Lesson 15

Microbiological diagnosis of gram-negative rods form of zoonotic bacterial infections (*Brucella, Francisiella, Yersinia*)

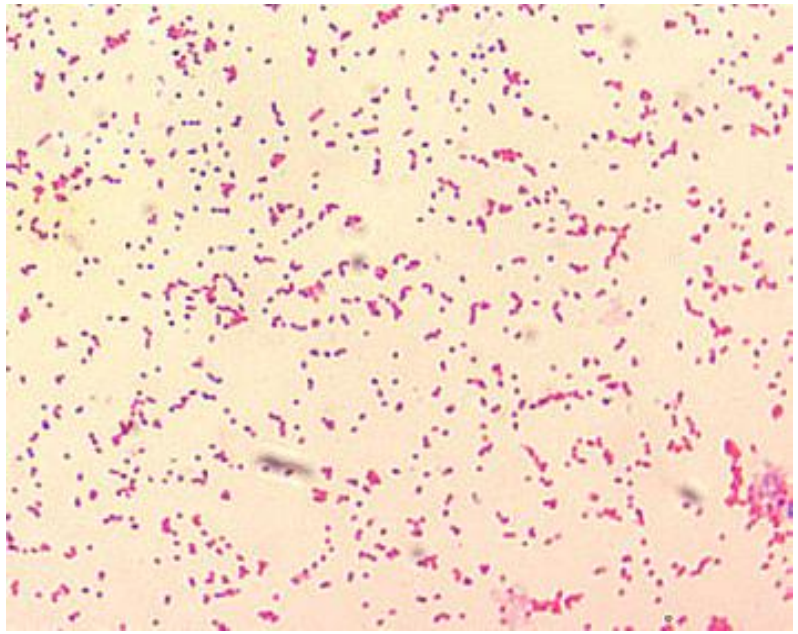
Genus *Brucella*

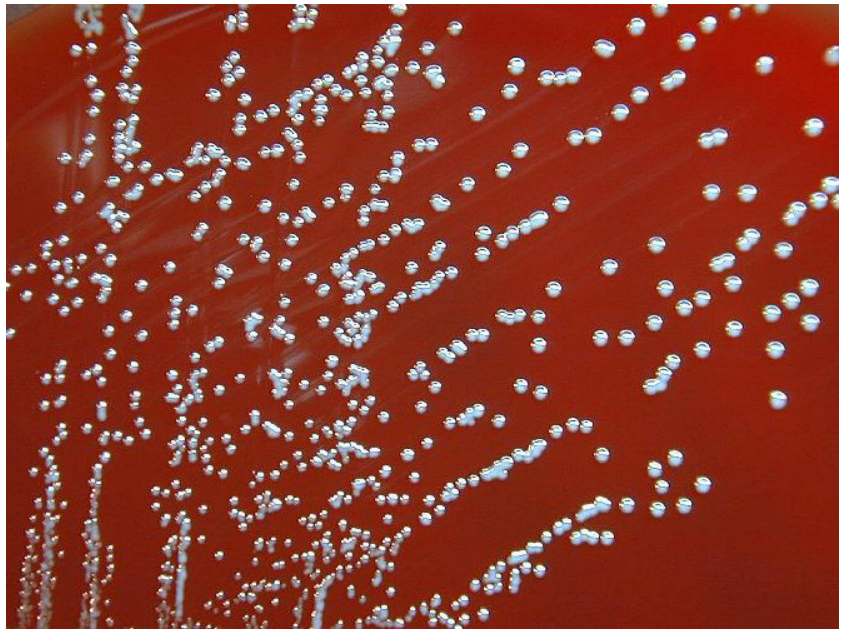
- ***Brucella melitensis***
- ***Brucella abortus***
- ***Brucella suis***
- ***B.ovis***
- ***B.canis***

Morpho-biological features

Brucella are small, immobile, gram-negative rods or cocobacilli 0.5-0.6x0.6-1.5 μm in size. Polymorphic. They do not form spores. Some species, when grown on a medium with immunological serum, form a delicate capsule. Under the action of antibiotics, L-forms are converted.

Brucella are aerobes, demanding on nutrient media. The optimal culture medium is liver agar. On dense nutrient media, they form small, convex, smooth, cloudy non-hemolytic S-colonies. Well cultivated in the yolk sac of the chicken embryo.





(smear from pure culture and colony of Brucella on liver agar)

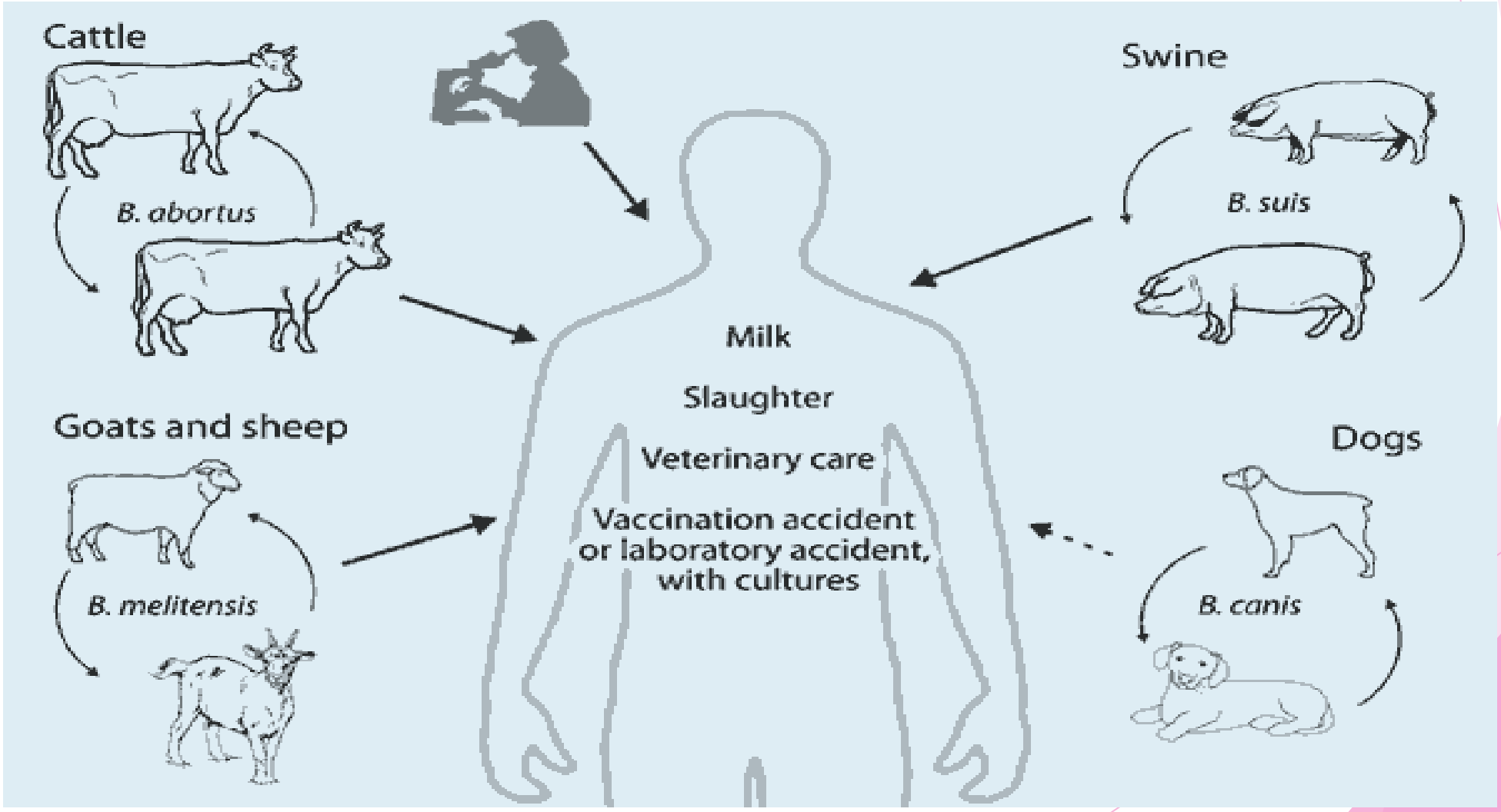
Intraspecific differentiation of Brucella

