Lesson 18

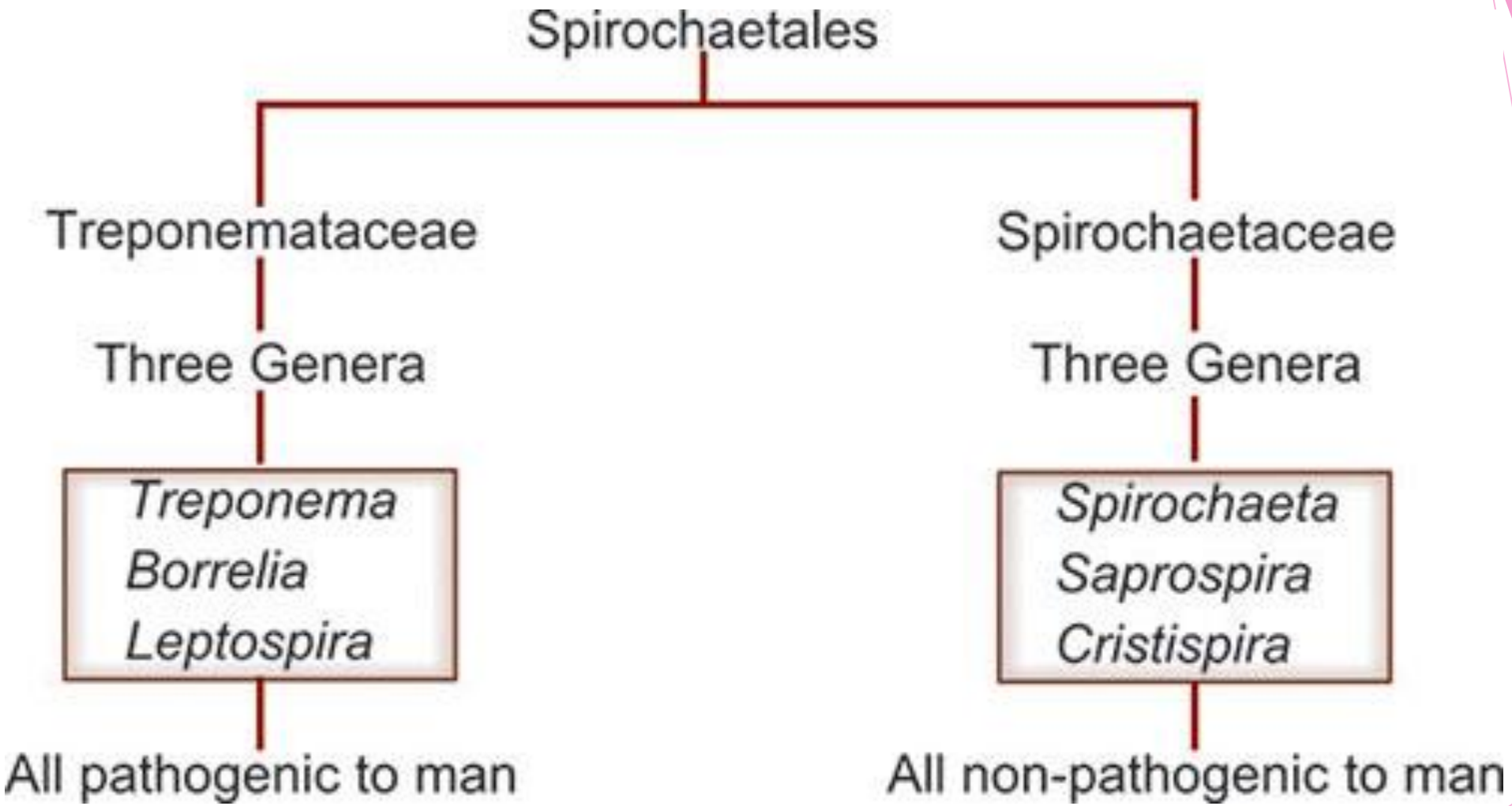
Microbiological diagnosis of infections caused by Treponema, Borrelia, Leptospira.

Spirochetes

- Spirochetes (speria-spiral, chaite-hair) are mobile spirally convoluted bacteria. According to morphological and some biological properties, spirochetes occupy an intermediate position between bacteria and protozoa. They belong to the order Spirochetales, which includes 2 families.

