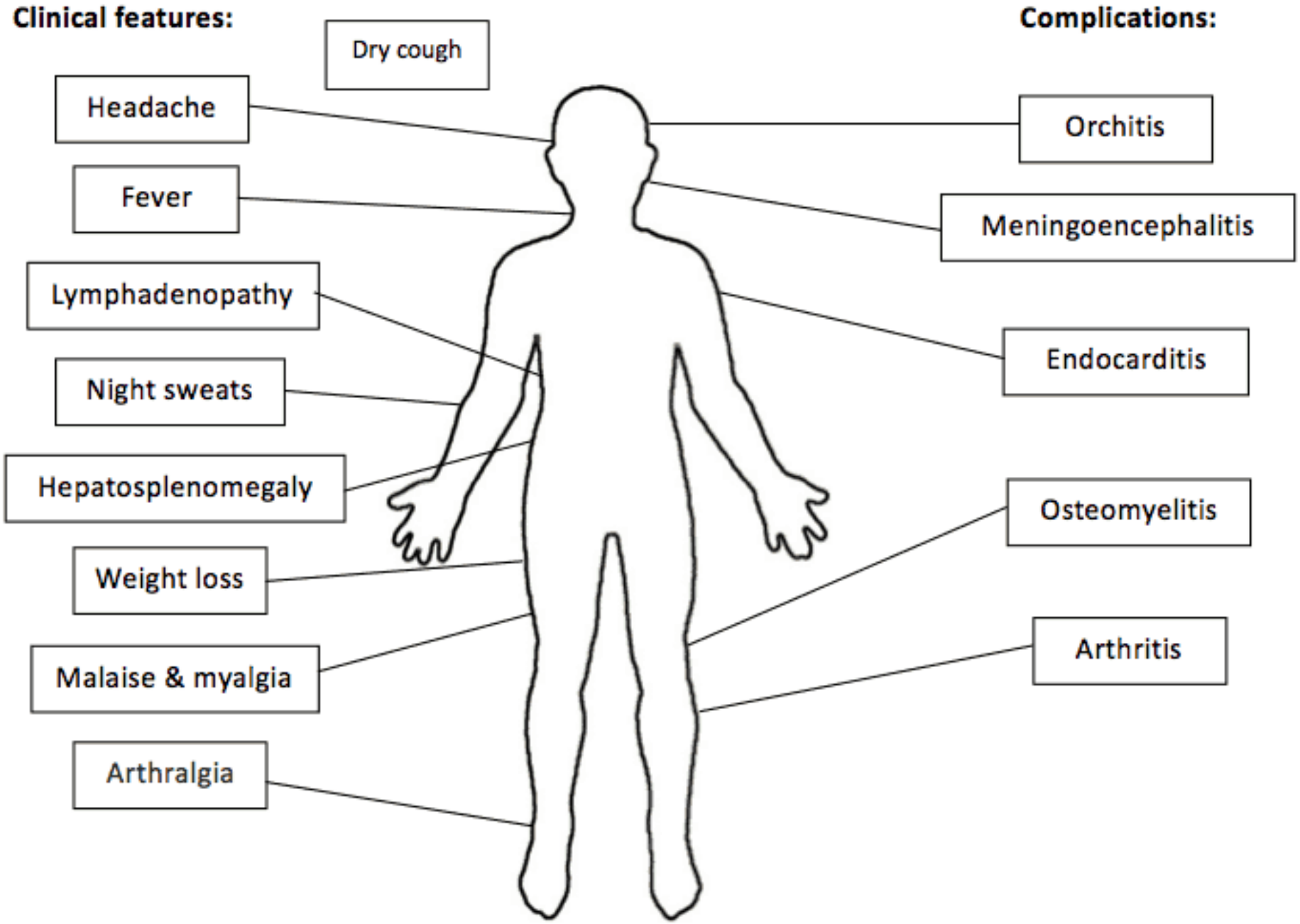
- Acute brucellosis is mostly due to Br melitensis.
- It is usually known as undulant fever, but this is misleading as only some cases show the undulant pattern
- It is associated with prolonged bacteraemia and irregular fever.
- The symptomatology is varied, consisting of muscular and articular pains, 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 features:
Dry cough
Headache
Fever
Lymphadenopathy
Night sweats
Hepatosplenomegaly
Weight loss
Malaise & myalgia
Arthralgia
Complications:
Orchitis
Meningoencephalitis
Endocarditis
Osteomyelitis
Arthritis

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

CLINICAL MANIFESTATIONS

- 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.

DIRECT DETECTION

- Conventional PCR.
- Real time PCR.
- Both these directly detect the Brucellae from clinical specimens.
- **MICROSCOPY –NO USE**
- Gram staining is not useful for demonstration of Brucella organisms in clinical specimens due to their small size and intracellular location.

CULTURE AND ISOLATION

Methods: **1.Castaneda's method, 2.Automated methods such as Bactec, 3. Lysis centrifugation system.**

- Blood culture is the most definitive method for the diagnosis of brucellosis.
- 5ml of Blood is inoculated into a bottle of 50 ml trypticase soy broth and incubated at 37 °C under 5-10% C02.
- Subcultures are made on solid media every 3-5 days, beginning on the fourth day. subcultures are made on solid media, every 3-5 days for 8 weeks before declaring the culture as negative.
- BACTEC cultures may become positive in 5 to 6 days.

CULTURE AND ISOLATION

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.

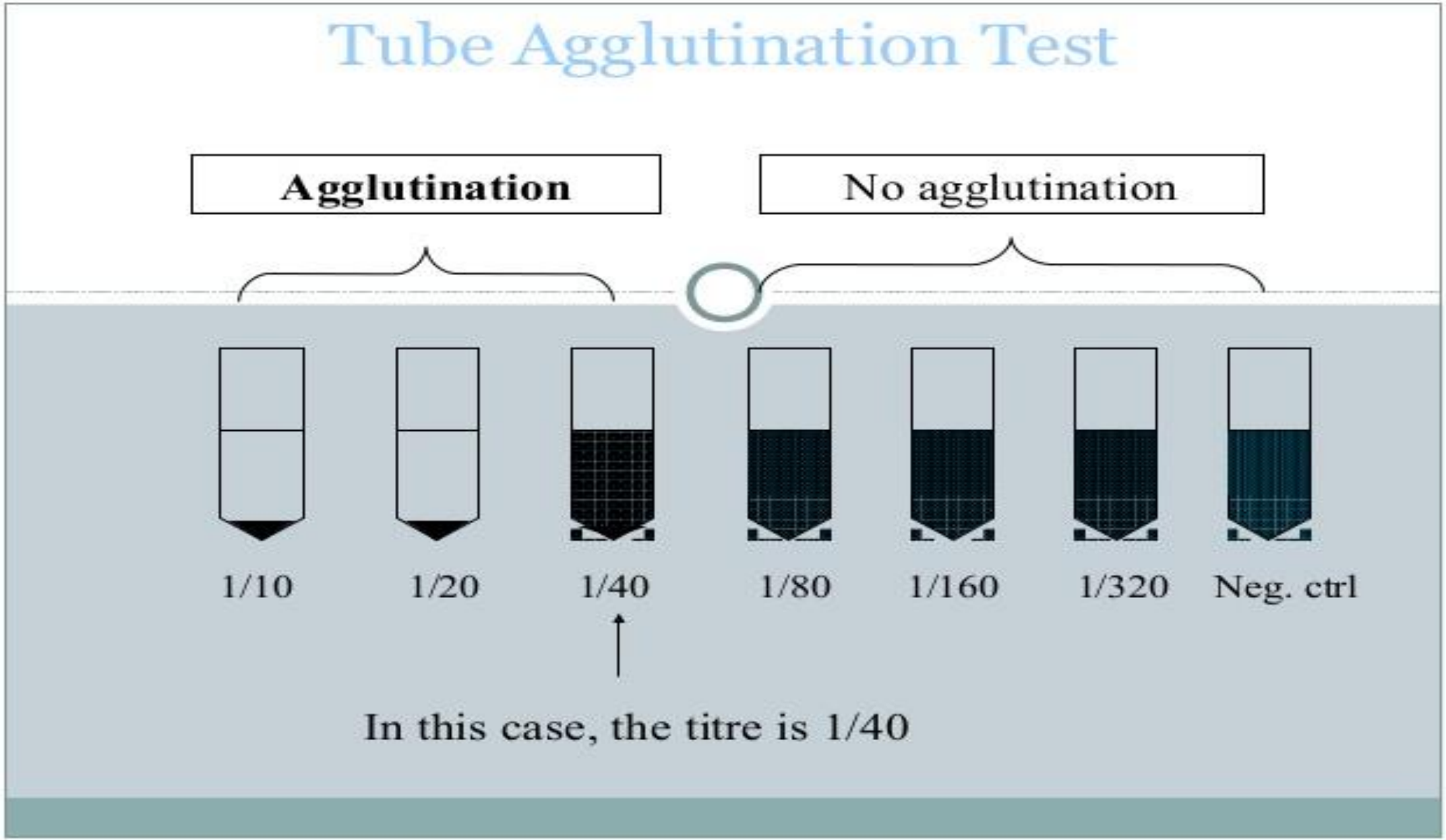
SEROLOGY

- 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.

Tube Agglutination Test

- Also known as the standard agglutination test or serum agglutination test (SAT)
- Test serum is diluted in a series of tubes (doubling dilutions)
- Constant defined amount of antigen is then added to each tube and tubes incubated for ~20h @37°C
- Particular antigen clumps at the bottom of the test tube
- Test is read at 50% agglutination
- Quantitative

Tube Agglutination Test



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.

SEROLOGY

- 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.**

Brucella skin test

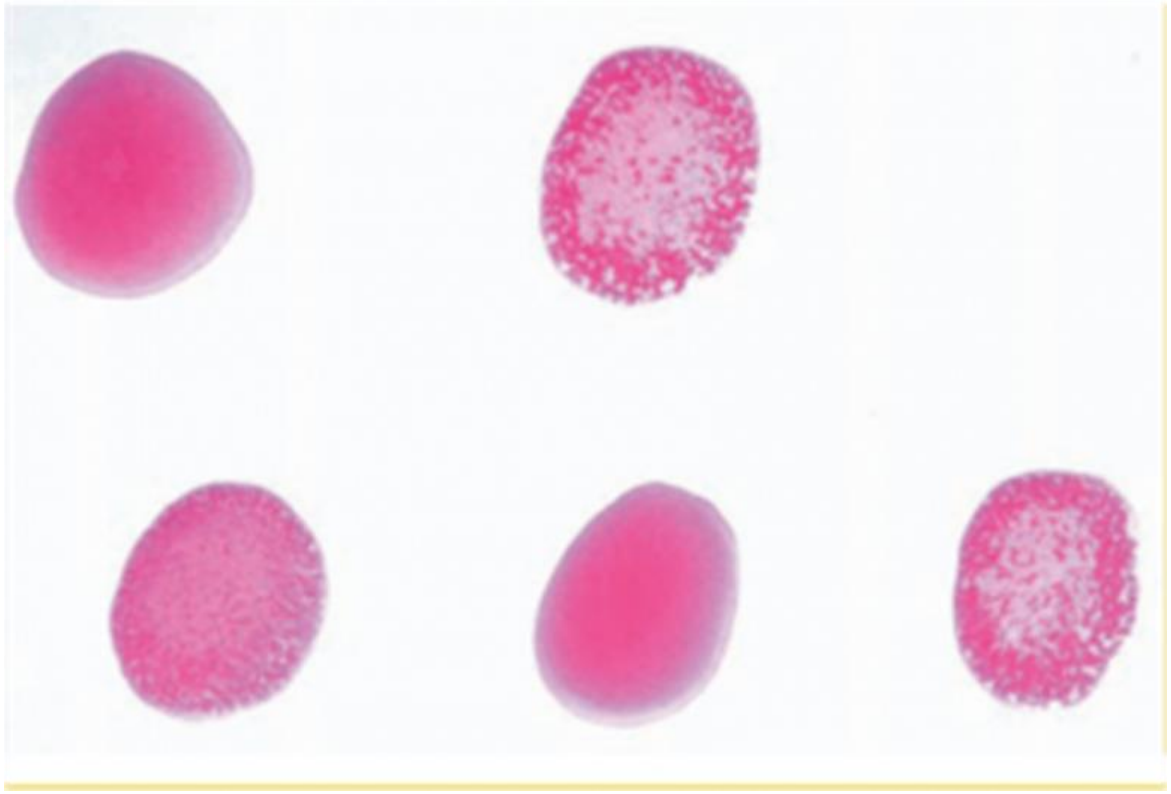
- Brucella skin test is a delayed type of hypersensitivity reaction to brucella antigen.
- In this test, brucellin, a protein extract of the bacteria, is used as an antigen and is administered intradermally.
- The presence of erythema and induration of 6 mm or more within 24 hours is suggestive of positive reaction.
- This test is positive only in chronic brucellosis but negative in acute brucellosis.
- Repeated negative skin test excludes brucellosis.

Laboratory diagnosis in animals

- These include:
- 1. *Rapid plate agglutination test and*
- *2.Rose Bengal card test.*

Rose bengal test

The tested serum (0.03 ml) is mixed with an equal volume of antigen on a glass plate.
After four minutes, agglutination is observed.
The reaction is considered positive when there is a visible precipitate.



Milk ring test

- This is a frequently used serological test for demonstration of antibodies in the milk of an animal.
- This is a screening test used to detect the presence of Brucella infection in infected cattle.
- In this test, a concentrated suspension **of killed B. abortus or B. melitensis stained with hematoxylin** is used as antigen.
- This test is performed by adding a drop of colored brucella antigen to a sample of whole milk in a test tube.
- Then it is mixed, and mixed suspension is incubated in a water bath at **70°C for 40-50 minutes**.
- In a positive test, if antibodies are present in the milk, the bacilli are agglutinated and raised with the cream to form a blue ring at the top, leaving the milk unstained.
- In a negative test, the milk remains uniformly blue without formation of any colored ring.

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.