View	Bacteriostatic effect of dyes		H2S Education
	magenta	thionine	
Brusella melitensis	+	+	-
Brusella abortus	+	-	+
Brusella suis	-	+	+

Antigenic structure

- They have somatic O and capsular K antigens. Different types of Brucella are similar in antigenic structure, which makes it difficult to differentiate species by agglutination reaction.
- Different types of Brucella differ in the quantitative ratio of the two main surface antigens: A (abortus) and M (melitensis). This ratio in B.abortus is 20:1, in B.melitensis - 1:20, in B.suis - 1:2.
- Brucella share a common antigen with V.cholerae

Source of infection and route of infection



Pathogenic factors:

Brucella are facultative intracellular parasites.

- ✓ high invasive ability
- ✓ capsule and endotoxin
- ✓ low molecular weight substances that inhibit the fusion of phagosomes with lysosomes

Source of infection and routes of transmission

Brucellosis is a zoonotic infection, the reservoir of the pathogen in nature is animals. The main source of infection for humans is farm and domestic animals.

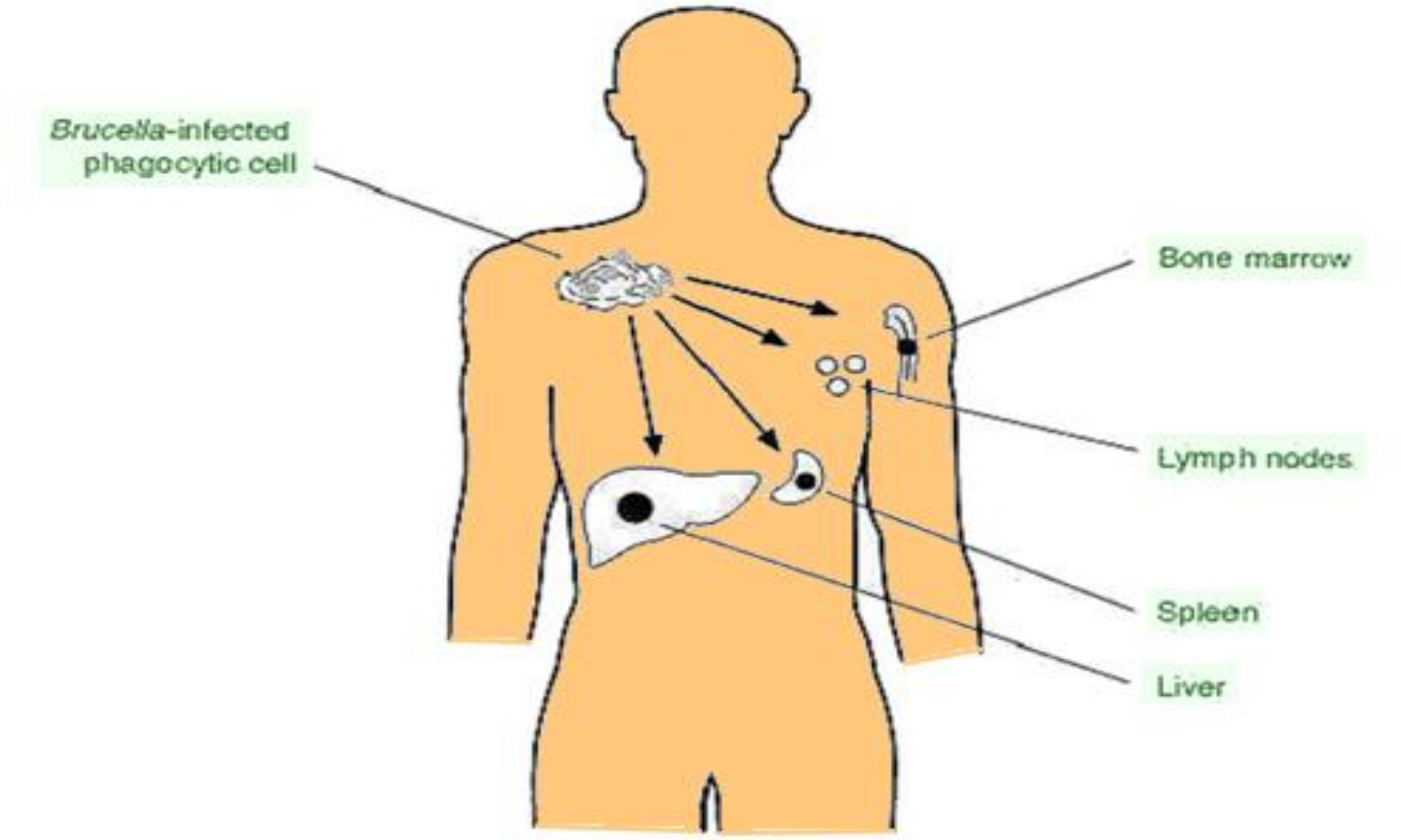
- Alimentary way
- contact way
- aerogenic way

A sick person is not contagious. However, laboratory workers become infected when working with pathological material taken from patients with brucellosis.