- The Spirochaetaceae family includes free-living, non-pathogenic forms.

- Representatives of the genera Treponema, Borrelia, Leptospira of the Treponemataceae family are pathogenic for humans.


Spirochaetales
Treponemataceae
Three Genera
Treponema
Borrelia
Leptospira
All pathogenic to man
Spirochaetaceae
Three Genera
Spirochaeta
Saprospira
Cristispira
All non-pathogenic to man

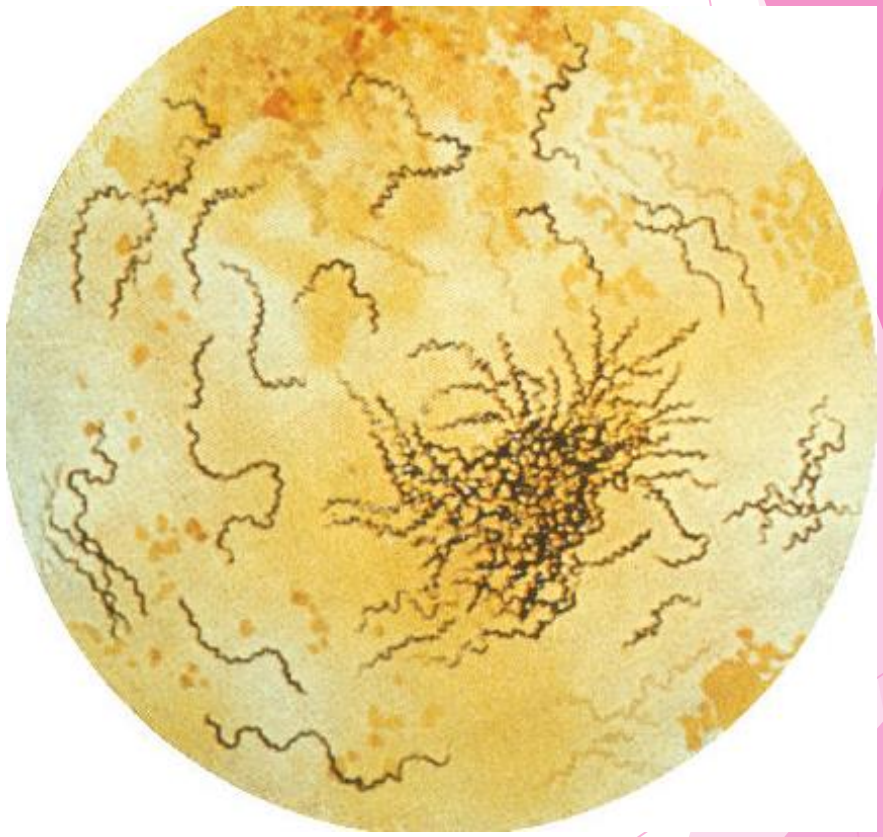
genus Treponema

- The genus Treponema (from the Latin trepo - bend, nema - thread) includes many species. Free-living forms of treponema do not occur in nature. They live in the oral cavity, gastrointestinal tract and genital organs of various animals.
- Some species of the genus Treponema –T. denticola, T. macrodenticum, T. orale, T. vincentii are part of the microflora of the oral cavity: T. vincentii in combination with fusobacteria causes Vincent's necrotizing angina.
- An important role in human pathology is played by the species T. pallidum. The species is divided into 3 subspecies: subspecies pallidum - the causative agent of syphilis, subspecies endemicum - the causative agent of endemic syphilis or bejeli, and subspecies pertenue - the causative agent of yaws. T. carateum causes pint disease in humans.