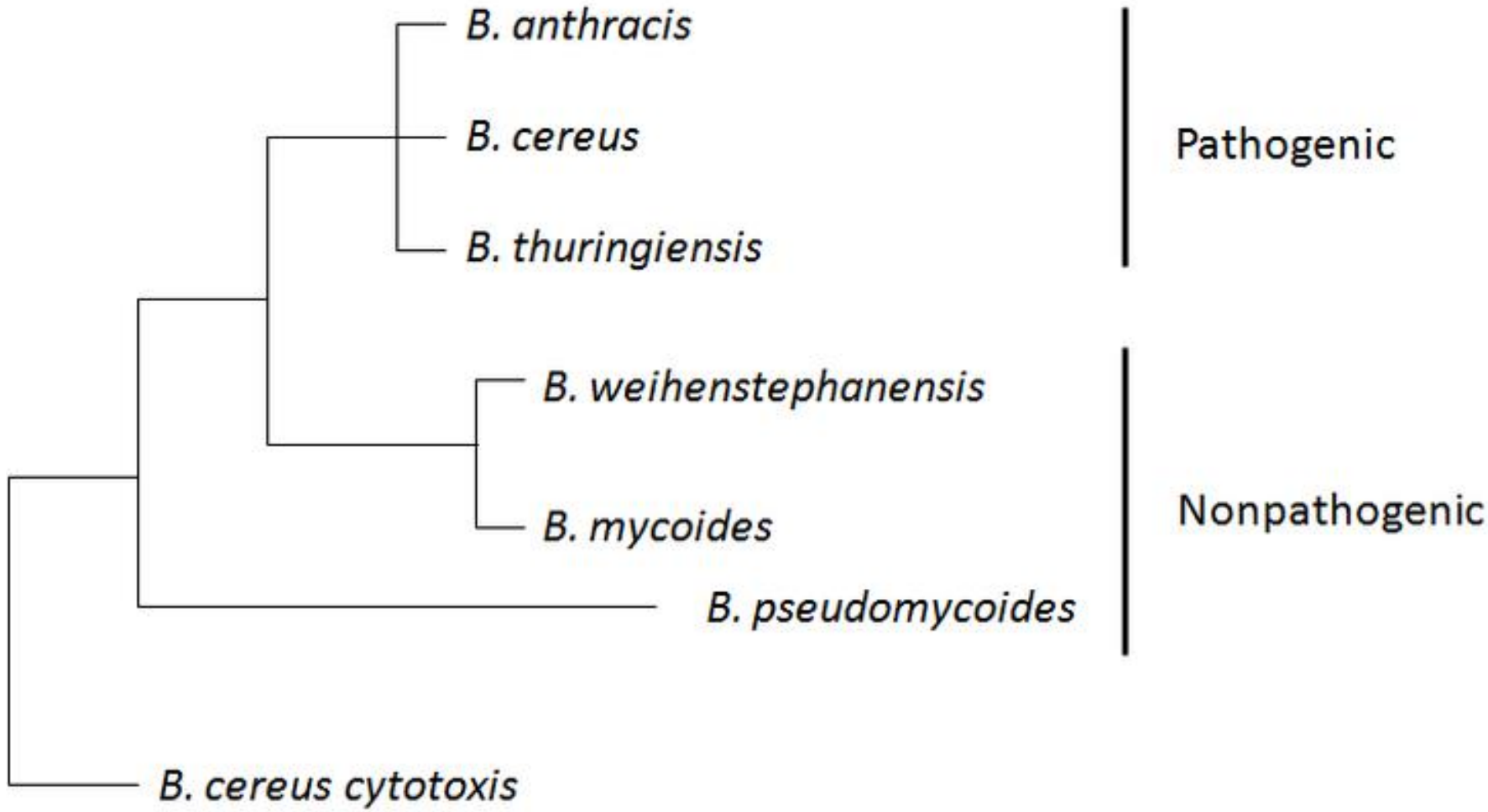
PREVENTION & CONTRPOL

- 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

Bacillus – Taxonomy

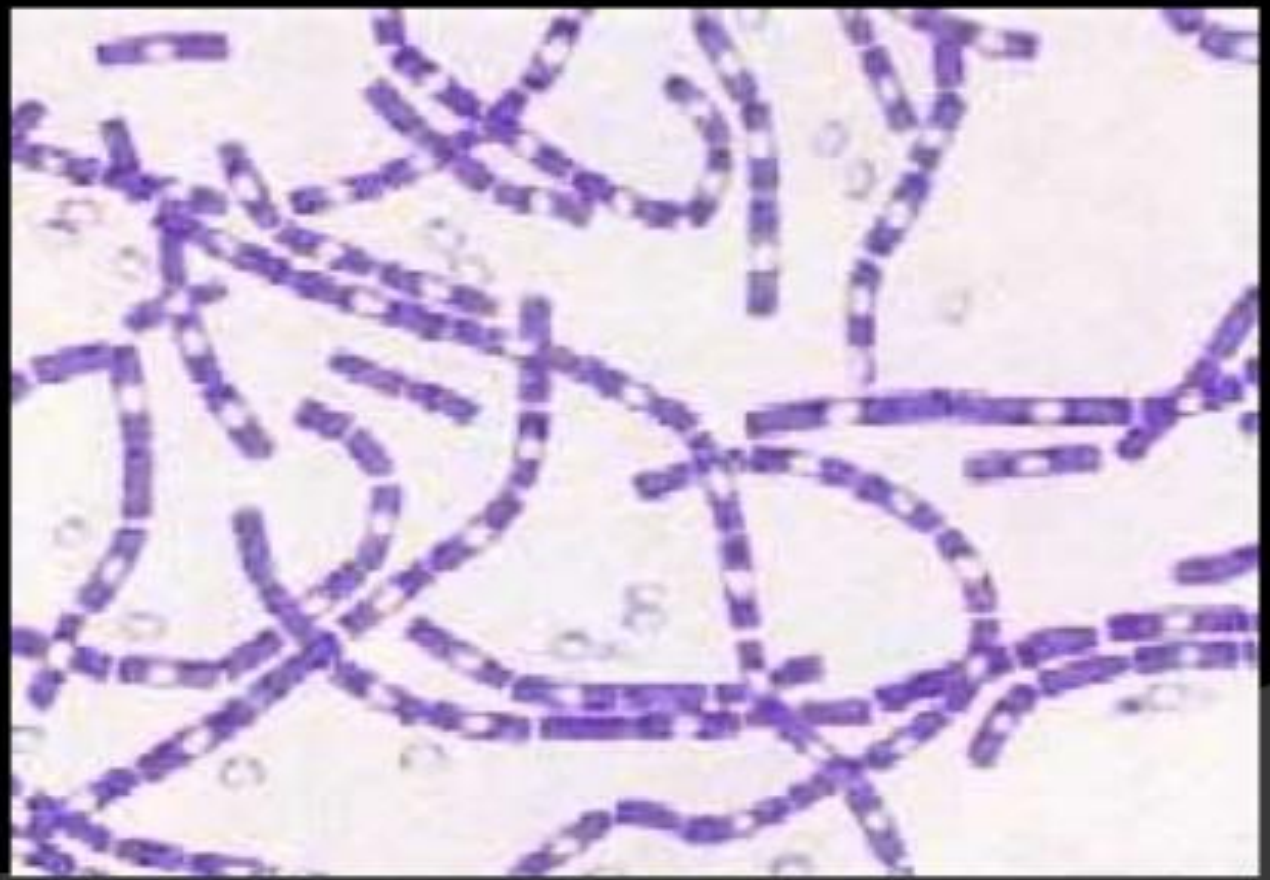


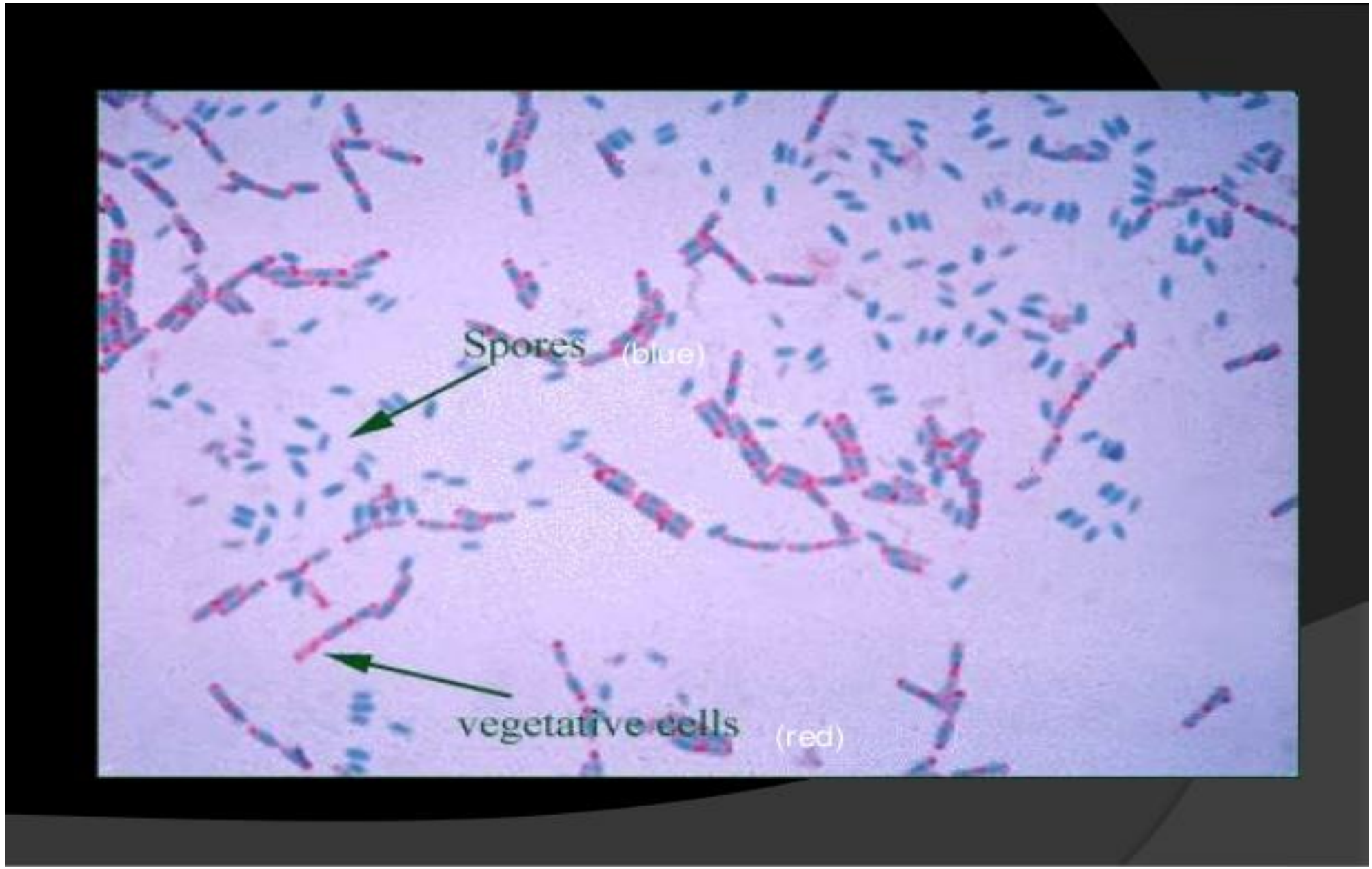
Antrax

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.

Bacillus anthracis: culture




Spores (blue)
vegetative cells (red)

CULTURAL CHARECTERISTICS

-Aerobic ,Facultative anaerobic

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

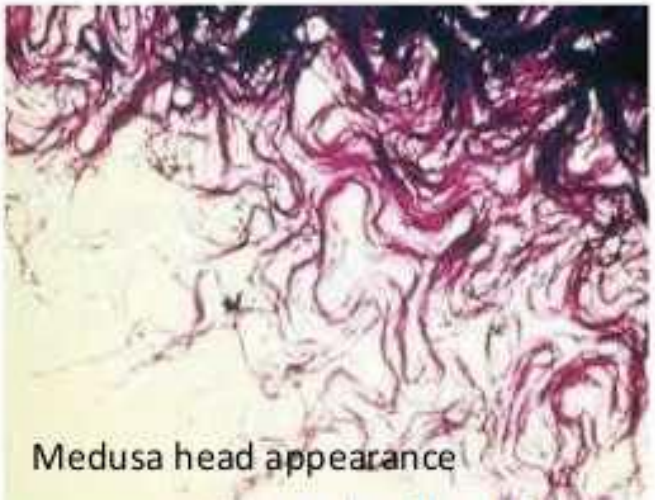
•Medusa head /Barrister's wig appearance of colonies under low magnification

-On BA: Non-hemolytic colonies

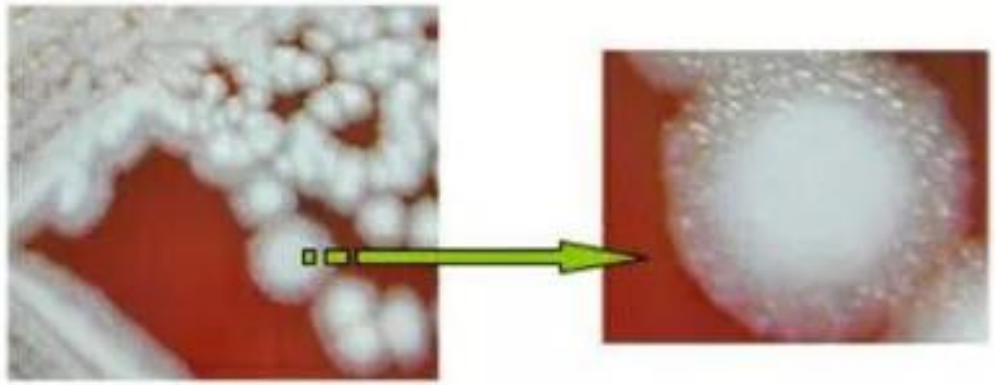
-Gelatin stab culture: "Inverted fir tree" appearance

-PLET medium: for selective isolation

CULTURAL CHARECTERISTICS



Medusa head appearance



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.

Transmission cont.

- 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.

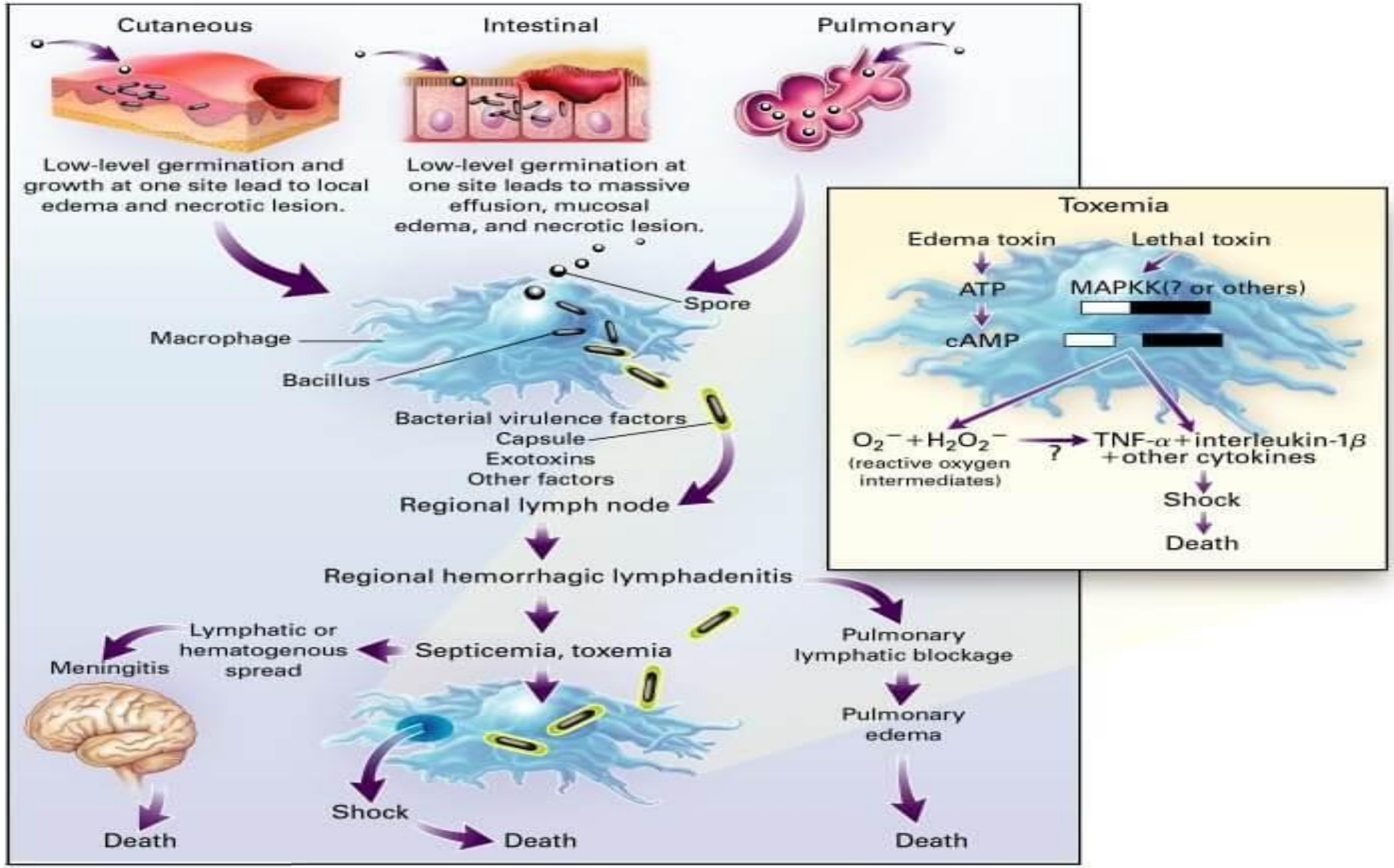
- The disease spreads among grazing animals through **contaminated soil and feed**; and among omnivorous and carnivorous animals through contaminated meat, bone meal or other feeds derived from infected carcases.

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

Transmission cont.