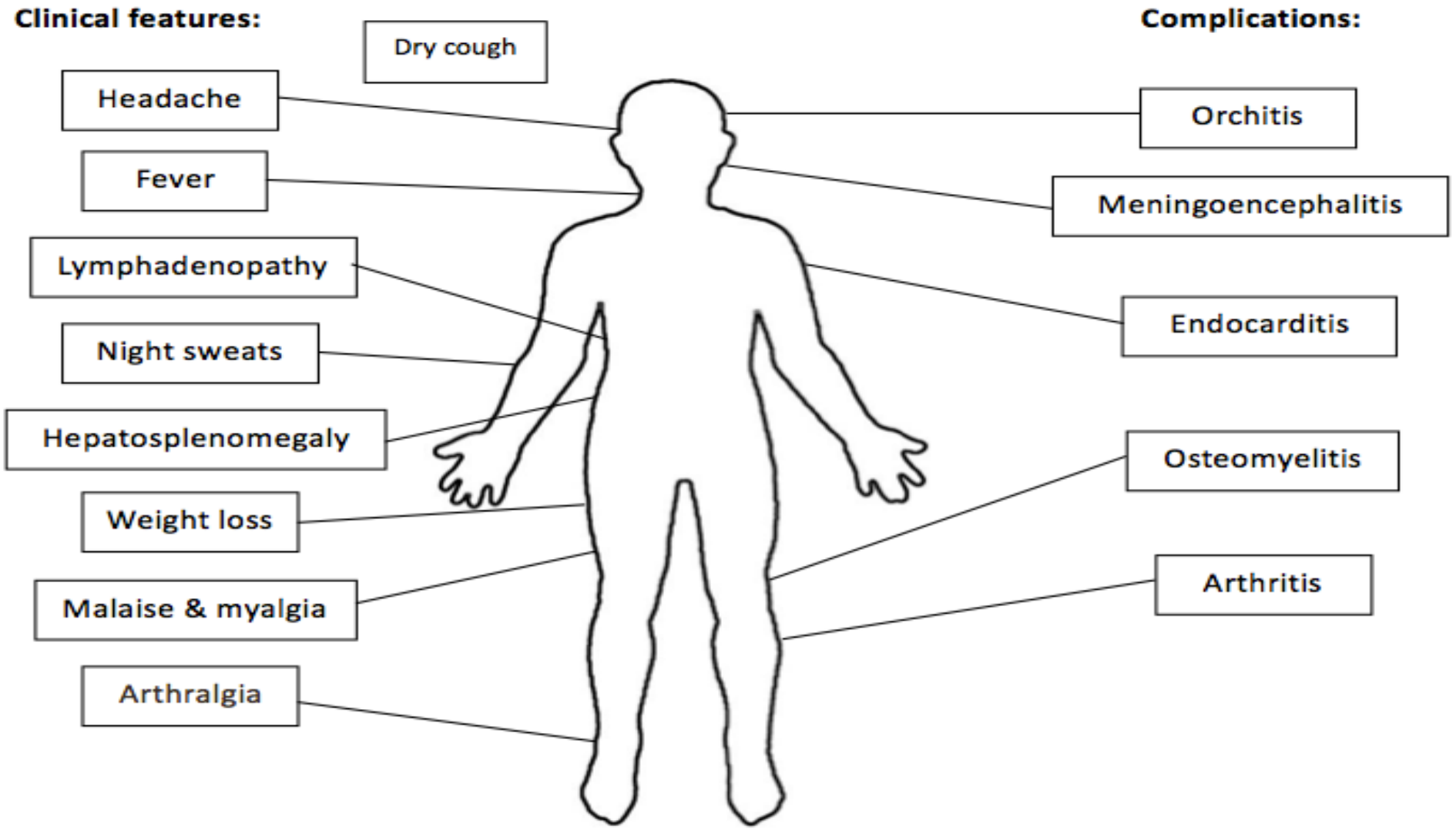
Brucellosis pathogenesis

- Brucella enter the body through the skin or mucous membranes, spread through the lymphatic tract and deposited in the lymph nodes. During the incubation period, the bacteria multiply in the macrophages of the regional lymph nodes.
- From the lymph nodes, Brucella enter the bloodstream and disseminate to the liver, spleen, and bone marrow. In the future, bacteria can enter the human mammary glands and appear in breast milk. In the organs of brucella, due to the ability to intracellular parasitism, they persist for a long time and form foci of necrosis, surrounded by infiltrates. The disease is characterized by a chronic course with a change of exacerbations and remissions. With exacerbations of the process, brucella again intensively multiply, enter the bloodstream, causing repeated waves of generalization.

Brucellosis pathogenesis



Clinical manifestations of brucellosis



Microbiological diagnostics

(research materials)

- blood
- bone marrow punctate
- urine
- excreta
- milk and dairy products
- pieces of organs

Microbiological diagnostics

- The bacteriological method is the inoculation of the test material in certain nutrient media, a pure culture of the pathogen is obtained after 3-4 weeks of incubation.
- Pathogens are isolated from blood (hemoculture), bone marrow punctate (myeloculture), urine (urine culture).
- To differentiate the obtained culture, the ability to form hydrogen sulfide, sensitivity to the bacteriostatic action of aniline dyes is used.

Microbiological diagnostics:

- The serological method is the main diagnostic method for brucellosis.
- Already on the 1st week of the disease, IgM appears in the patient's blood serum, reaching the maximum titer by the 3rd week and is found in the chronic form of brucellosis.
- On the 3rd week of the disease, IgG and IgA appear, reaching a maximum titer by the 6-8th week, which are found in the chronic course of brucellosis.

Microbiological diagnostics:
(serological method)

- The Hedelson reaction (approximate, microagglutination on glass) allows you to determine antibodies in the patient's blood serum.
- Using the Wright reaction (extended agglutination), the antibody titer is determined.
- A high antibody titer (above 1:80) is noted in acute brucellosis.
- The Coombs reaction is used to determine incomplete blocking antibodies. ELISA allows you to determine antibodies IgA, IgG, IgM.

Microbiological diagnostics:

- The biological method is based on infection (subcutaneously) of laboratory animals - white mice and guinea pigs.
- An allergic test (Burne test) is used to detect delayed-type hypersensitivity to Brucella. In the middle third of the forearm, a patient is injected intradermally with 0.1 ml (a protein extract from a Brucella culture). A positive reaction is considered infiltrate, redness 4-6 cm in size, which occur after 24-48 hours.