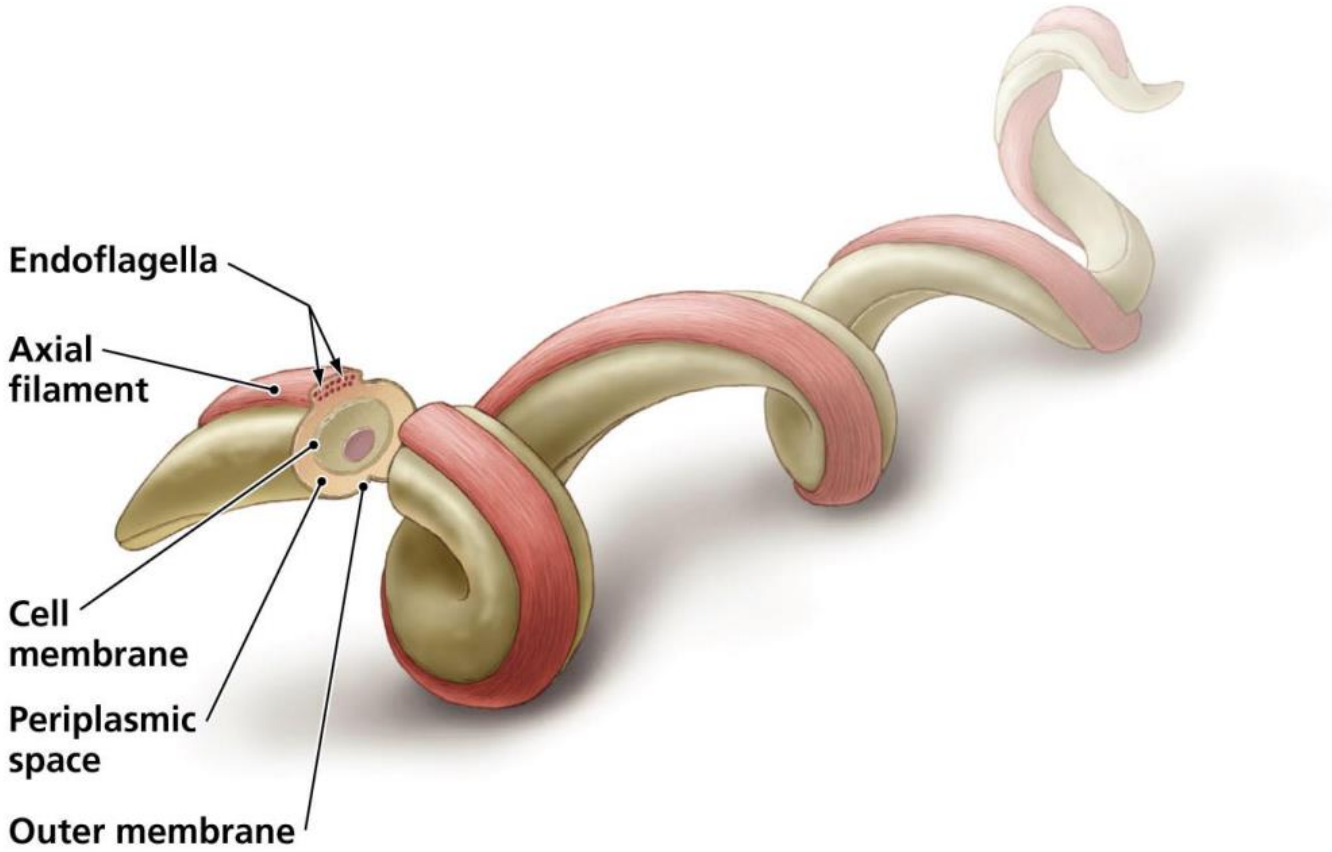
The causative agent of syphilis (Treponema pallidum)

- Morpho-biological properties. T.pallidum - spiral bacteria 5-15 microns long, 0.2 microns thick, having 8-12 uniform curls.
- The distance between the curls is 1 μm. These are typical treponemas in morphology. Slightly stained with aniline dyes. According to Romanovsky-Giemsa, they are painted in a pale pink color (hence the name of the species is pale spirochete, from Latin pallidum - pale).
- Detected during impregnation with silver (silver plating method according to Morozov)
- Mobile spirochetes in native smears are detected using phase-contrast and dark-field microscopy.

Treponema pallidum



Treponema pallidum



Treponema pallidum

dark field microscopy

Treponema pallidum

- In freshly prepared preparations, spirochetes have active mobility - they perform rotational, translational, flexion and helical movements, which are detected by dark-field microscopy.
- Flexion movements at right angles without losing the spiral shape is an important differential feature of the genus Treponema. The name of the genus "Treponema" is associated precisely with this feature (Latin means "bending thread").