- 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

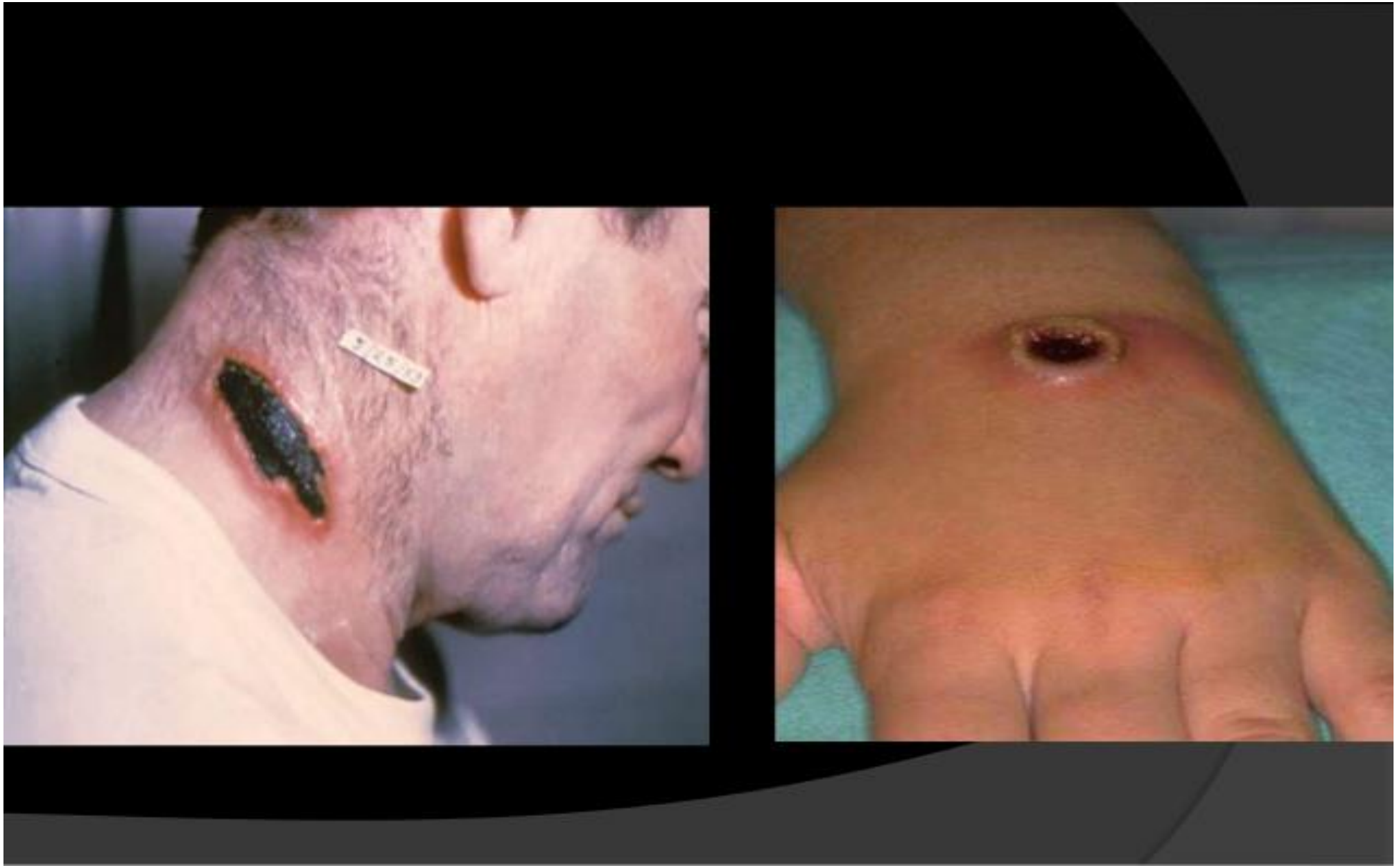
- Ninety percent of cases are cutaneous anthrax

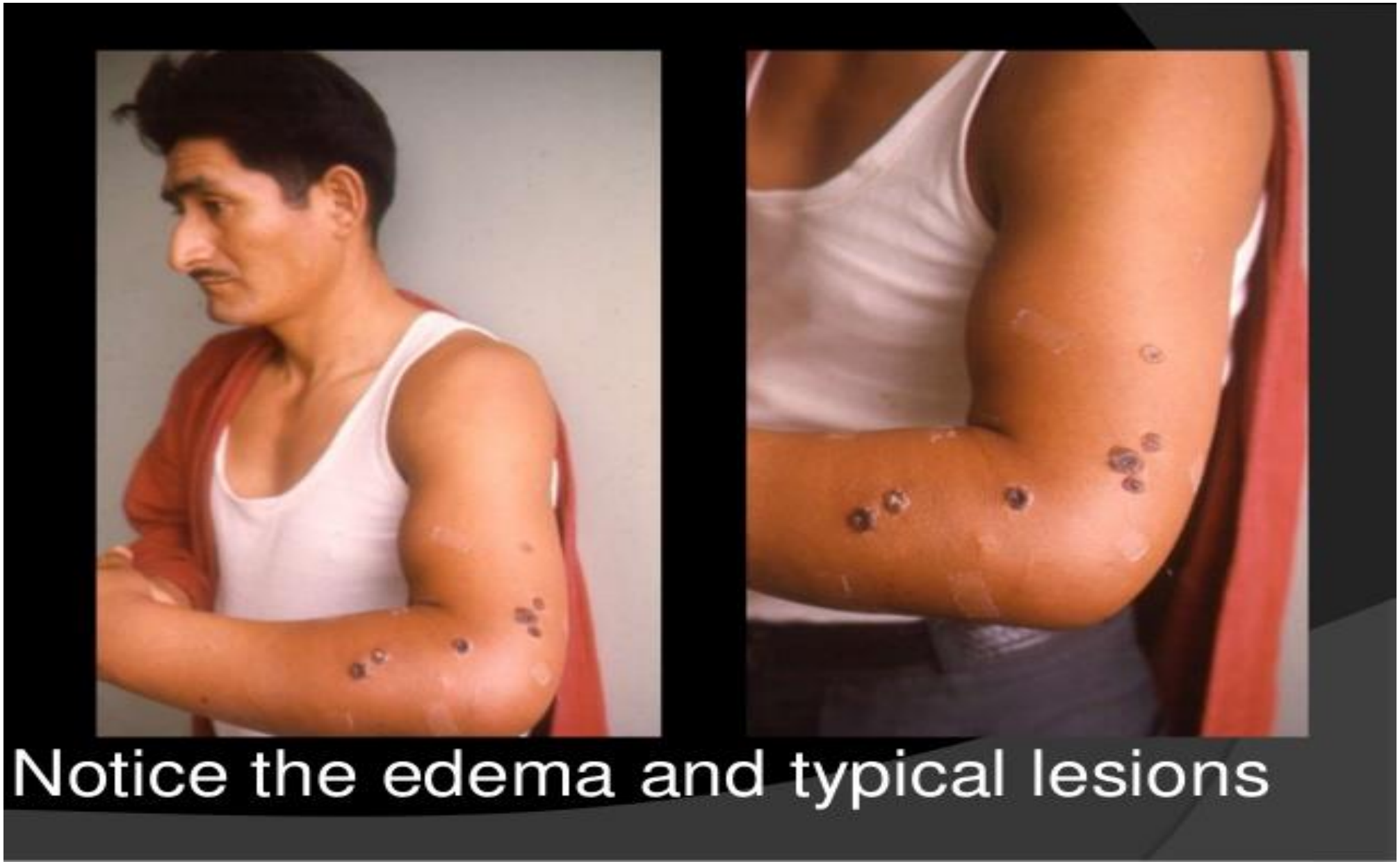
Cutaneous Anthrax

- 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

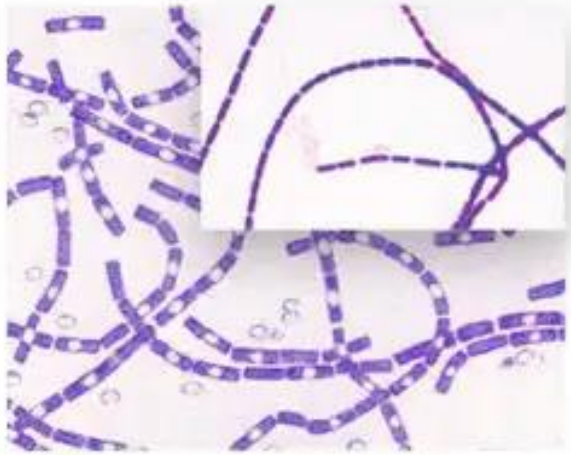
- Head: usually no complication
- Face: severe, superinfection; gangrene near eye
- Neck, breast or chest wall: massive edema, over thorax and sometimes involving scrotum
- Shoulders, arms: may be multiple, small lesions
- Forearms, fingers: atypical on palms
- General symptoms, fever, chills, depend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

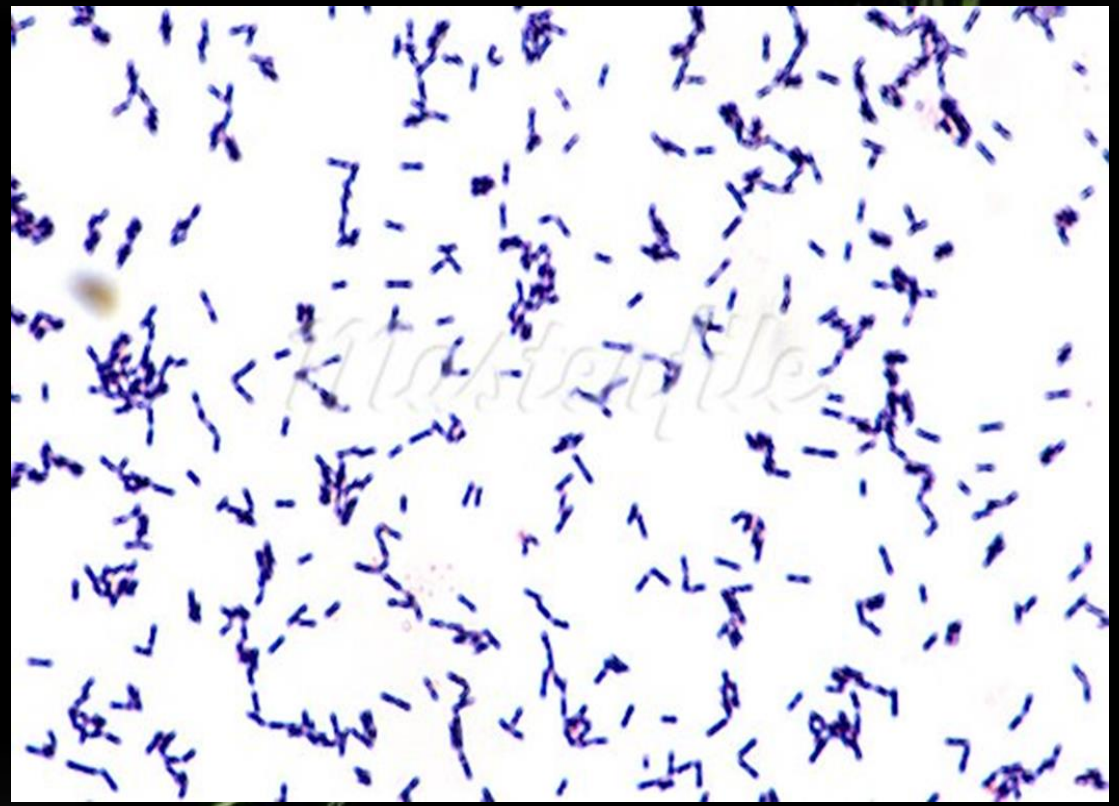
LISTERIA MONOCYTOGENES

- 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.

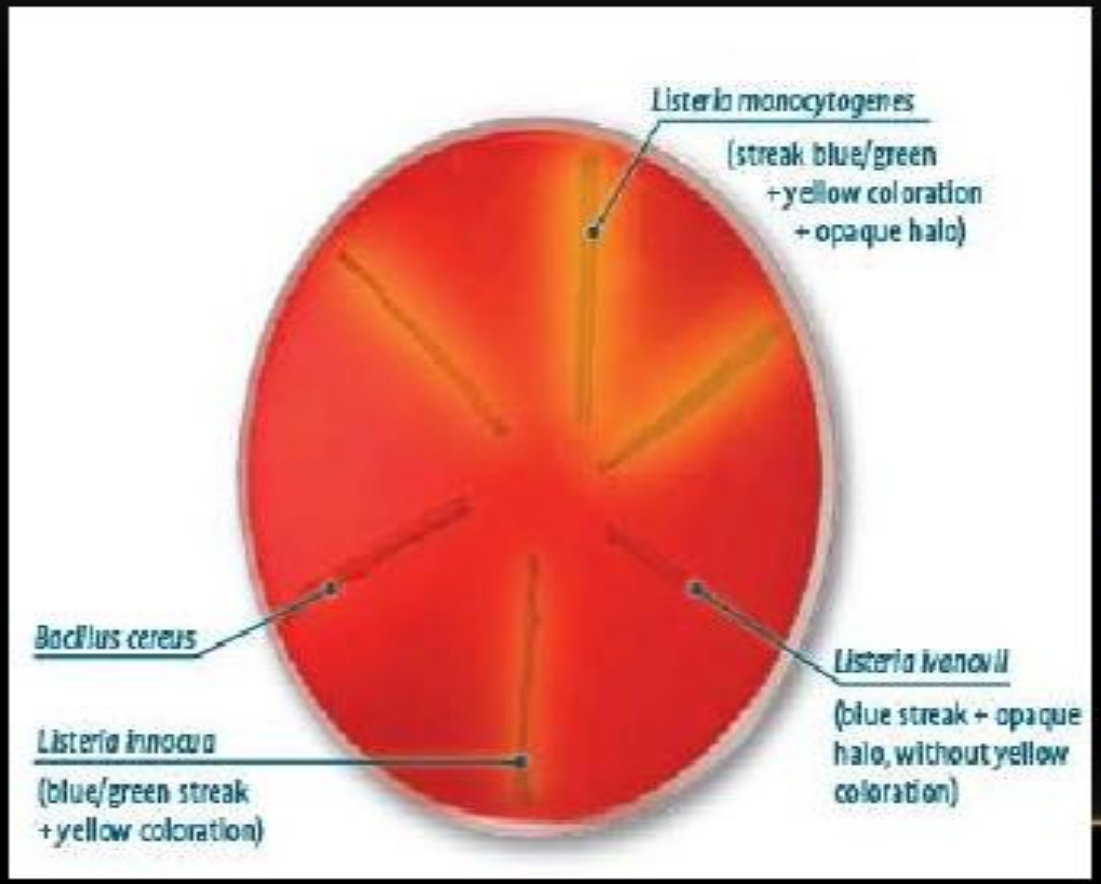


CULTURE AND GROWTH CHARACTERISTICS

- 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)

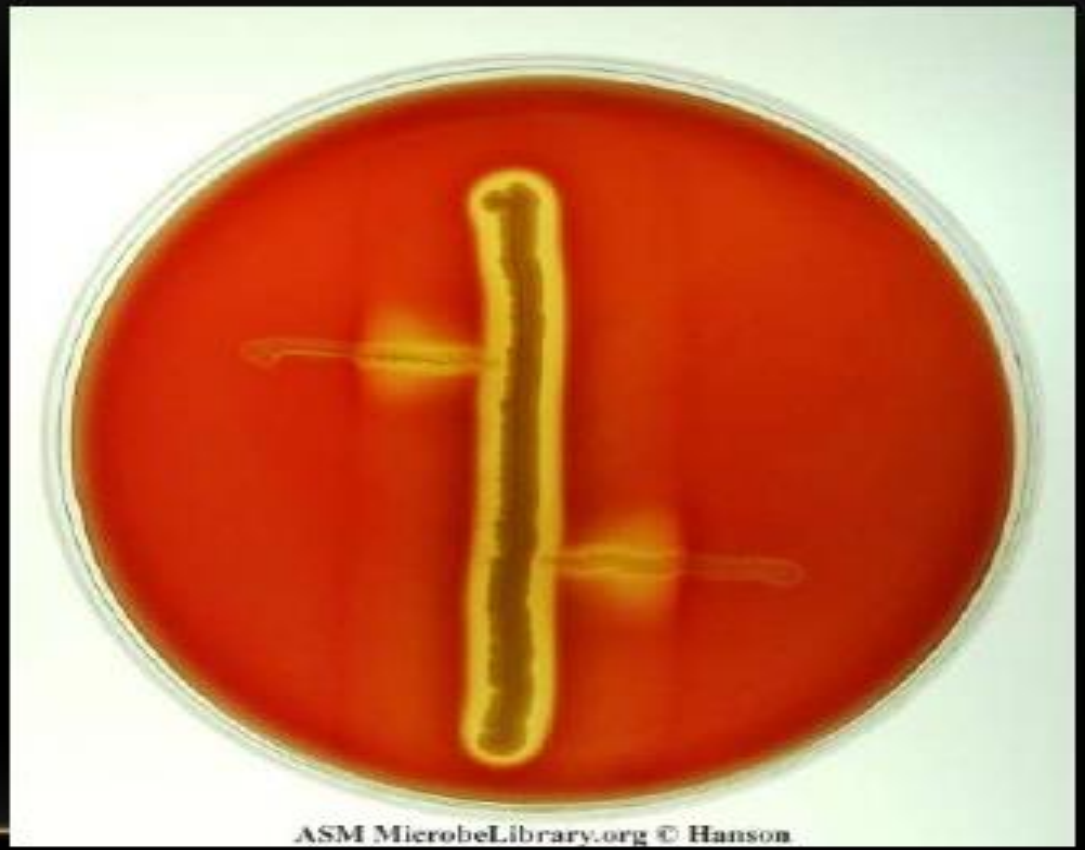
BIOCHEMICAL REACTIONS



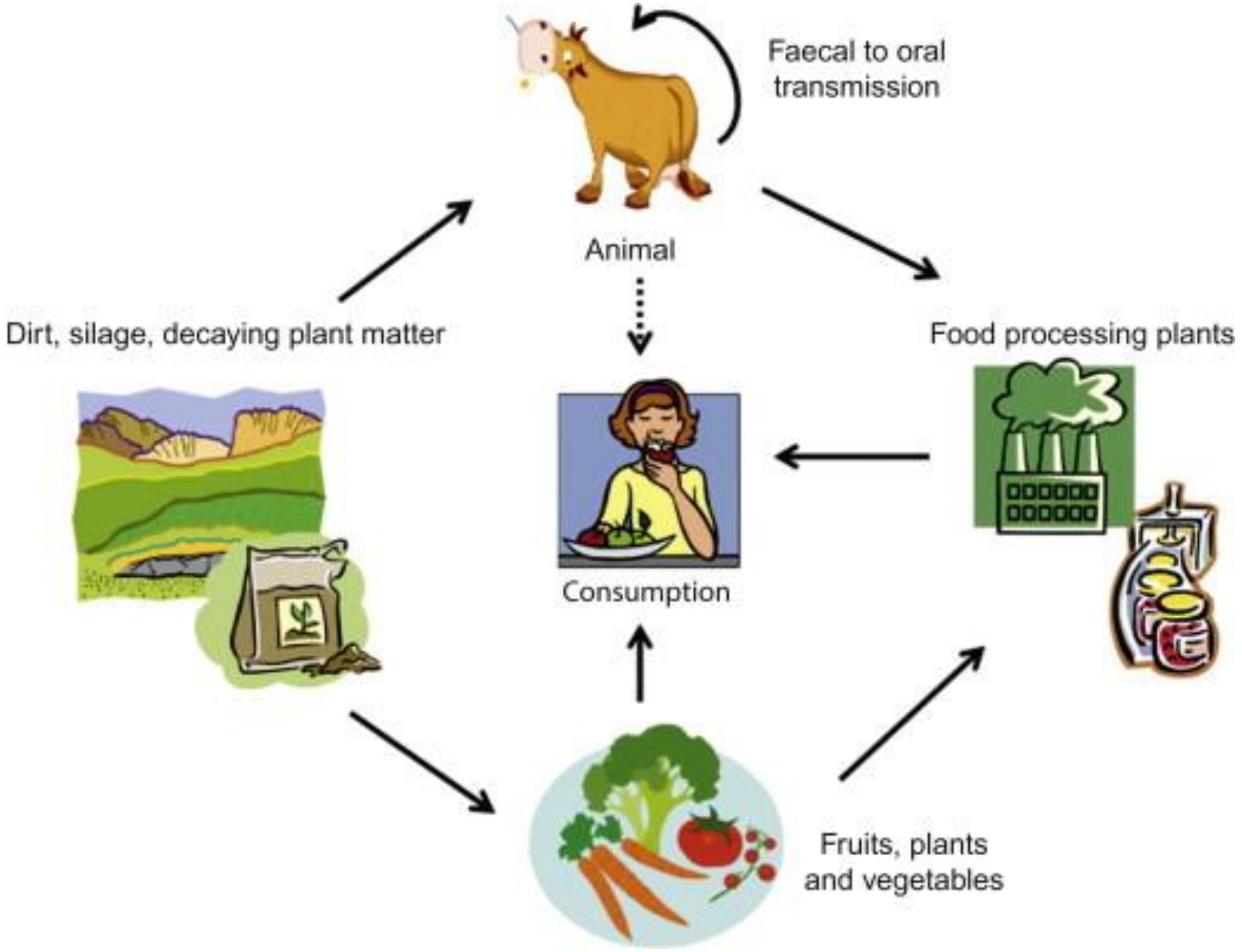
- Bacteria are facultative anaerobic microbes
- Catalase + motile
- Listeria produce acid and not gas in various sugar fermentation tests