Prevention

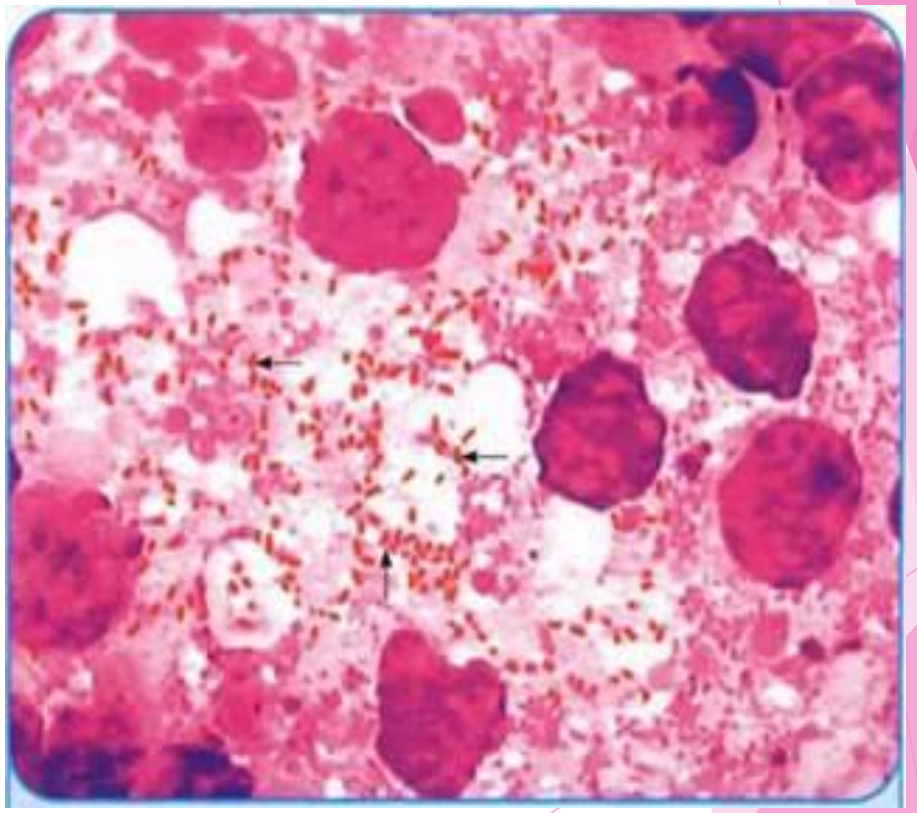
- People engaged in animal husbandry are vaccinated according to epidemic indications using a live brucellosis vaccine.
- The vaccine is reactogenic and does not have a high protective effect.

Treatment

- Brucella are sensitive to tetracycline and streptomycin.
- In the chronic form of brucellosis, specific immunotherapy with a killed therapeutic brucellosis vaccine or brucellin is used.

The causative agent of tularemia Morpho-biological properties

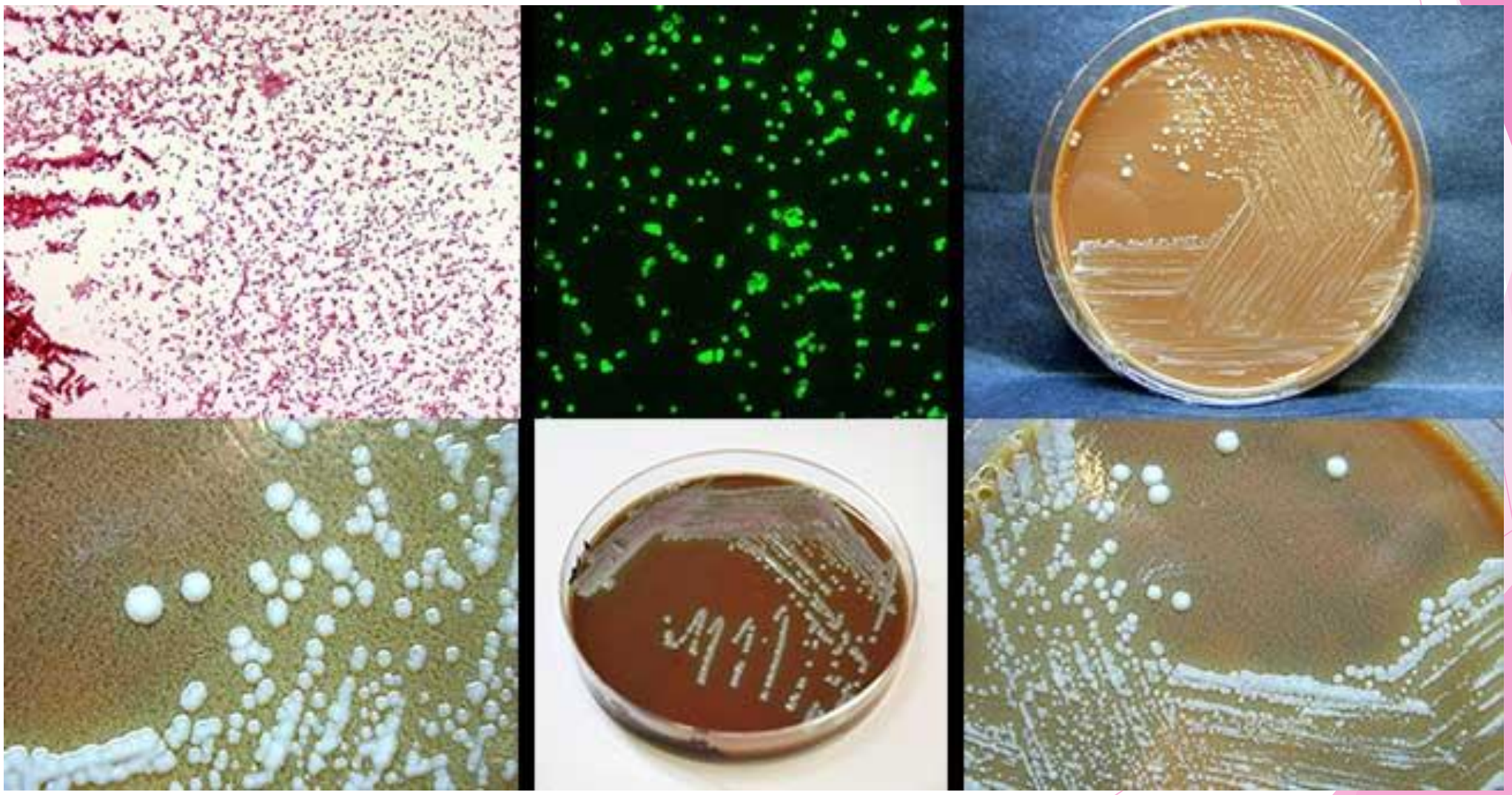
Francisella tularensis - small gram-negative coccobacilli 0.3-0.6x0.1-0.2 μm in size. They are characterized by polymorphism: they can have spherical, filamentous, and other shapes that pass through bacterial filters. They do not form spores, are immobile, and can form a tender capsule.



Francisella tularensis

- Facultative anaerobe. Does not grow on simple nutrient media. Cultivated on yolk media (McCoy's medium) or on media with the addition of blood and cysteine (Francis' medium) at a temperature of 37-38C.
- On dense nutrient media within 4-14 days forms small colonies of milky-white color with a diameter of 1-3 mm.
- Virulent strains form S-colonies. When cultivated on artificial nutrient media, virulent S-forms are converted into avirulent and non-immunogenic R-forms.
- The causative agent of tularemia is well cultivated in the yolk sac of the chicken embryo.

Francisella tularensis



Source of infection and route of infection

- Tularemia is a natural focal disease. The source of infection in natural conditions are mainly small rodents (field mice, water rats, muskrats, etc.) and hares. On the territory of natural foci, sheep, pigs, and cattle can become infected with tularemia.