Treponema pallidum

dark field microscopy

Treponema pallidum

- The causative agent of syphilis is a microaerophile. Virulent strains of T.pallidum are not cultivated either on artificial nutrient media or in cell culture.
- Non-virulent strains (eg Reiter's strain) can be cultured in vitro under anaerobic conditions at 35°C in agar containing amino acids, vitamins, salts, minerals and serum albumins.
- On the 3-5th day of cultivation, small, smooth colonies appear.
- Cultivation leads to a loss of virulent and a change in antigenic properties.

Antigenic structure

- The antigenic structure is not well understood.
- In the body, specific antibodies are formed to the pathogen, which are detected by indirect immunofluorescence, as well as by the immobilization reaction.
- Antibodies have the ability to destroy bacteria and bind complement with the participation of treponema.
- In the course of the disease, nonspecific antibodies are also formed - reagins, which can be detected in a flocculation reaction with cardiolipin extracted from a bovine heart. This reaction is used in the diagnosis of syphilis.

Resistance to environmental factors

- T.pallidum is sensitive to desiccation, sunlight, and disinfectants.
- When heated, it instantly dies. In blood preparations at a temperature of 40C for 24 hours remains viable.
- Under unfavorable conditions, it forms cysts and L-forms.

Pathogenicity factors

- The pathogenicity of T.pallidum is associated with active mobility. Due to this, the pathogen easily passes through the skin and mucous membranes, and penetrate into the bloodstream.
- Receptors for fibronectin and collagen ensure its adhesion to the interstitial tissue.
- Does not form toxin.
- Lipoproteins are involved in the development of immunopathological processes

Source of infection and routes of transmission

- Under natural conditions, only a person gets sick.
- Infection occurs, as a rule, by sexual contact, less often by contact and household routes.
- During pregnancy, infection with syphilis occurs transplacental (from mother to fetus), as a result, intrauterine death occurs or the child is born with signs of syphilis.
- Infection is possible as a result of blood transfusion, especially when transfusing fresh specimens.

Pathogenesis and clinical manifestations of syphilis

- The entry gates of infection are the skin and mucous membranes.
- The causative agent can penetrate through intact skin, through microtraumas of the mucous membranes or skin, and multiply in tissues and partially in regional lymph nodes.
- Syphilis is a disease with a cyclic course, occurring in several periods.

Primary period of syphilis.

- Due to the fact that with syphilis the base of the ulcer has a hard consistency, it was called "hard chancre" (French, chancre - wound).
- Hard chancre develops as a result of reactive vasculitis, which is accompanied by massive death of epithelial cells due to blockage of microcapillaries.
- The dense consistency of the chancre is the result of a productive infiltrative process involving lymphocytes and plasma cells. There is no pus in the wound, the bottom of the wound is clean with a varnish surface, the edges are serrated.
- Due to the fact that the chancre contains a large number of spirochetes, patients during this period are most contagious. Despite the fact that after some time the hard chancre heals, after 2-10 weeks the second stage of the disease begins.

Syphilis (hard chancre)

Syphilis - II period

- A red maculopapular rash develops on various parts of the body, including on the arms and legs, and pale warts appear on the anogenital organs and oral mucosa. During this period, the development of syphilitic meningitis, chorioretinitis, hepatitis, immunocomplex type of nephritis, periostitis is possible.

- Because the rash is rich in spirochetes, patients are contagious during this period. Although these elements heal on their own, they may reappear after 3-5 years, however, after this period, the tertiary period of the disease begins.
- Approximately in 30% of cases, spontaneous cure can occur, in 30% of cases the disease remains in a latent form and is detected only in positive serological reactions. In other cases, the disease passes into the tertiary period.
- It should be noted that both the primary and secondary periods of syphilis can be asymptomatic - have a subclinical course. In such cases, the disease manifests itself with signs of the tertiary period.