CAMP POSITIVE LISTERIA

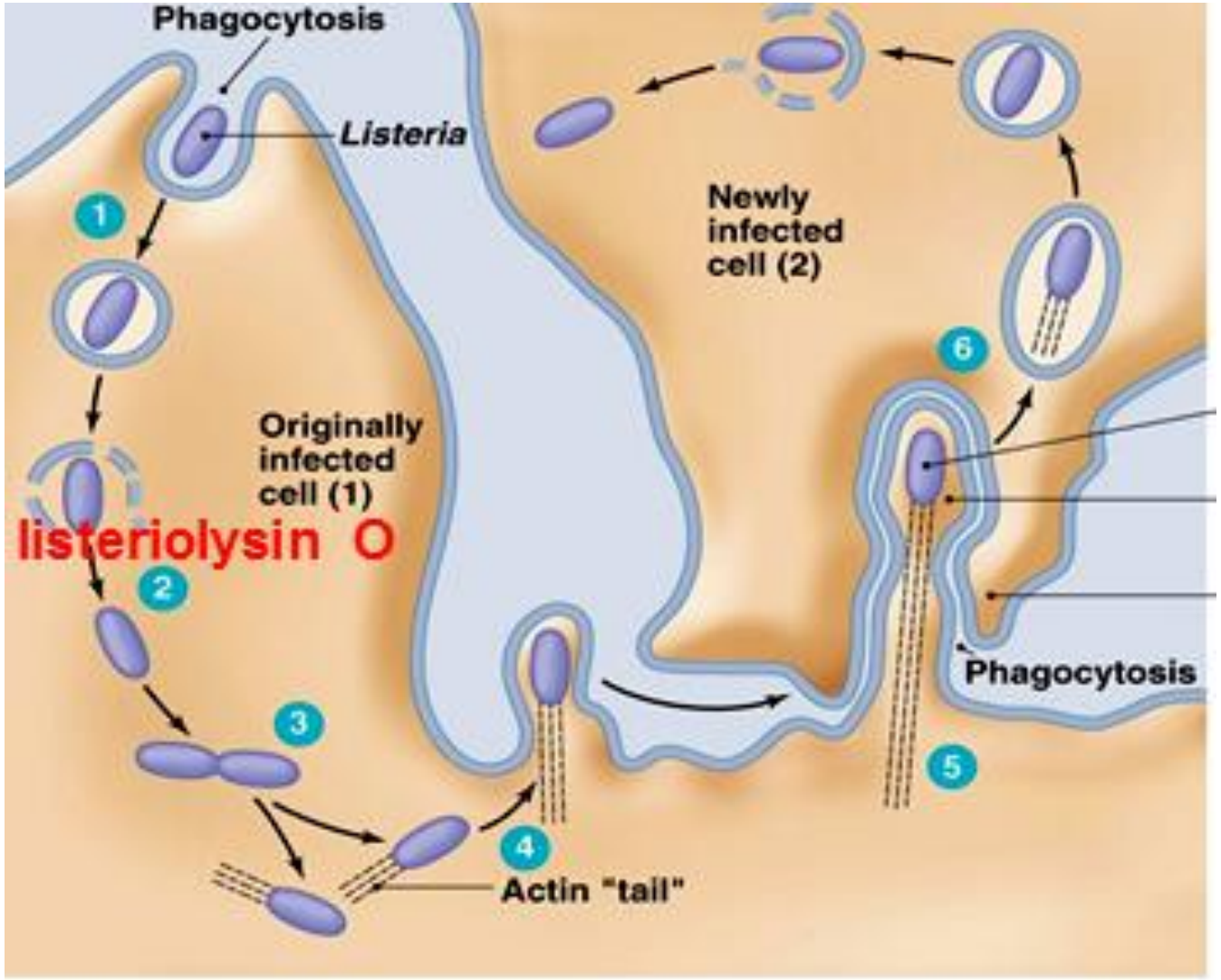
- CAMP-positive Listeria monocytogenes inoculated at right angles to b-hemolytic Staphylococcus aureus. Note the arrow-shaped zone of weak enhanced hemolysis indicating a positive CAMP test. Gloves are worn when working with L. Monocytogenes. (Anne Hanson, University of Maine)



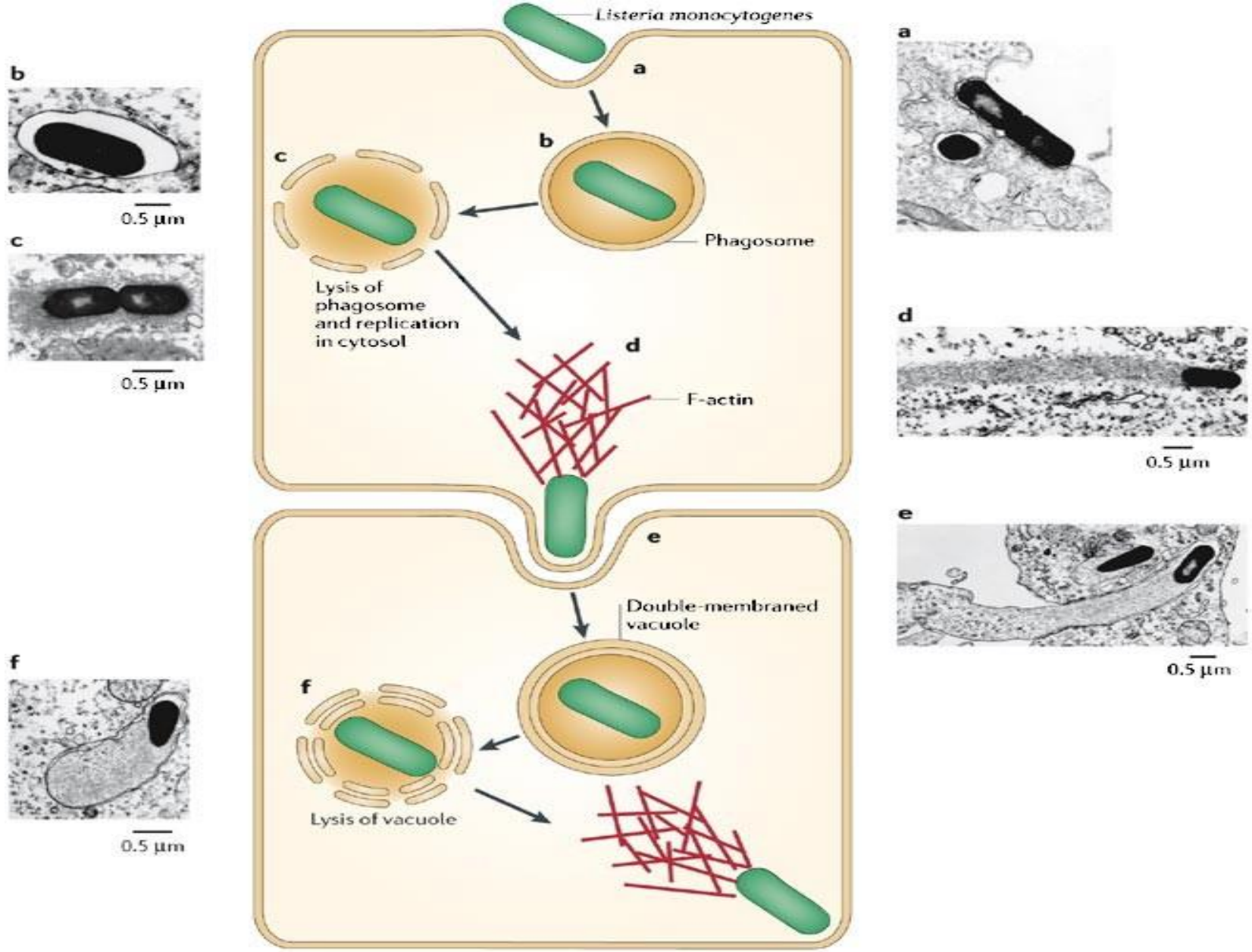
Mode of transmission



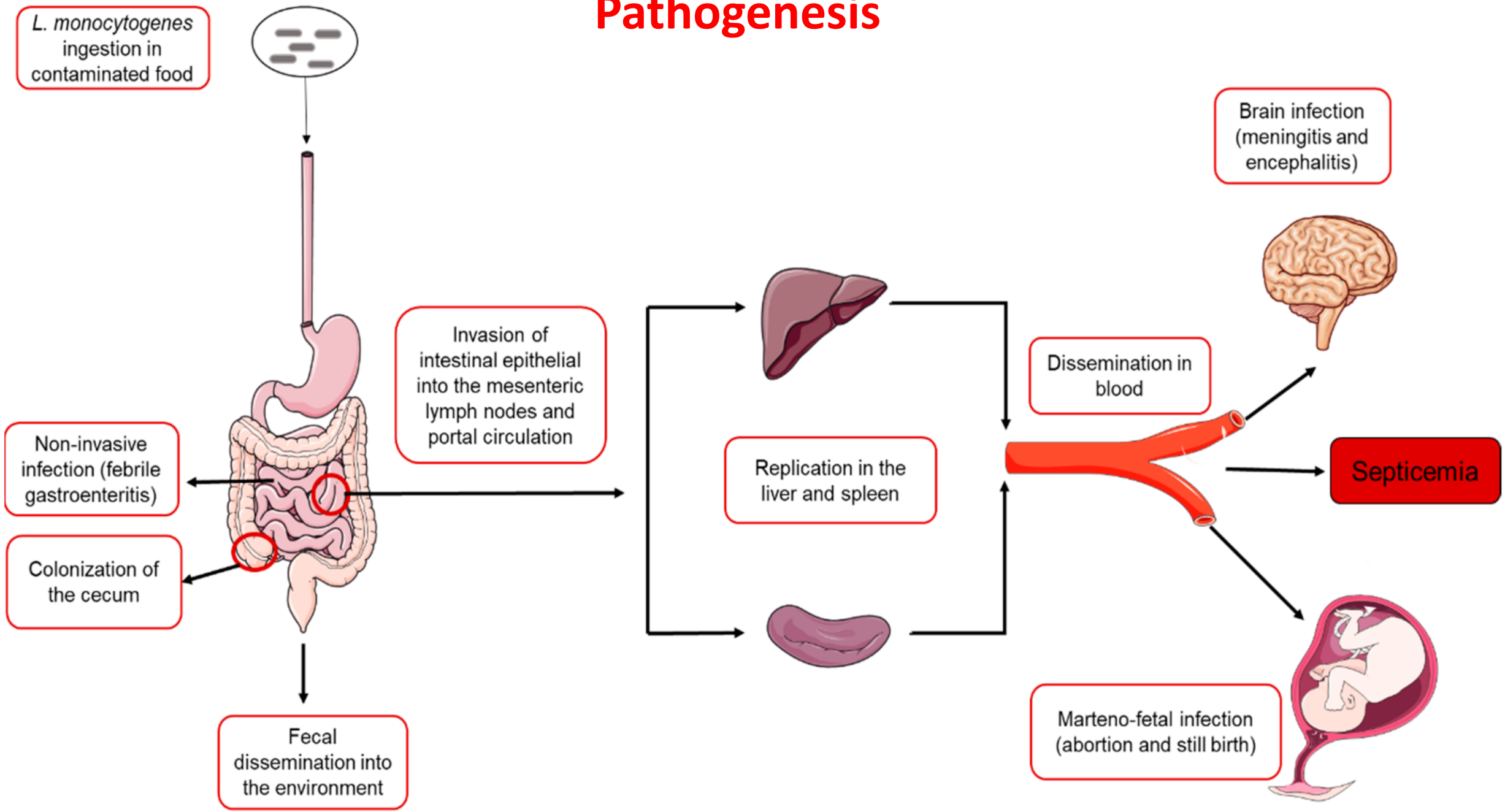
Listeria monocytogenes
mechanism of transfer from one cell to another



Listeria monocytogenes - schematic illustration and electron micrographs of the life cycle



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.

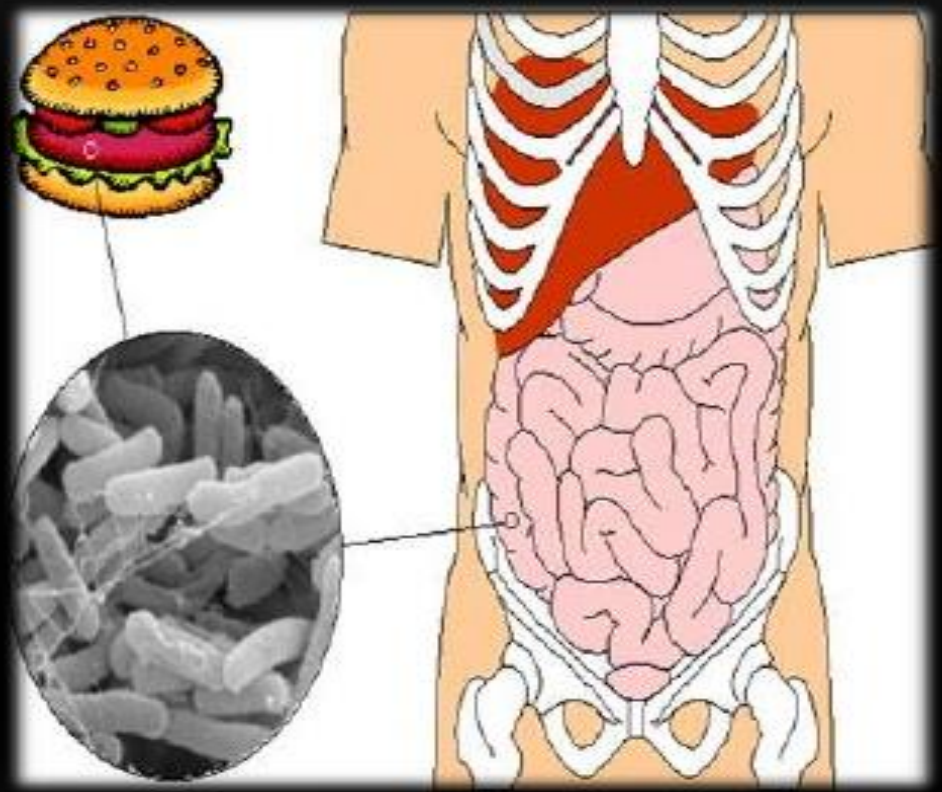
SYMPTOMS

- 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.