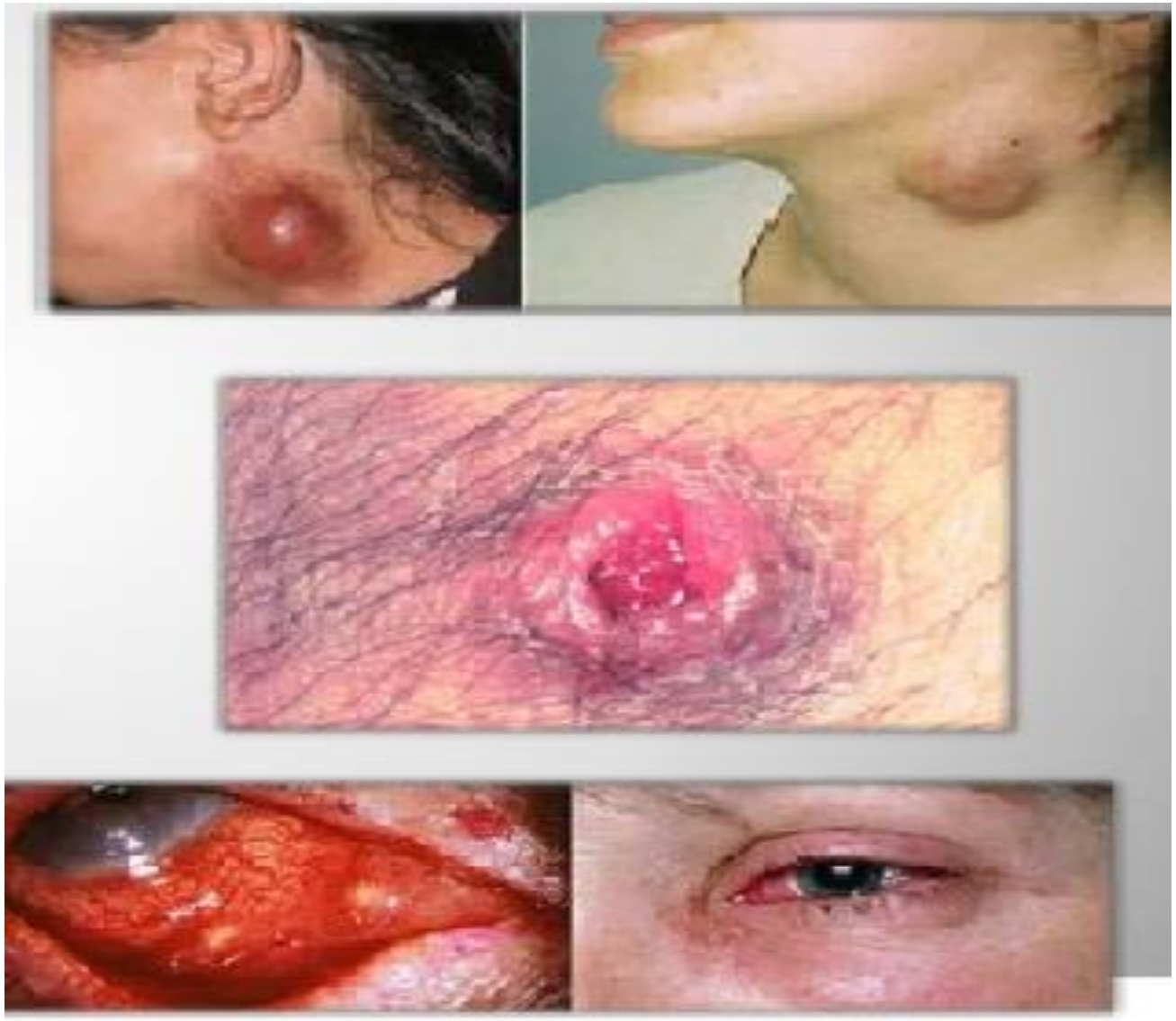
Disease pathogenesis

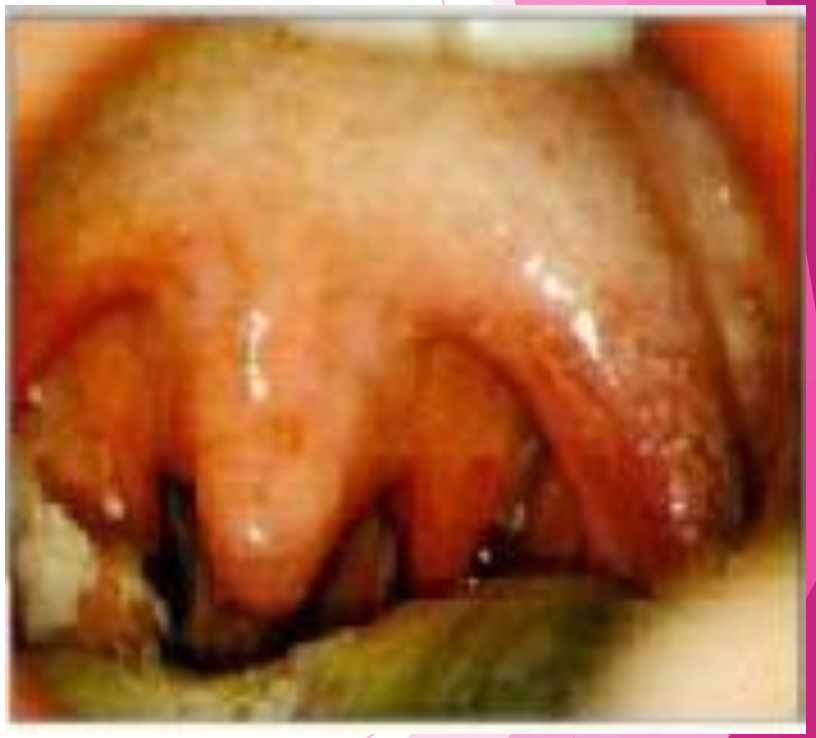
- The causative agent of tularemia - F.tularensis enters the human body through the skin, mucous membranes of the eyes, respiratory tract, gastrointestinal tract.
- After penetration into the body, the pathogen spreads with the lymph flow. Despite the active absorption of bacteria by phagocytes, complete destruction does not occur, which creates prerequisites for the deposition of bacteria in the lymph nodes. Some of the pathogens die, which is accompanied by the release of endotoxin, which acts on the lymph nodes with the development of primary foci and the formation of tularemia buboes.

Disease pathogenesis

- With tularemia, as well as with plague, primary (deposition of the pathogen in the lymph nodes) and secondary buboes (generalized form) develop. Periodically, from the formed primary foci, the pathogen penetrates into the lymph and bloodstream, which leads to the spread of bacteria to the liver, spleen, lungs, bone marrow and other organs, forming secondary foci.