Syphilis - II period

Syphilis - III period

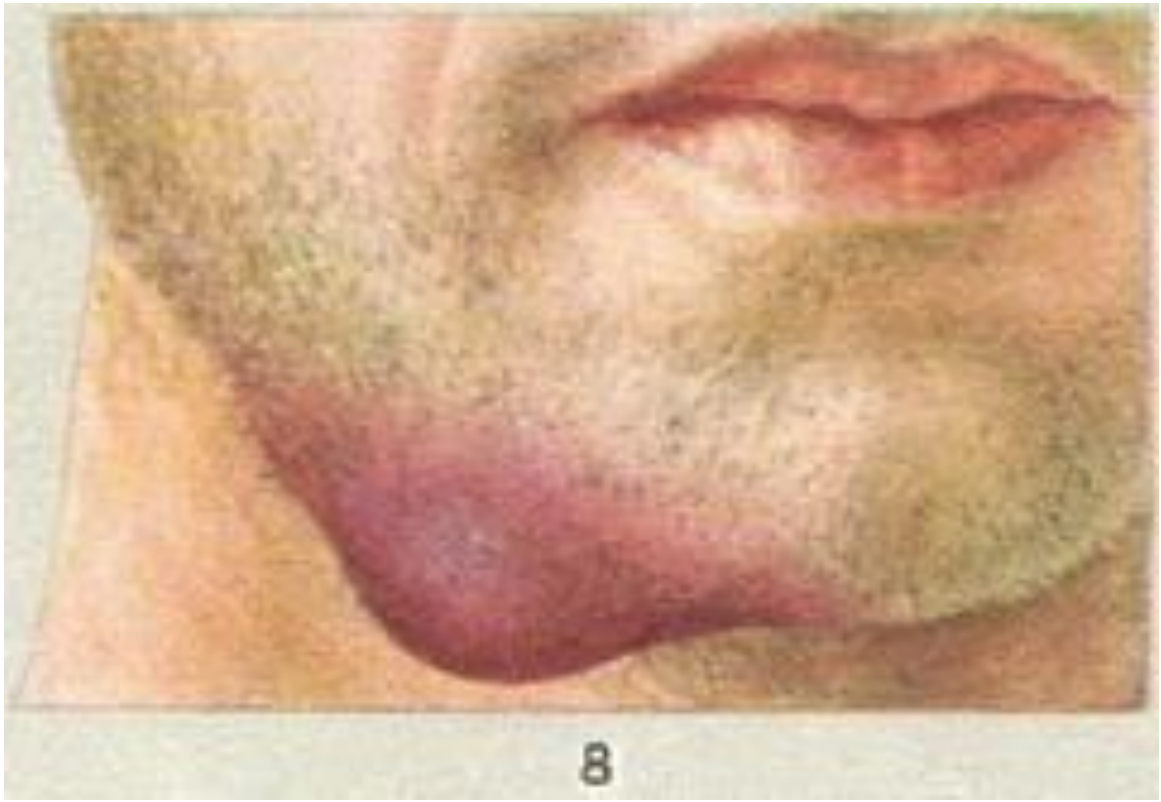
- It is manifested by the formation of syphilitic granulomas - gum on the skin, bones and liver. They are the result of the development of an immunopathological process and a response to treponemas that persist in the body.

- Gummas tend to disintegrate with subsequent general destructive changes in damaged organs and tissues.

- There may be degenerative changes in the central nervous system (meningovascular syphilis, paresis, dorsal tabes dorsalis), as well as changes in the cardiovascular system, manifested by aortitis, aortic aneurysm, aortic valve insufficiency.

- At the third stage, the disease is not contagious; in some cases, treponemas are found in the central nervous system.

Syphilis - III period



Congenital syphilis

- Treponema, circulating in the blood of a sick mother, in the second trimester of pregnancy can infect the fetus through the placenta.
- The outcome of intrauterine infection depends on the number of treponemas that infect the fetus.
- A high infectious dose can lead to stillbirth and abortion. In other cases, congenital syphilis occurs.

- Since the pathogen passes directly through the placenta into the fetal circulation, the symptoms of congenital syphilis are similar to those of the second stage of syphilis in adults. The lesions on the skin are rich in treponema, the appearance of the newborn is similar to the senile one - the skin of the face is wrinkled, there is a lack of weight and malnutrition.
- Sometimes there are Hutchinson's triad - keratitis, barrel-shaped teeth, signs of deafness.

Congenital syphilis

Immunity

- As with all sexually transmitted diseases, the immunity that develops after syphilis does not protect against relapse. In the case of re-infection with syphilis, a hard chancre is not observed, the disease begins with signs of the second period. Therefore, the immunity formed during syphilis is sometimes called "immunity to the chancre.“
- Superinfection with T. pallidum is impossible both in the active period of the disease and in latent syphilis. However, after effective treatment, those who have been ill again can get sick with syphilis.