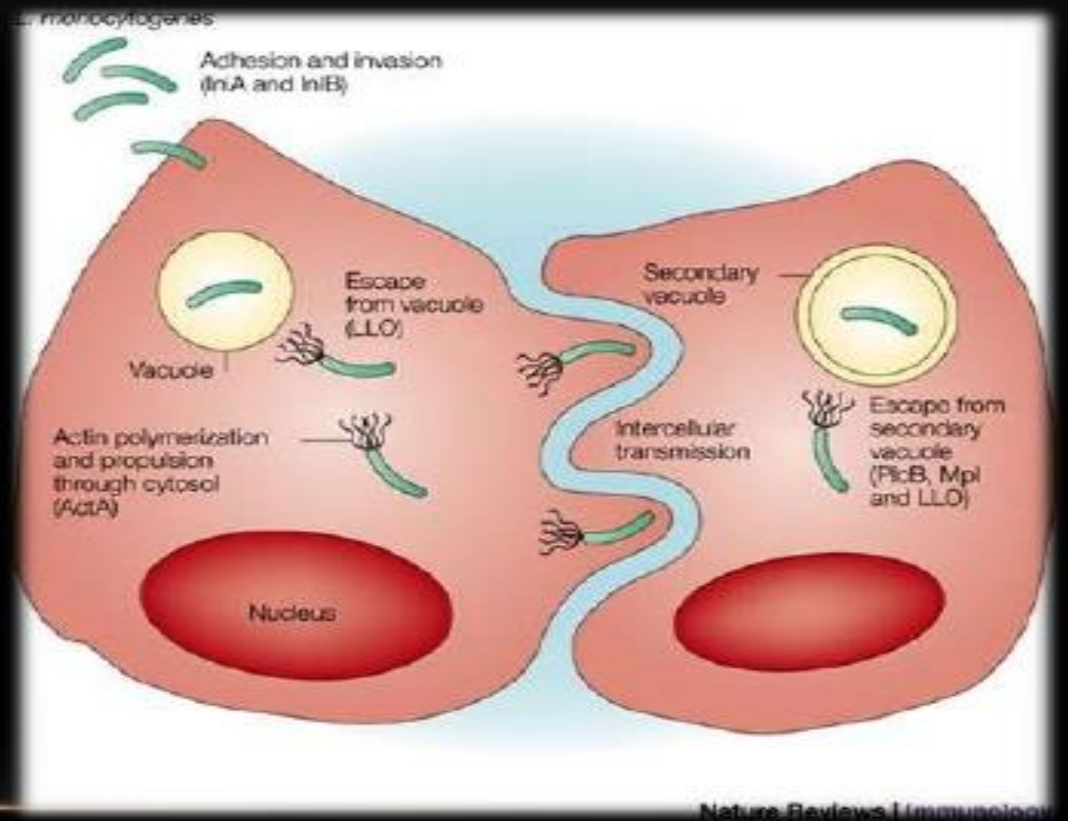
COMMON PRESENTING MANIFESTATION OF LISTERIOSIS

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



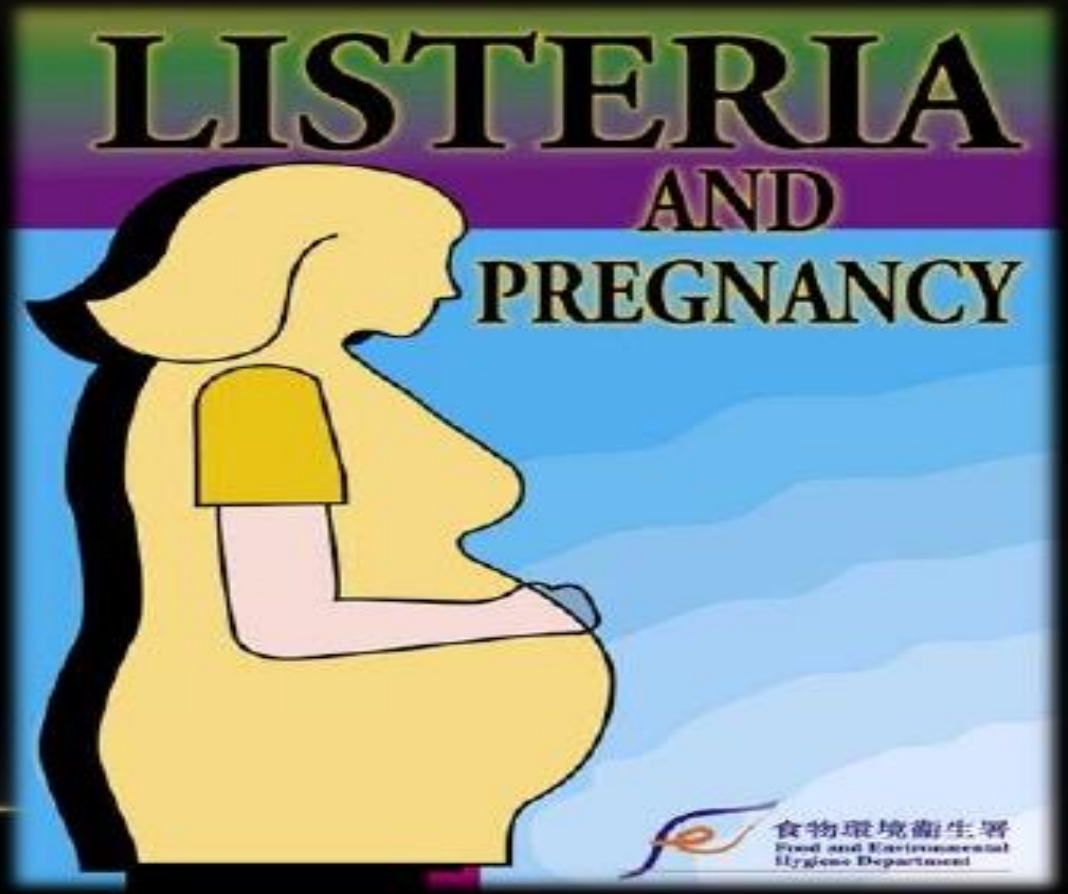
LISTERIOSIS IN ADULTS

- 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

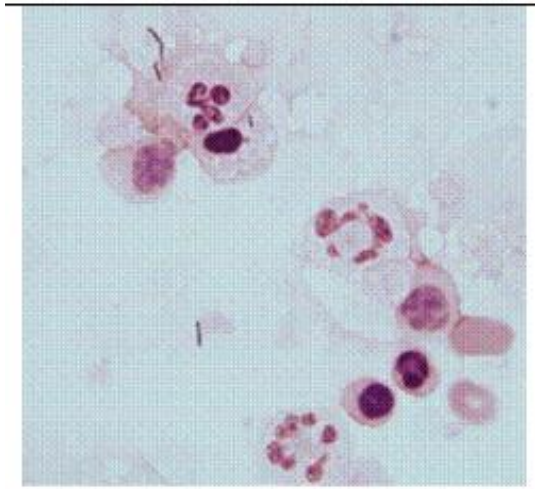
- Diagnosis dependent on isolation of Organisms in cultures obtained on CSF, Blood, and other fluids

Listeria monocytogenes

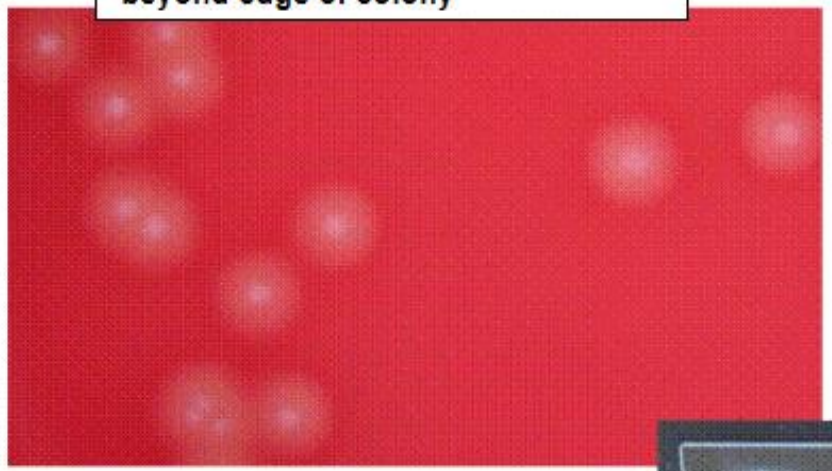
Major pathogen in CSF and Blood only

@ Ellen Jo Baron 2007

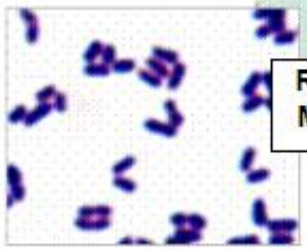
CSF showing PMNs, Monocytes, & Gram positive rods (may be intracellular)



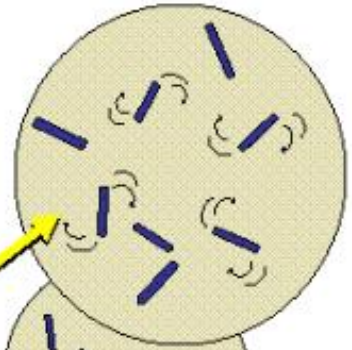
Beta hemolysis does NOT extend beyond edge of colony



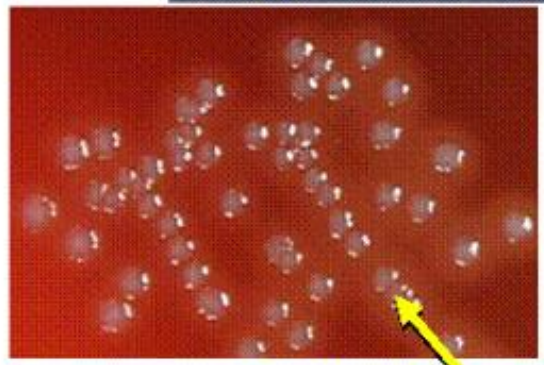
Colony looks like Group B streptococci. Differentiate from Group B strep by Gram stain and positive catalase reaction



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*

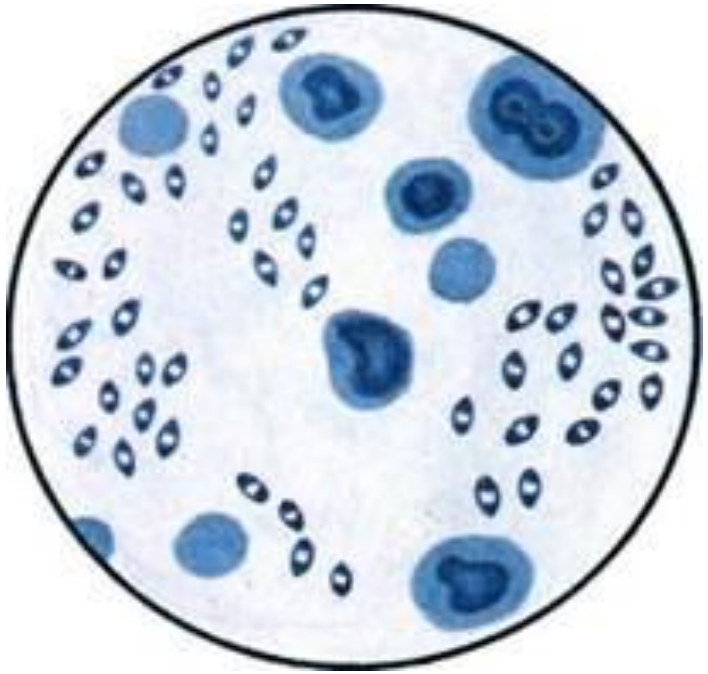
SPECIES : *pestis, enterocolitica, pseudotuberclosis*

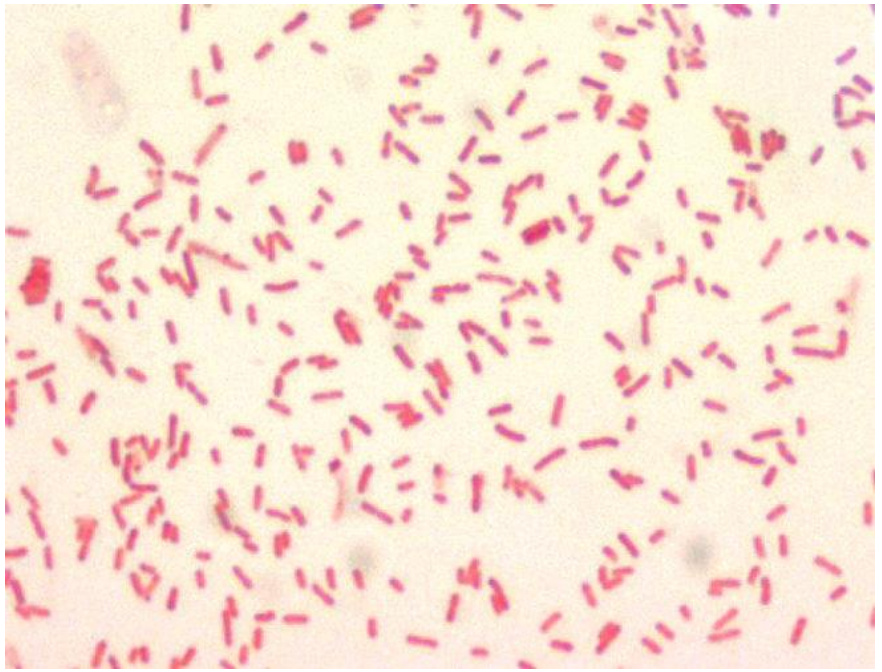
Yersinia Pestis

- *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

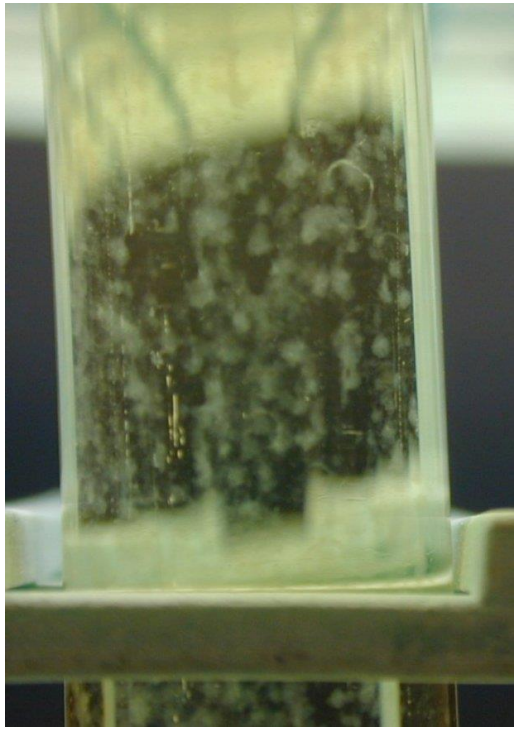
Yersinia pestis
cultural characteristics

- 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.

Yersinia pestis

Yersinia pestis
cultural characteristics

- 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.

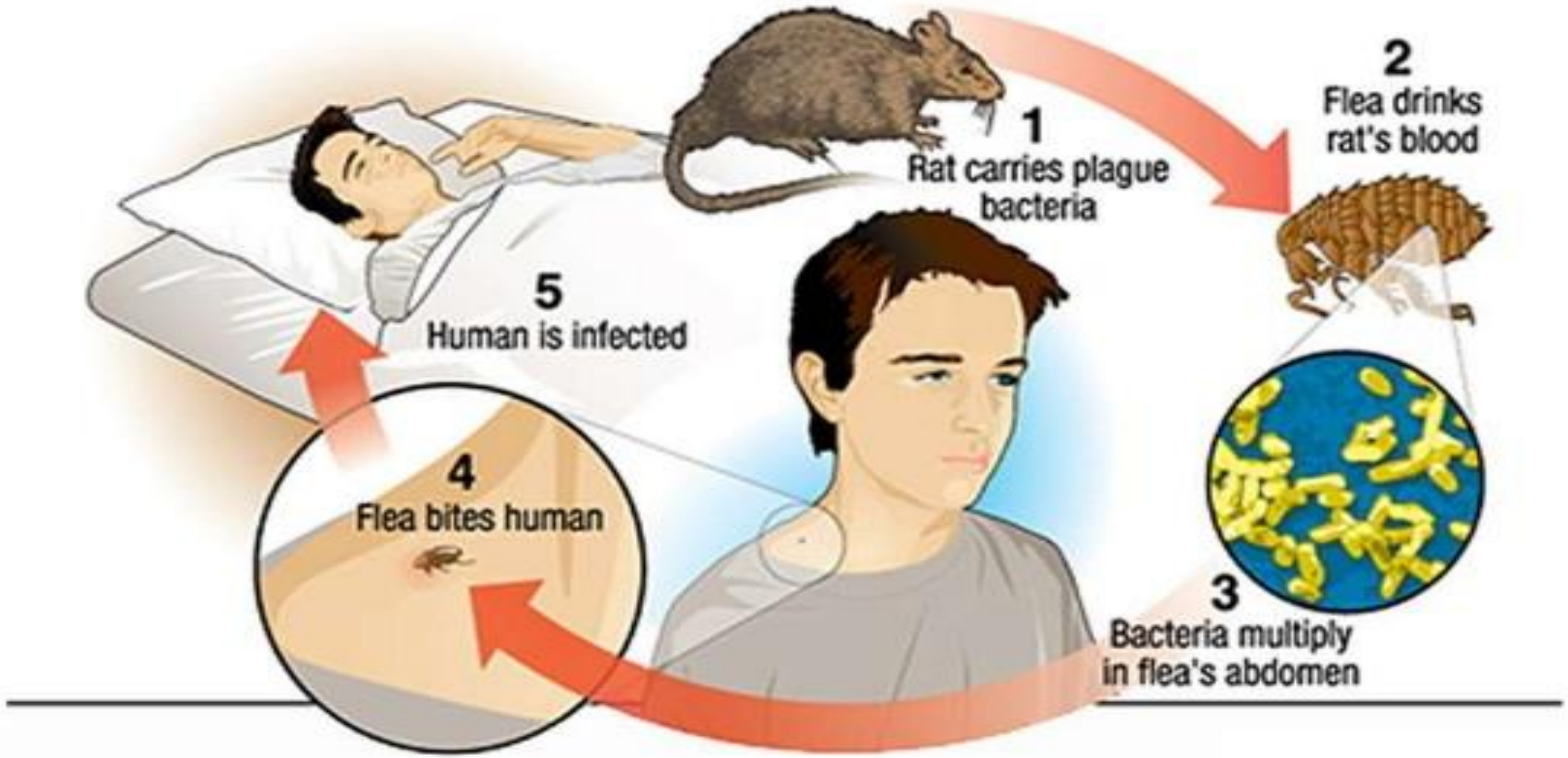
Transmission ...