Clinical manifestations of the bubonic form of tularemia:
(bubonic, ulcerative-bubonic, eye-bubonic, etc.)





Microbiological diagnostics

- Bacteriological method - although it is possible to isolate the pathogen from pathological material (blood, punctate from bubo, conjunctival discharge, plaque from the throat, sputum, etc.), the bacteriological method in the diagnosis of tularemia rarely gives positive results.
- Serological method - RA. A suspension of bacteria killed by formalin is used as a diagnosticum. A positive result of the reaction at a serum dilution of 1:160 or more is detected in persons with tularemia and who have had an infection.
- Allergic test - put a skin or intradermal test using a suspension of bacteria killed by heat (tularin). An infiltrate with a diameter of at least 5 mm is taken as a positive result.

Treatment

- Streptomycin
- Gentamicin
- Tetracycline

F.tularensis is not sensitive to beta-lactam drugs!

Prevention

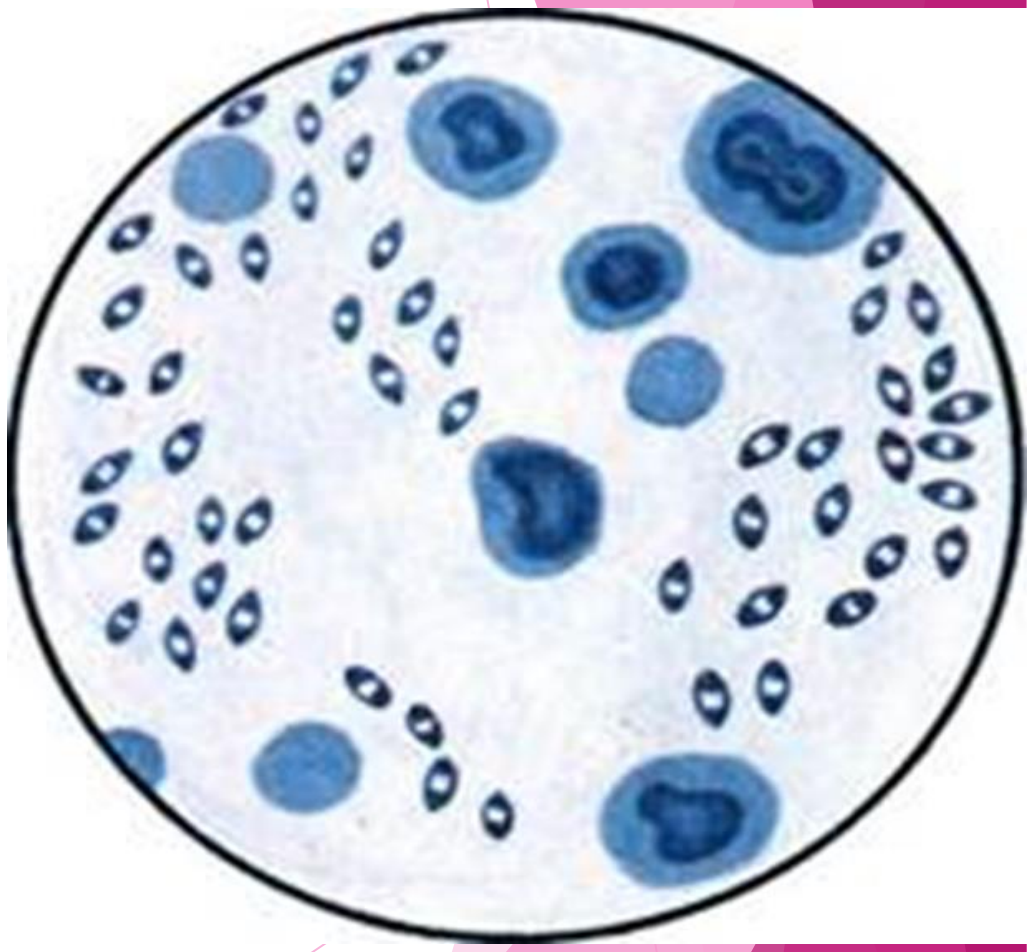
- Non-specific prophylaxis (rodent control)
- specific prophylaxis. Vaccination is carried out for persons at risk using a live attenuated tularemia vaccine.

Genus Yersinia

Family: Enterobacteriaceae

Genus: Yersinia

Species: *Y.pestis, Y.enterocolitica, Y.pseudotuberculosis*



Morpho-biological properties:

Y. pestis - Gram-negative, immobile ovoid rods, 0.4-0.7x1-2 μm in size. They form a delicate capsule.

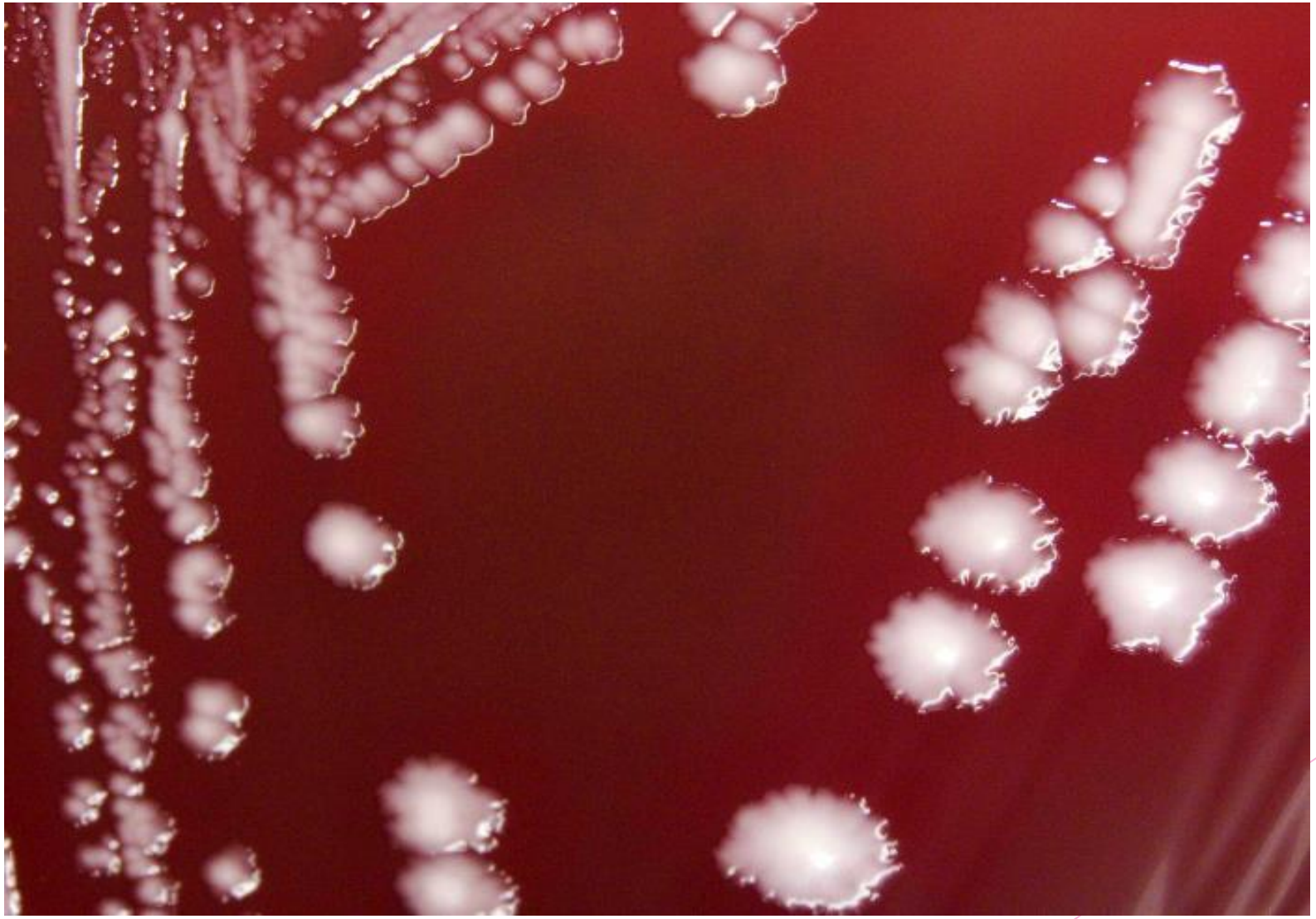
Yersinia pestis

Cultural properties - facultative anaerobe, the elective medium of which is casein medium and blood clot hydrolyzate.

On dense nutrient media, they form colonies with uneven edges. After 48 hours of incubation, virulent bacteria form R-shaped colonies resembling lacy handkerchiefs. Less virulent bacteria form S-shaped colonies.

On liquid media, they grow in the form of a film, from which threads descend, resembling cave stalactites.

Yersinia pestis
(colonies on blood agar)



Differentiation into species within the genus Yersinia

Index	Y. pestis	Y.enterocolitica	Y.pseudotuberculosis
Mobility	-	+	+
Indole products	-	±	-
Hydrolysis of gelatin	-	-	-
Voges–Proskauer reaction	-	±	-
Rhamnoza	-	-	+
sucrose	-	+	-
Dextrin	+	-	-
Esculin	+	±	-
Urease	-	+	+
Lysine decarboxylase	-	-	-
Ornitidine decarboxylase	-	+	-

Antigenic structure and pathogenicity factors:

- The plague bacillus has a complex of antigens, many of which are pathogenicity factors.
- O-antigen (endotoxin) - toxic to humans and animals.
- Fraction I (F1-antigen) is a capsular antigen that protects bacteria from phagocytosis, has no toxic effect, and does not show immunogenic properties.
- Fraction II (F2-antigen), an antagonist of adrenergic receptors, is represented by a protein-like substance localized intracellularly. Causes shock and death in laboratory animals: LD50 in mice less than 1 mg (also toxic to rats). Mouse toxin has the ability to inhibit the respiratory activity of mitochondria, lowering the activity of NADP-reductase.

Antigenic structure and pathogenicity factors:

- The V/W (Vi) antigen consists of a protein (V-fraction) and a lipoprotein (W-fraction). It exhibits antiphagocytic properties and promotes intracellular reproduction of bacteria. Strains containing only V/W-Ar are virulent in mice.
- Plasminogen activator is a protease that activates the lysis of fibrin clots that prevent dissemination of the pathogen, and inactivates the C3b and C5a components of the complement.
- In addition, Y.pestis synthesizes aggression enzymes such as plasmacoagulase, hemolysin, lecithinase, hyaluronidase and RNase.

Source of infection and routes of transmission:

- The source of infection is sick animals, in particular rodents.
- Plague epidemics among humans usually follow epizootics among animals.
- Human susceptibility to plague is very high. The contagiousness index approaches one.

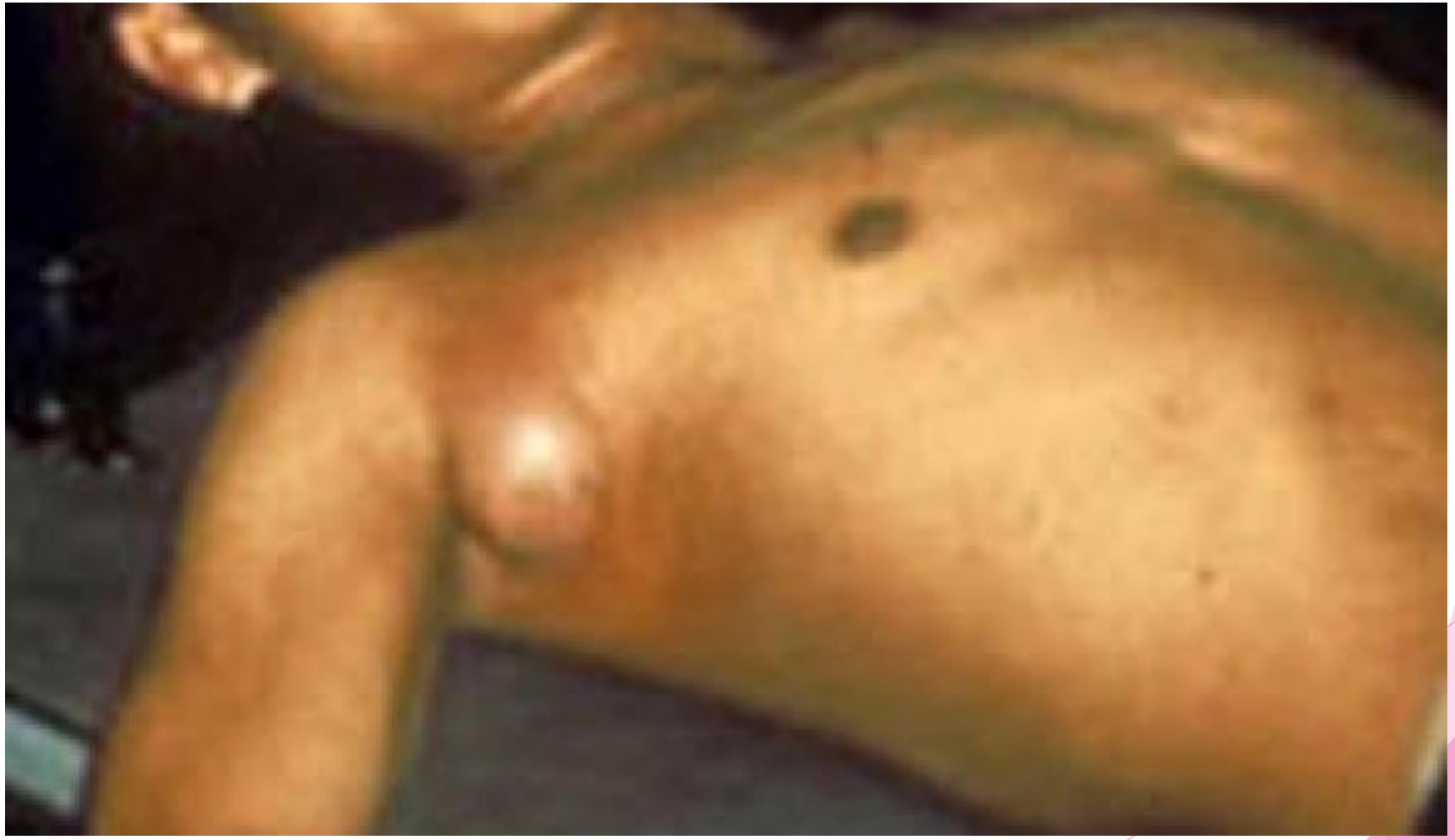
Transmission routes:

- *Transmissible route (through the bites of infected fleas)*
- *Contact route (when cutting skins and meat of infected animals)*
- *Alimentary route (food contaminated with plague microbes)*
- *Airborne droplet (from patients with pneumonic plague)*

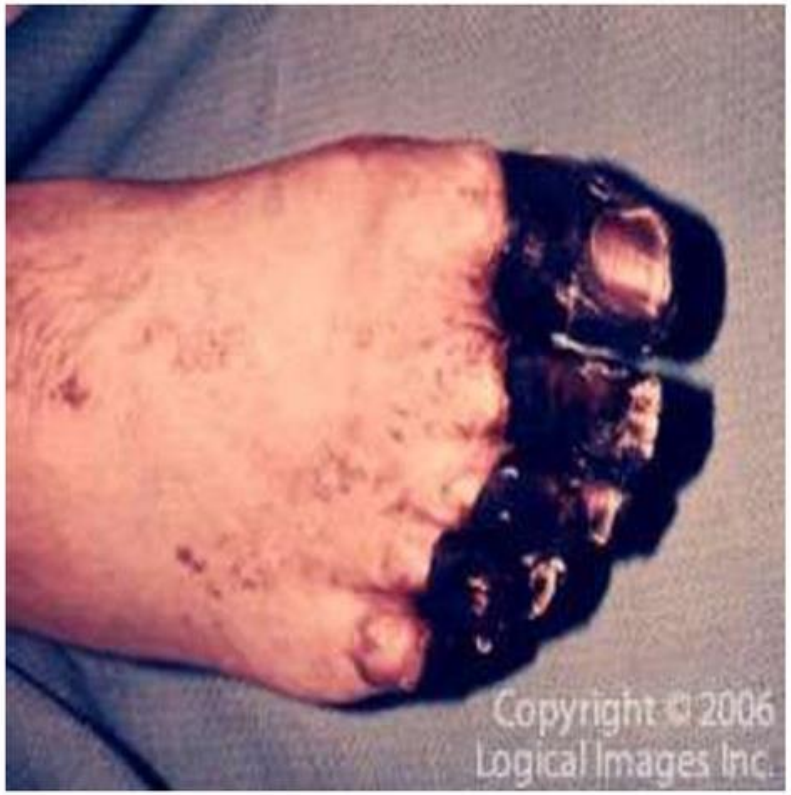
Pathogenesis and clinical manifestations of plague:

- Cutaneous plague
- bubonic form of plague
- Skin-bubonic form
- Secondary buboes
- Primary septic form
- Secondary septic form
- Primary pulmonary form
- Secondary pulmonary form
- intestinal form

Bubonic form of plague



Septic form of plague



Microbiological diagnostics:

- All activities are carried out in specialized laboratories. Depending on the clinical form, the materials for the study are bubo punctates, sputum, blood, urine, vomit, feces, cadaveric material, etc.
- Research methods: microscopic, bacteriological, biological and serological.
- Microscopic method: microscopy of smears stained with Gram, Giemsa and methylene blue.
- Immunofluorescence reaction (RIF) is used as express diagnostics.

Microbiological diagnostics:

- *Bacteriological method*. Pathological material is sown on nutrient media; Identification of isolated cultures is carried out on the basis of morphological, cultural, biochemical properties and sensitivity to the bacteriophage.
- *Serological method* (ELISA)
- *A biological test* is carried out by infecting guinea pigs and rats.
- *Molecular genetic method* (PCR)

Prevention

- Non-specific prophylaxis is aimed at preventing infection of people and the occurrence of epizootics in natural foci.
- If plague is detected, quarantine measures are taken. For persons who have been in contact with patients, prophylactic treatment with tetracycline is prescribed.
- For specific prophylaxis, vaccines from microorganisms killed by formalin are used. Active immunization is carried out to persons who are in endemic foci and work with the pathogen.

Treatment

Streptomycin is used to treat plague patients. An alternative drug is tetracycline, which can be used in combination with streptomycin.

Y.enterocolitica

Morpho-biological properties:

Y.enterocolitica - gram-negative rods, 1.8-2.7x0.3-0.7 µm in size, mobile, do not form spores and capsules. Facultative anaerobes grow well on normal nutrient media. On meat-peptone agar form small shiny colonies in the form of dew drops. Glucose and sucrose are fermented to acid, do not break down rhamnose, and produce urease.

Antigenic structure - has O-, H-antigens

Pathogenic factors - produce enterotoxin

Pathogenesis and clinical manifestations of intestinal yersiniosis

- Intestinal yersiniosis is an infectious disease with intestinal damage and the development of mesenteric lymphadenitis.
- The main symptom is gastroenteritis. Joint damage - polyarthritis.
- Etiological agent of ankylosing spondylitis

Y.pseudotuberculosis

Morpho-biological properties:

Y.pseudotuberculosis - gram-negative motile bacteria 0.8-2x0.4-0.6 microns in size do not form spores, form a capsule. Facultative anaerobe, grows well on simple nutrient media. On dense nutrient media at a temperature of 28ºC it forms colonies in the S-form, at a temperature of 37ºC - colonies in the R-form. Forms a film on liquid media. Biochemically inactive: they ferment rhamnose, produce urease, do not ferment sucrose.

Antigenic structure - has O-, H-antigens.

Pathogenic factors - endotoxin (LPS).

Pathogenesis and clinical manifestations of pseudotuberculosis

- Y.pseudotuberculosis - having invaded the intestinal mucosa with transcytosis through M-proteins, it enters the mesenteric lymph nodes, causing mesenteric lymphadenitis.
- Clinical symptoms - pain in the epigastric region, symptoms of peritoneal irritation, which mimic the symptoms of acute appendicitis.

Microbiological diagnostics

- Research materials - feces, blood
- bacteriological method. Pathological material is sown on nutrient media (MPA, Endo, Mac Conkey). After the isolation of a pure culture, identification is carried out on the basis of morphological, cultural, biochemical properties.
- Serological method - RPHA or ELISA