Immunity

- Humoral immunity is manifested by the formation of antibodies to the pathogen in the body. Non-specific antibodies (non-treponemal antibodies) are formed, called "reagins", represented by IgM and IgG antibodies, which are formed primarily to the lipoid antigen of the pathogen. As the number of treponemas in the body decreases, the titer of these antibodies decreases.
- Later, specific antibodies (treponemal antibodies) to the protein antigen are formed. Regardless of the presence of treponema in the body, they persist for a long time.
- Cellular immunity is a delayed-type hypersensitivity reaction that causes the formation of syphilitic gums.

Microbiological diagnostics

- Various research methods are used depending on the stage of the disease, mainly microscopic and serological.
- Microscopic method. It is based on the isolation of treponema from a detachable hard chancre in primary and rashes in secondary syphilis. Native preparations are examined in dark-field microscopy, preparations stained by the Giemsa method and silvering - under a light microscope. It should be remembered that a few hours after the start of antibiotic treatment, treponema is not microscopically detected. - Treponemas can also be detected by immunofluorescence. To do this, smears treated with antitreponemal antibodies labeled with fluorochromes are examined under a fluorescent microscope.

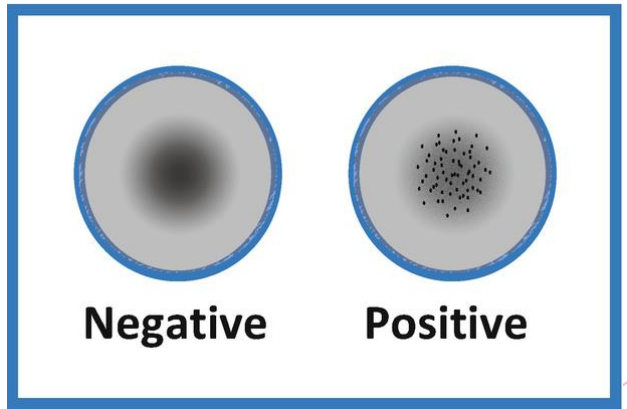
Microbiological diagnostics

The serological method of research is aimed at identifying treponemal and non-treponemal antibodies in the patient's serum.

Study of non-treponemal antibodies

- The most commonly used antigen is cardiolipin, derived from bovine heart muscle. Purified cardiolipin from a chemical point of view is diphosphatidylglycerol. The addition of lecithin and cholesterol accelerates the reaction of the cardiolipin antigen with non-specific reagents.
- The most commonly used test is the VDRL test (English, veneral disease research laboratory) and the RPR test (English, rapid plasma reagin), sometimes the TRUST test (English, toluidine red unheated serum test).
- The principle of these tests is based on the precipitation (flocculation) of the cardiolipin antigen by the reagins of the patient's serum. The results of the VDRL test are assessed microscopically, and in the RPR and TRUST tests, the precipitation reaction is visible to the naked eye as the antigens are adsorbed onto the colored particles.

Study of non-treponemal antibodies



Study of non-treponemal antibodies

- The RPR test is positive at high titers starting from the 2-3rd week of the disease, especially in the second stage of the disease. These tests are negative 6-18 months after effective treatment for syphilis.
- The RPR test can also be performed with the patient's cerebrospinal fluid, in which case it is positive starting from the 4-8th week of the disease. Reagins cannot cross the blood-brain barrier, so it is assumed that these antibodies are also formed in the central nervous system.

- The RPR test may be positive in about 1% of healthy people, in addition, with malaria, leprosy, measles, infectious mononucleosis, collagenoses (systemic lupus erythematosus, rheumatoid arthritis, etc.), as well as after vaccination (false positive reaction).