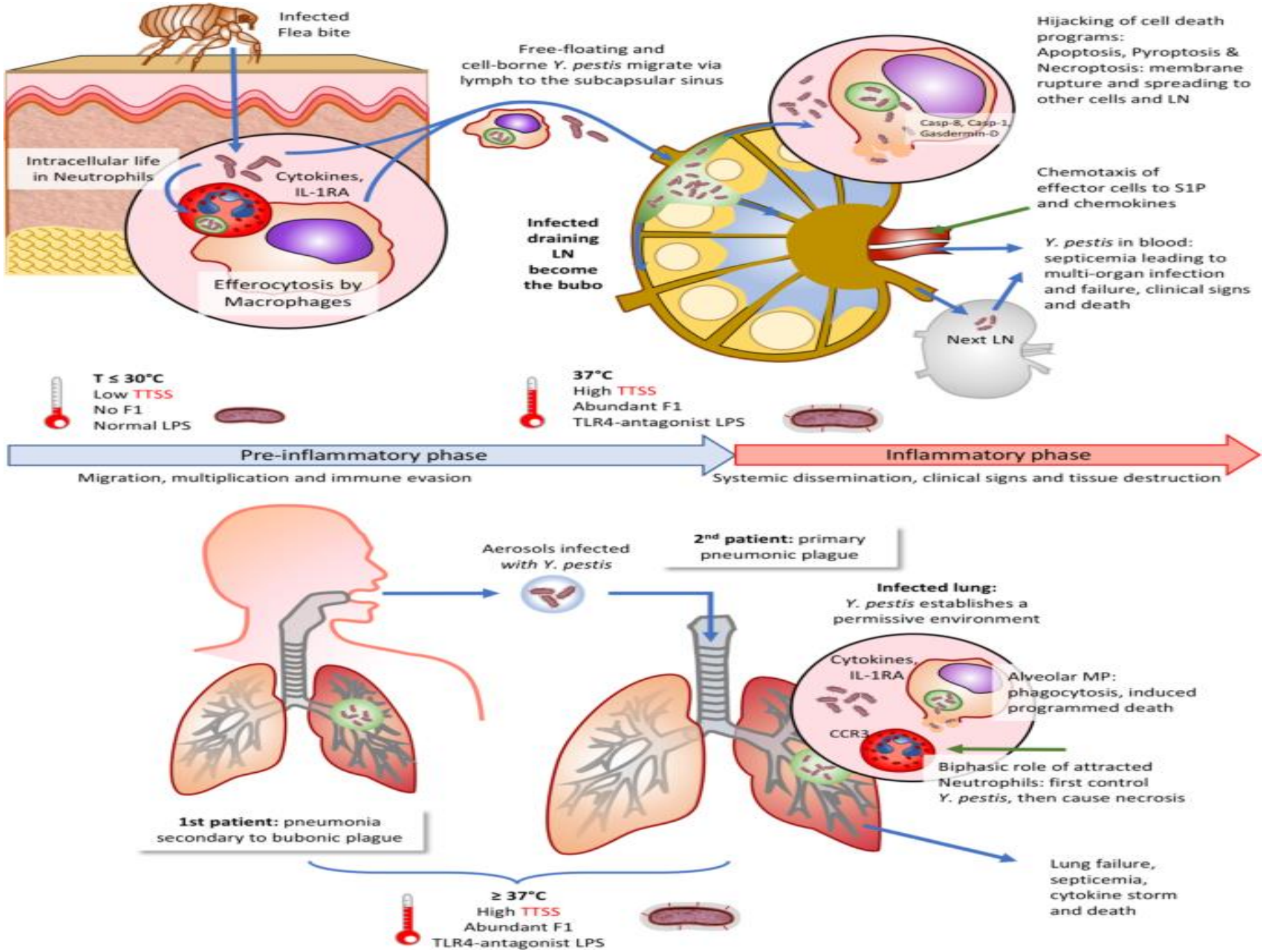
- **Air droplet** : coughing or sneezing on another person
- **Direct physical contact** : touching an infected person, including sexual contact .
- **Indirect contact** : usually by touching contaminated soil or a contaminated surface
- **Fecal–oral transmission** : usually from contaminated food or 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




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
Infected
draining
LN
become
the bubo
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
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
CCR3
Alveolar MP:
phagocytosis, induced
programmed death
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

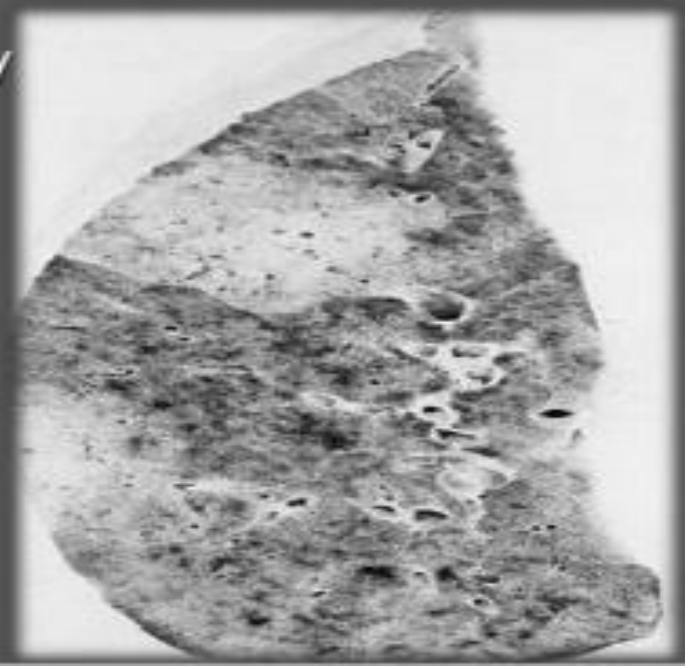
- **Bubonic Plague**
- **Pneumonic Plague**
- **Septicemic plague**
  - *Meningeal plague*
  - *Cellulocutaneous plague*

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 .

Symptoms

Bubonic plague

- Sudden, high fever
- Headache
- Chills
- Body Aches
- Swollen, painful lymph nodes at the groin and armpits (buboes)

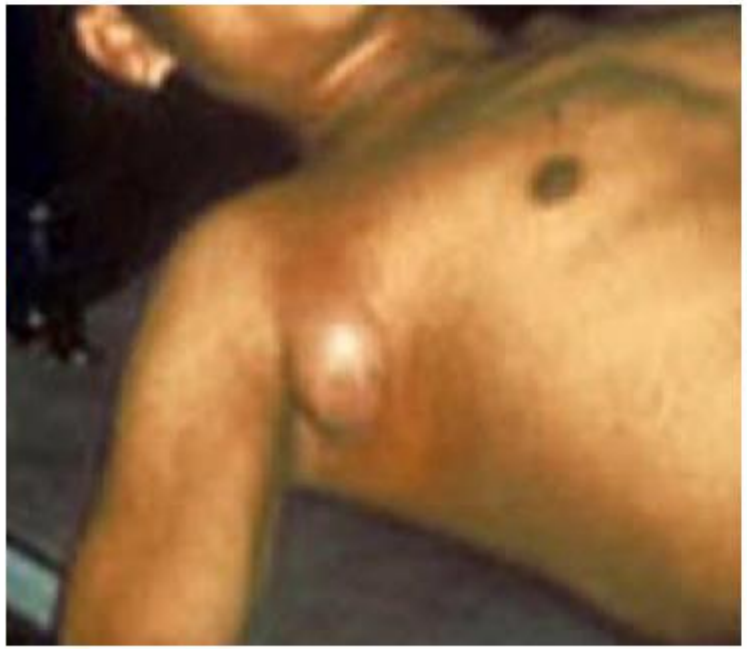
Pneumonic plague

- Sudden pneumonia
- Bloody, watery mucus
- Respiratory failure

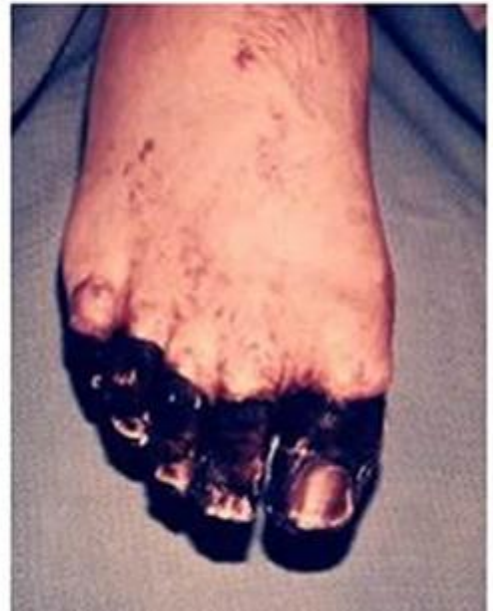
Septicemic plague

- Fever
- Chills
- Body Aches
- Severe abdominal pain
- Shock
- Blackened skin at the extremities

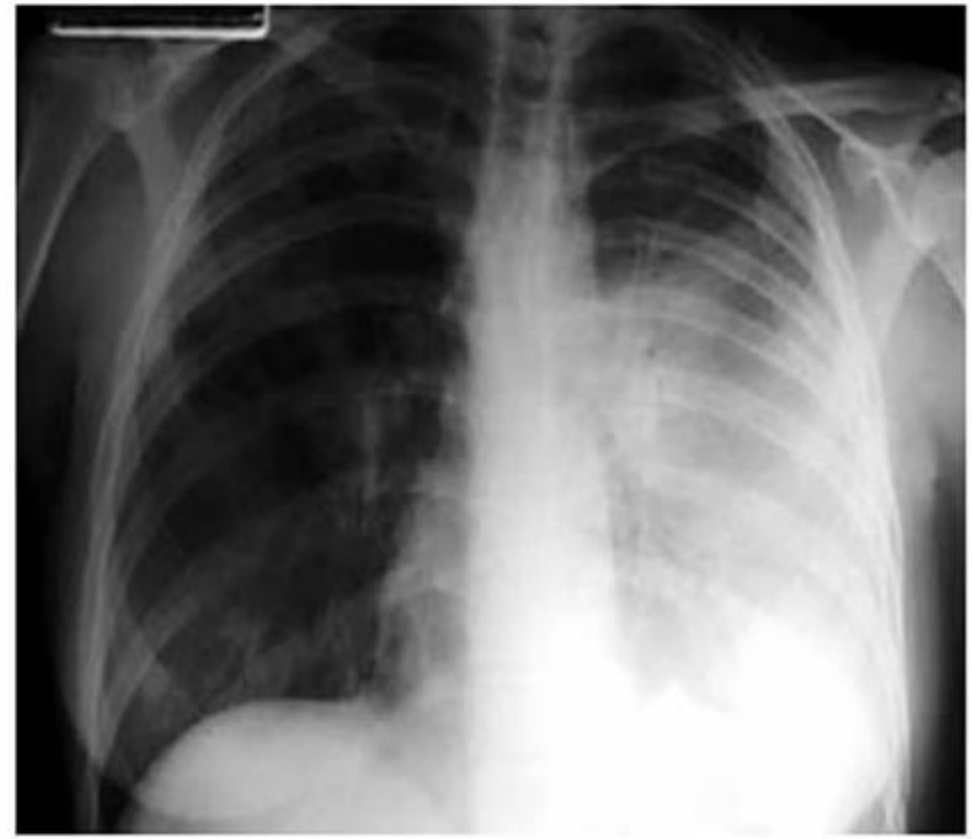
Bubonic



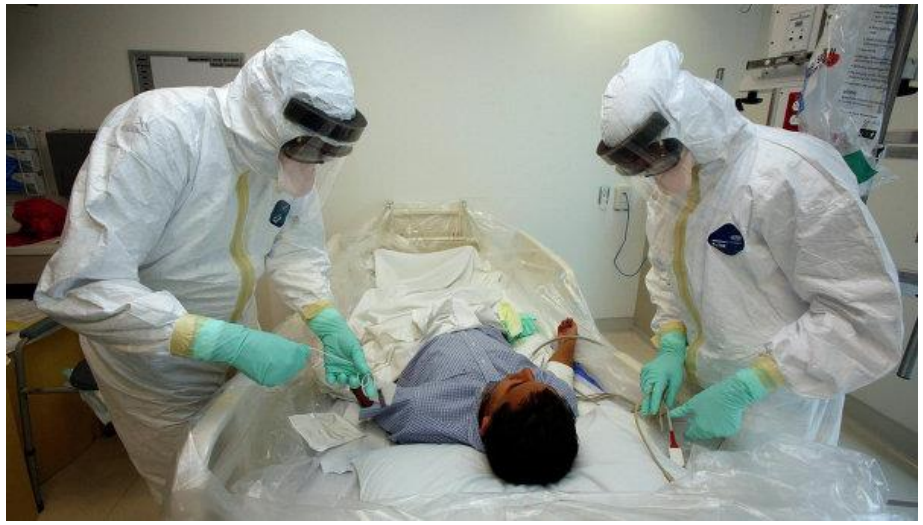
Septicaemic



Pneumonic



Diagnosis of plague is carried out in a special laboratory.



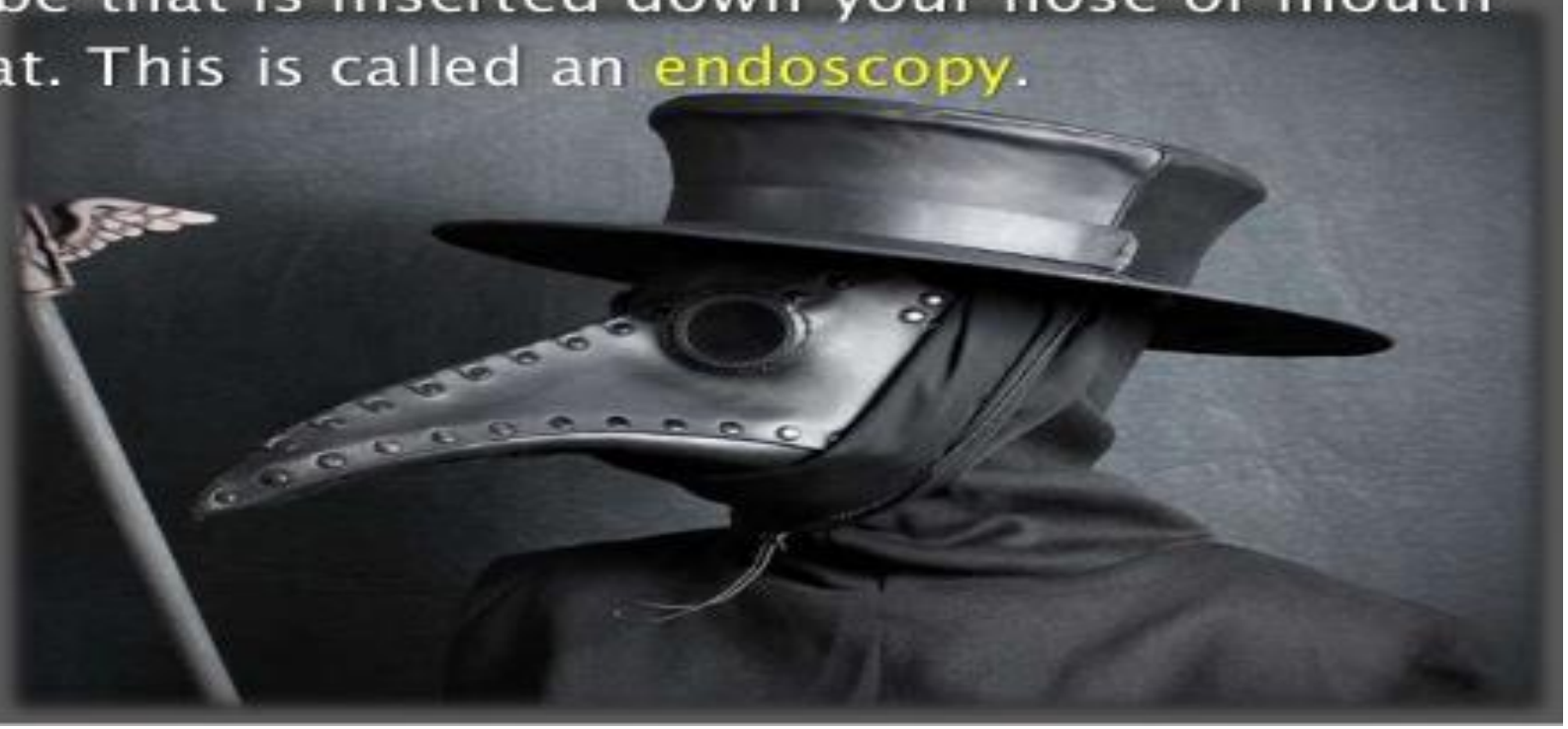
Diagnostic techniques

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.