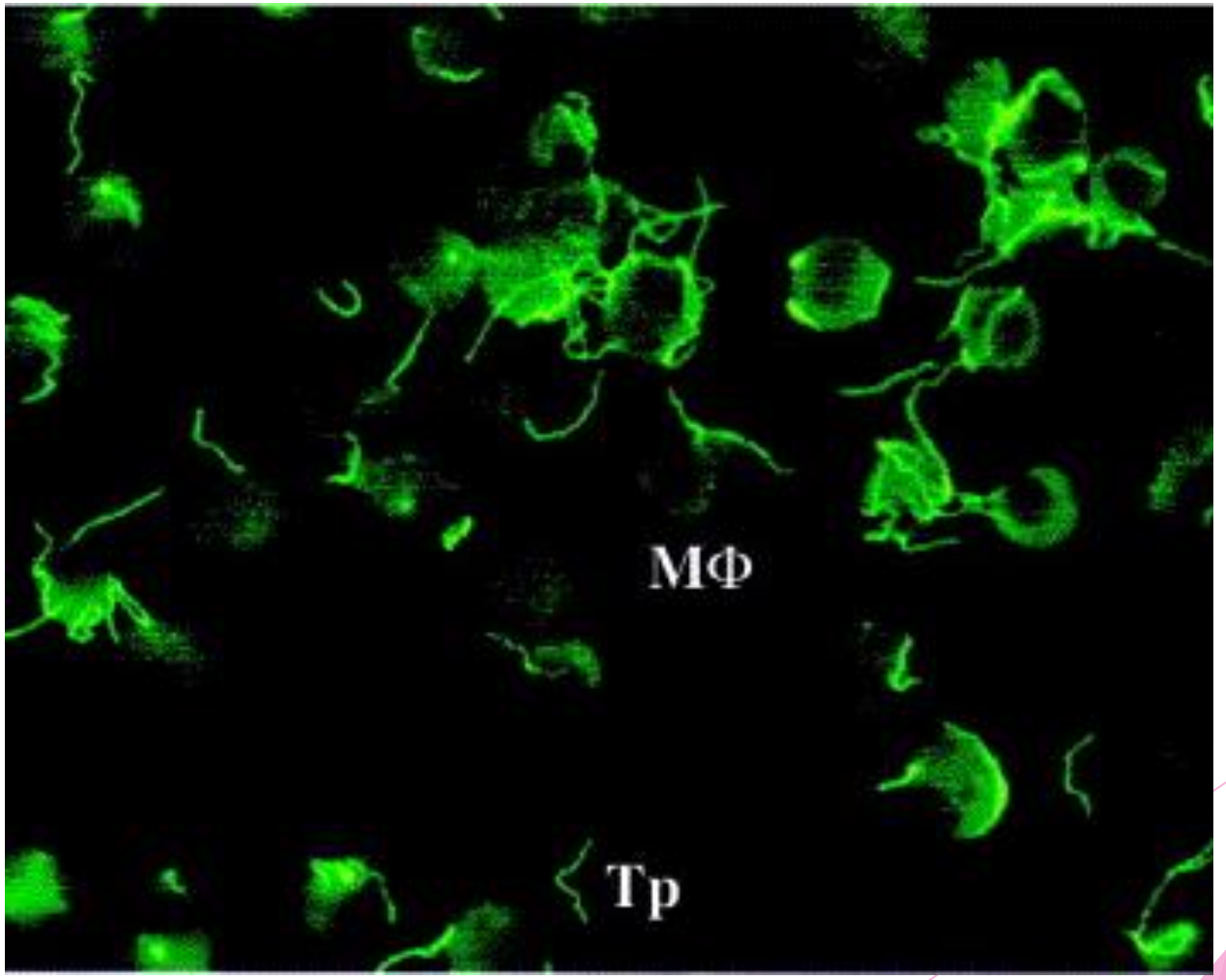
Treponemal antibody testing

- The detection of treponemal antibodies in the blood serum is considered a specific test.
- They have high sensitivity and specificity and are diagnostic confirmatory tests.

Indirect immunofluorescence reaction

- An indirect immunofluorescence reaction is performed using killed T. pallidum, patient serum, and labeled anti-immunoglobulin serum.
- If antibodies to treponemas are present in the blood serum, they interact with treponemas, and the resulting complex, in turn, binds to labeled antibodies to human immunoglobulins, which can be observed as a glow in a fluorescent microscope.
- This reaction is highly sensitive and specific, positive from the initial period of the disease, and usually positive even many years after effective treatment of syphilis. Therefore, this reaction is not used to assess the effectiveness of treatment.