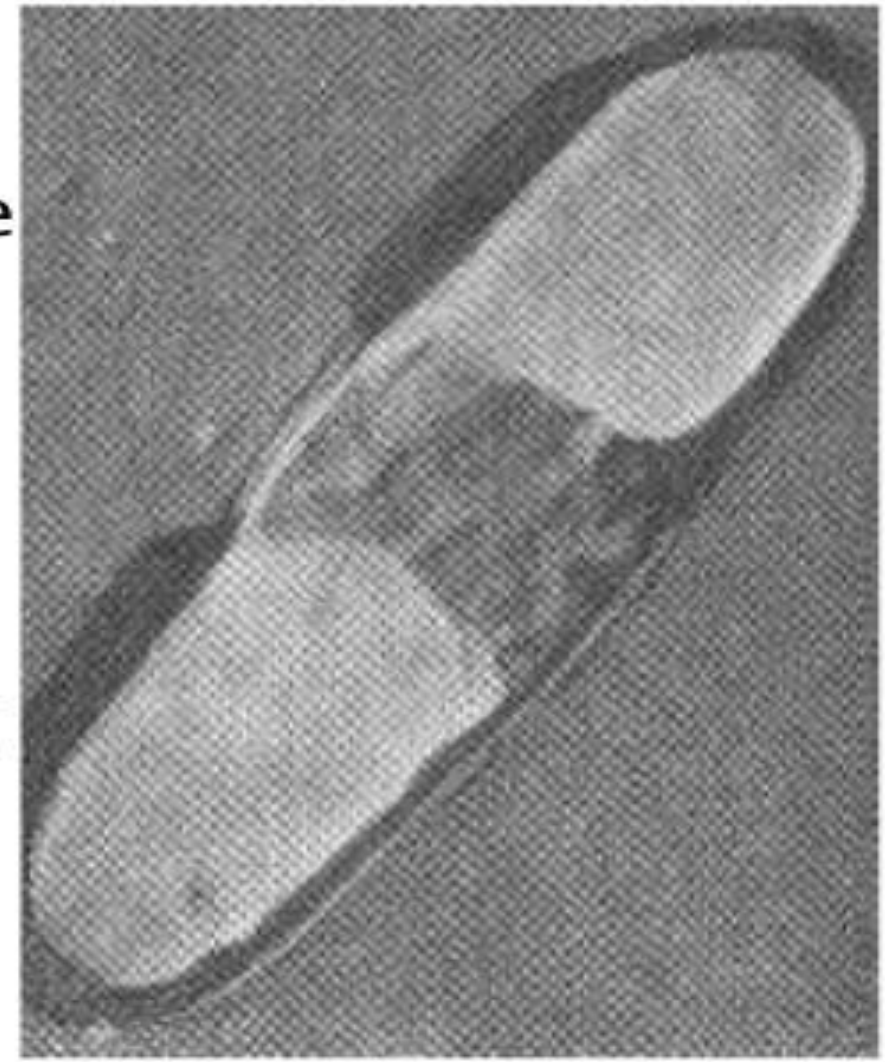
3. To check for pneumonic plague, fluid will be extracted from your airways by a tube that is inserted down your nose or mouth and down your throat. This is called an endoscopy.

4. By using X-ray .



LABORATORY DIAGNOSIS

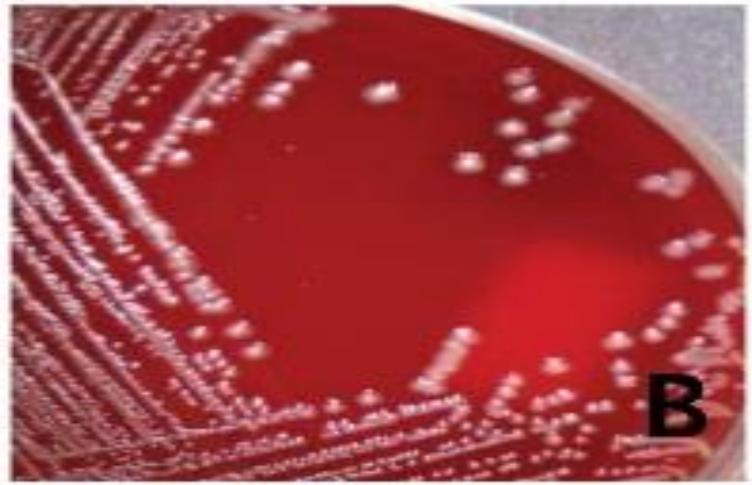
- The organism gives a bipolar "closed-safety pin" appearance on Giemsa, Wright, or Wayson stains (but not on Gram stain) (Guarner *et al.*, 2002).
- The organism also may be identified via immunohistochemical staining using a monoclonal anti-F1 *Y. pestis* antibody on formalin-fixed tissue samples
-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

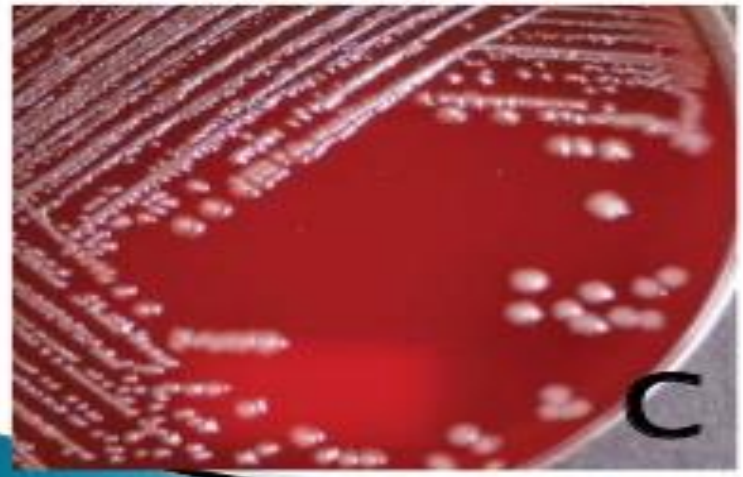


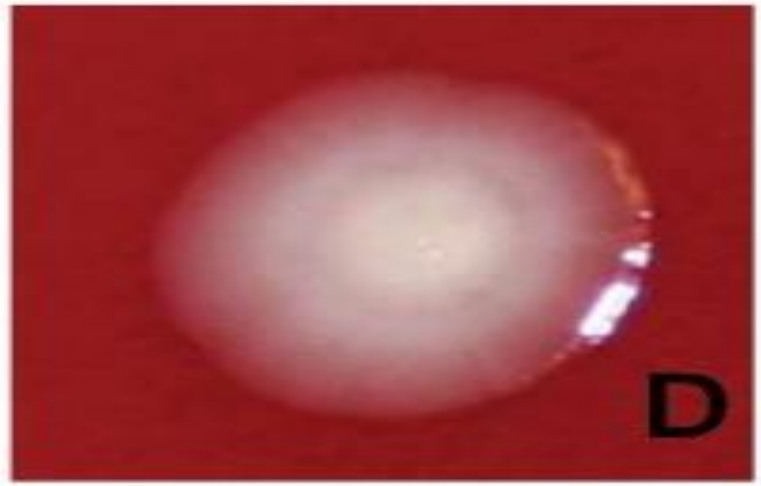
• Colonies are smooth, opaque, and round but may have irregular edges. Under magnification, colonies can be smooth or finely granular and might have a raised center with a flat periphery ("fried egg" appearance) or a "hammered copper" appearance (Brubaker, 1991).

• 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 **colonies grown on CIN agar**







Yersinia pestis growth on BA at

(A) 48 h,

(B) 72 h,

(C) 96 h,

(D) 96 h "Fried egg"

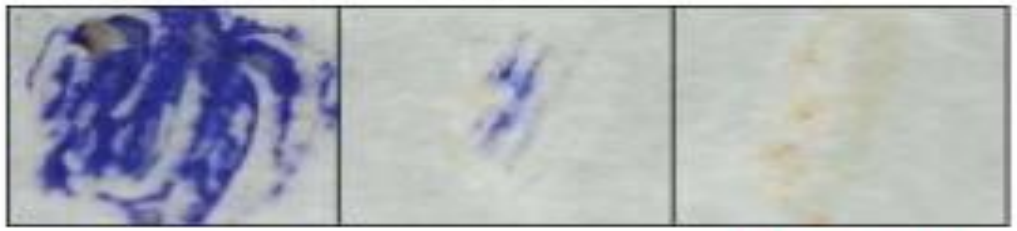
Plate 2. wadsworth center , 2007.

BIOCHEMICAL TESTS

CATALASE TEST

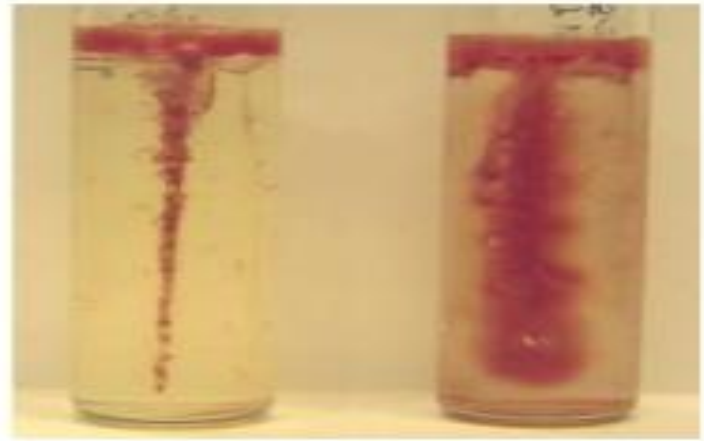
POSITIVE WEAKLY POSITIVE NEGATIVE

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.

Vaccination

- 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

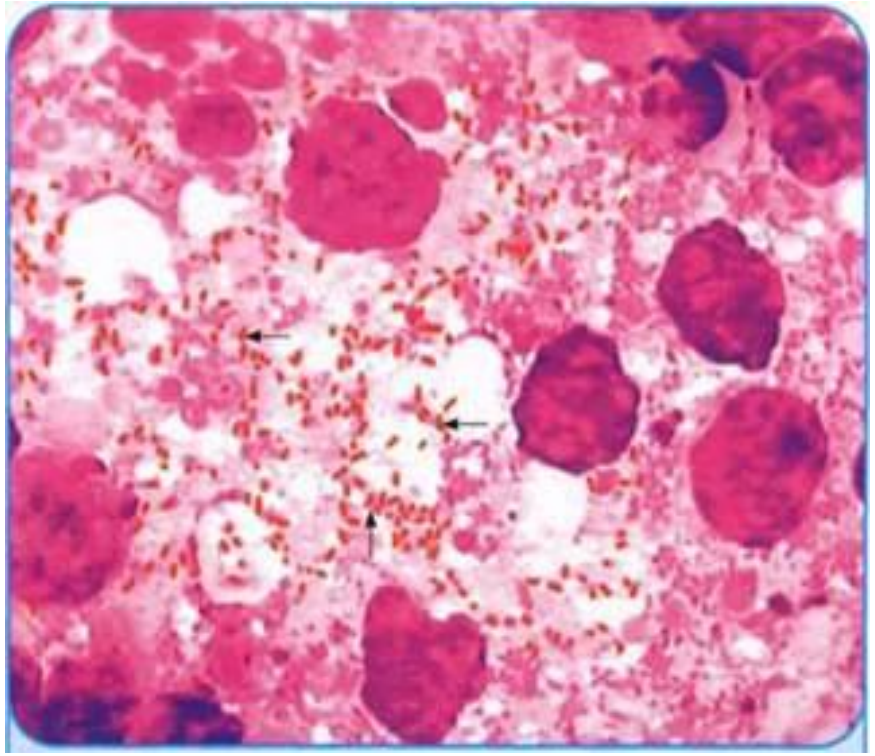
- (Domain): Bacteria
- (Kingdom): Pseudomanadota
- (Class): Gammaproteobacteria
- (Order): Thiotrichales
- (Family): Francisellaceae
- (Genus): Francisella
- (Species): *F.tularensis*

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

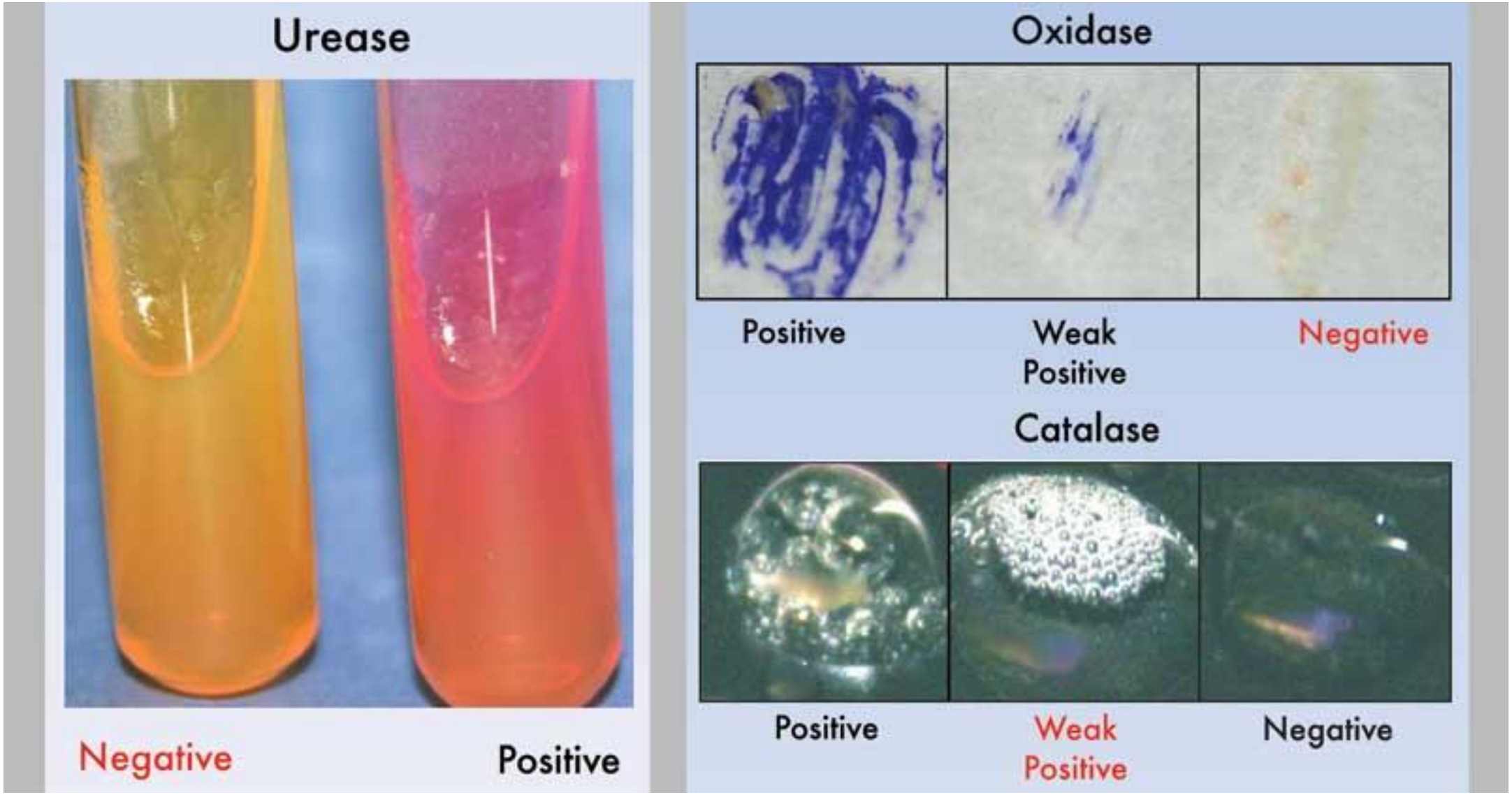
- Small, weakly staining gram-negative coccobacillus 0.2 to 0.2 – 0.7 um in size.
- Nonmotile, displays bipolar staining with Giemsa stain, obligate anaerobe, and is weakly catalase positive.
- Young cultures are relatively uniform in appearance while older cultures display extreme pleomorphism.
- Carbohydrates 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

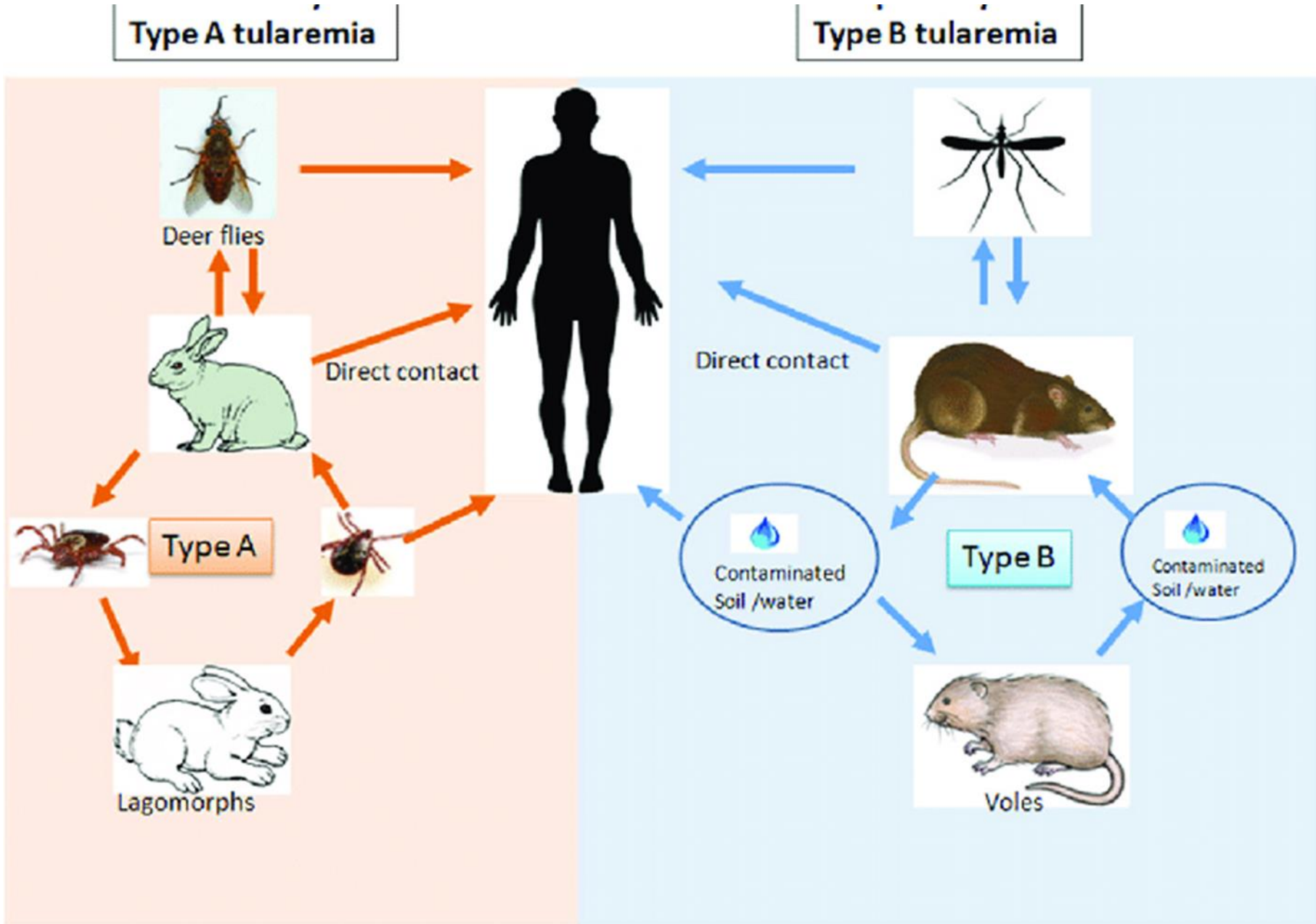
- Optimal growth at 37^0 C, growth range 24^0 to 39^0 C. Survival rate is best at lower temperatures.
- Slow growing with a requirement for iron and cysteine or cystine.
- No growth on routine culture media but small colony growth after 2 - 4 days on glucose-cysteine-blood agar or peptone-cysteine agar.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

Francisella tularensis - biochemical activity

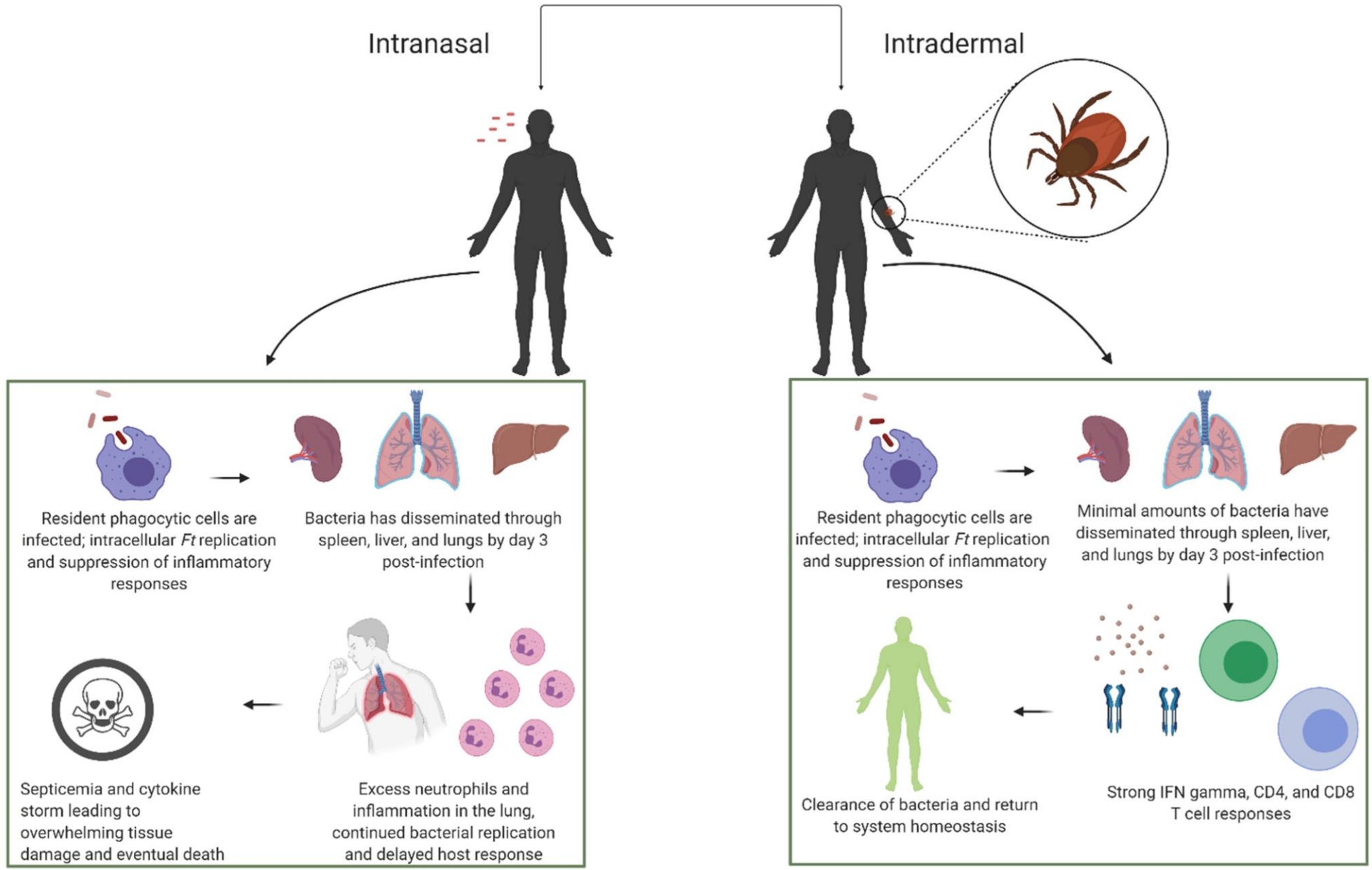


Etiology

- 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.

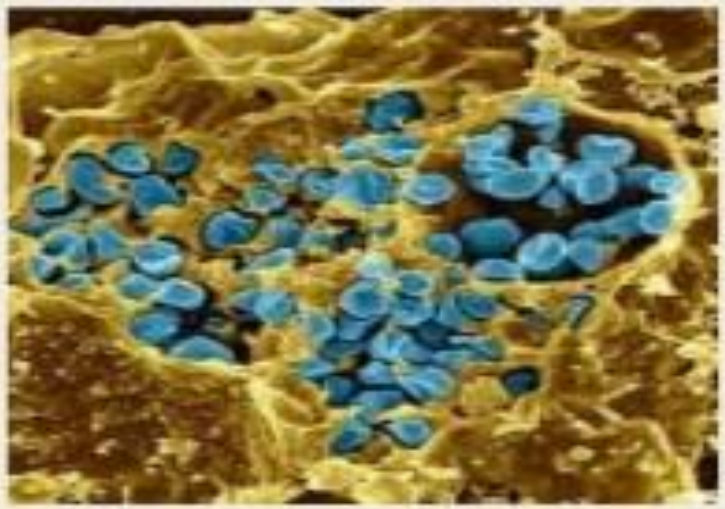
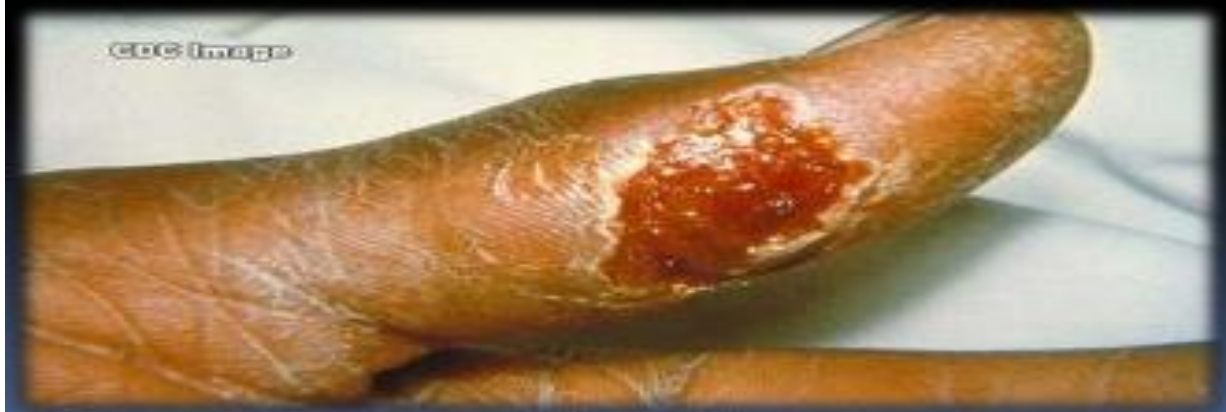


Fig : Francisella tularensis bacteria (blue) infecting a macrophage (yellow)

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*.

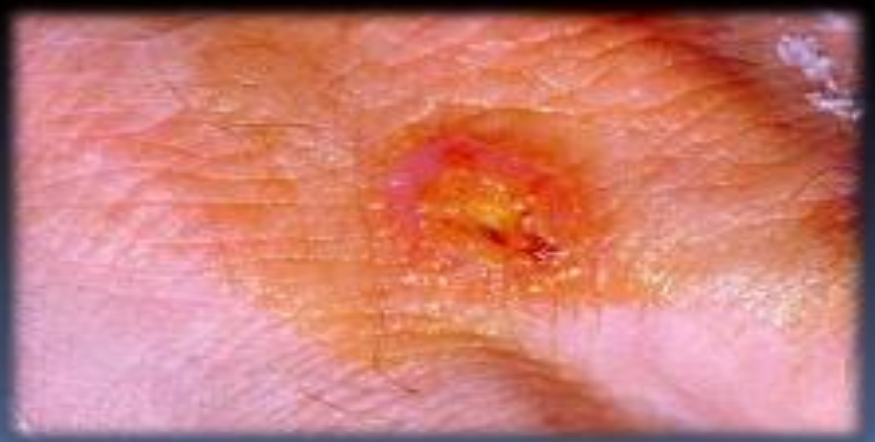


Epidemiology

- 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* .

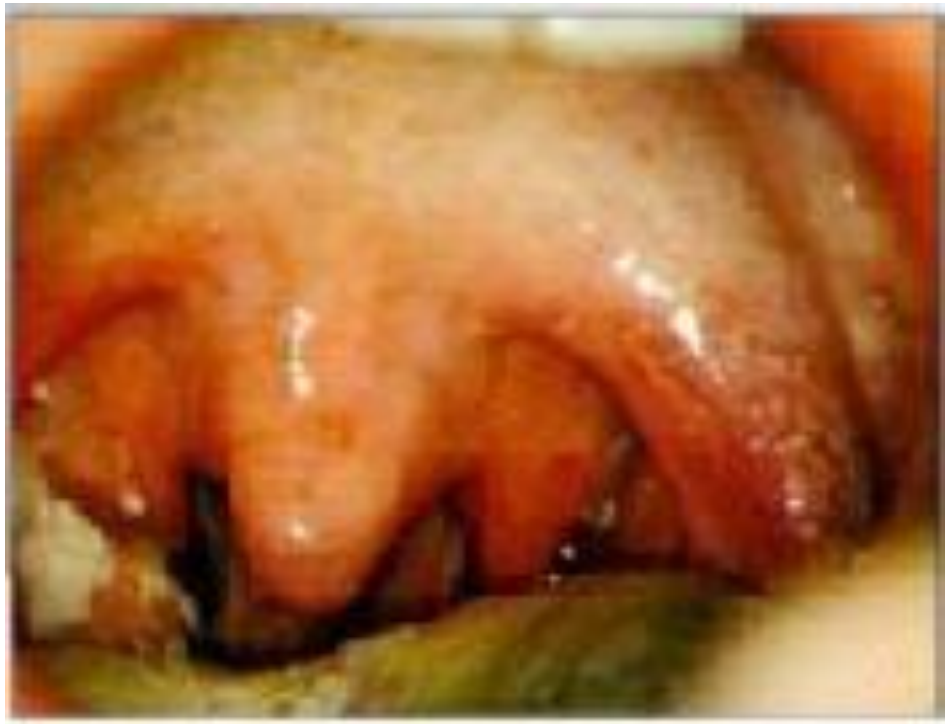
Symptoms

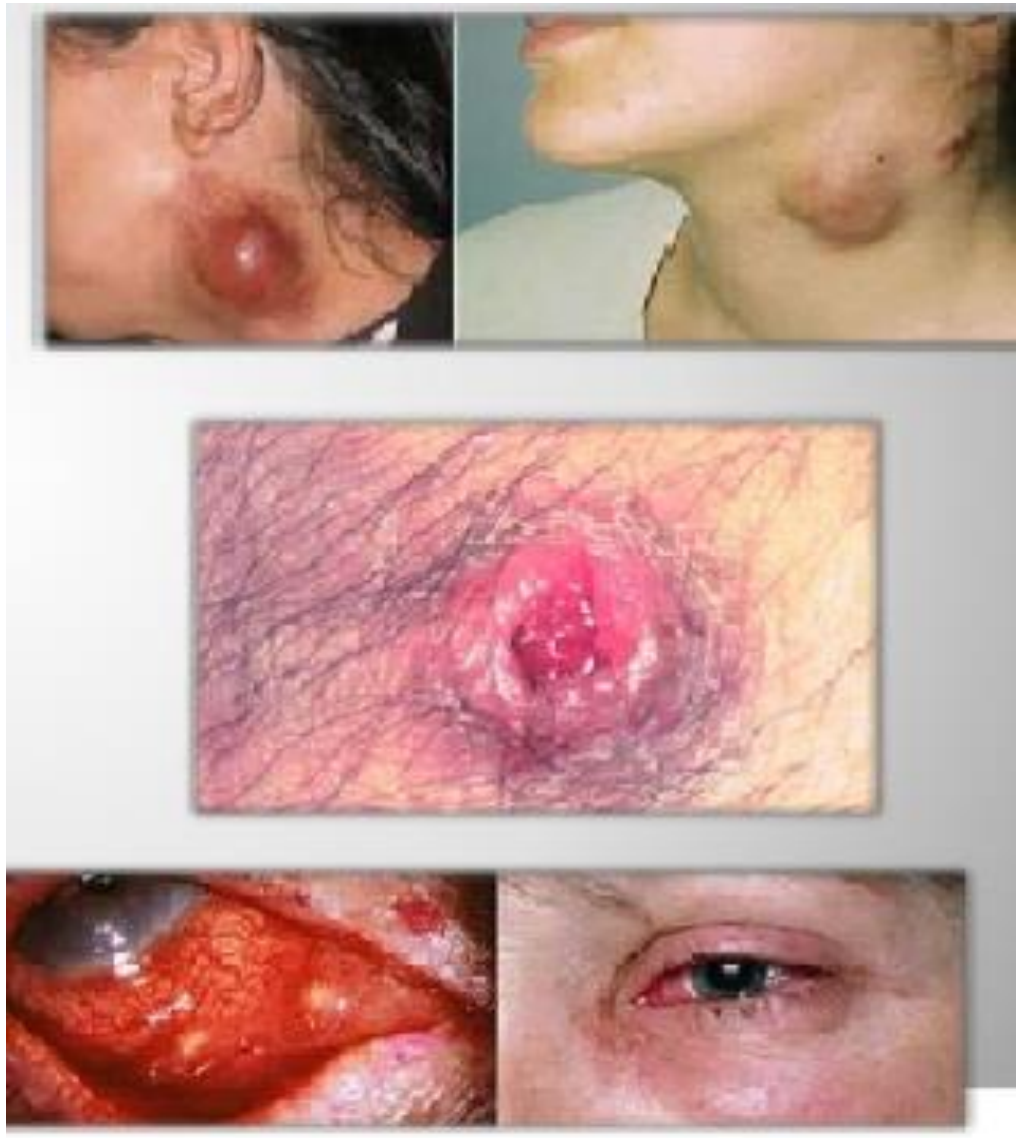
- **Ulceroglandular tularemia** is the most common variety of the disease. Symptoms can include:
- Lymph glands that are painful
- swollen
- Fever
- Chills
- Headache
- Fatigue
- **Oculoglandular tularemia** affects the eyes. Symptoms can include:
- Pain, swelling, or discharge in the eye
- Redness in 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.*