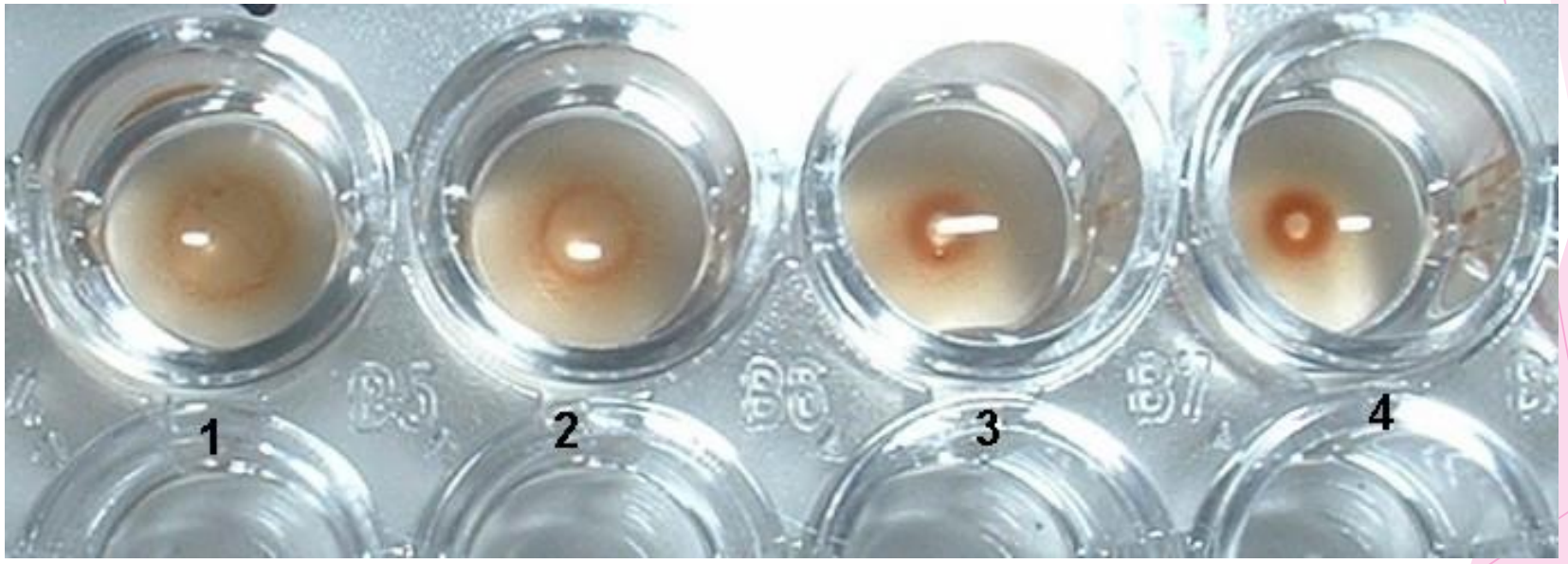
Indirect immunofluorescence reaction in the diagnosis of syphilis



Passive agglutination reactions (hemagglutination)

- T. pallidum hemagglutination (TPHA) and T. pallidum microhemagglutination (MHA-TP) reactions are used.
- Red blood cells, on the surface of which T. pallidum antigens are adsorbed, react with diluted patient serum containing antibodies, resulting in agglutination of red blood cells
- The sensitivity and specificity of these tests is as high as that of the indirect immunofluorescence test.

Passive hemagglutination test (PPGA) in the diagnosis of syphilis



Treatment

- T. pallidum is sensitive to benzylpenicillin (penicillin G), its concentration of 0.003 TI/ml has antitreponemal activity. Therefore, penicillin is the drug of choice in the treatment of syphilis. - disease lasting less than one year can be treated with benzathine-penicillin or bicillin - administered intramuscularly 3 times a week.
- In some cases, erythromycin and tetracycline are also used.

Genus Borrelia

- The genus Borrelia includes spirochetes 10-30 x 0.3-0.6 µm in size with 3-10 large irregular whorls.
- The motor apparatus consists of 15-20 flagella.
- Intensively stained with aniline dyes, gram-negative, stained blue-violet according to the Giemsa method.
- Dark field microscopy makes it easy to identify moving spirochetes.

Genus Borrelia (cultivation)

- Borrelia are strict anaerobes,

- Cultivated in complex nutrient media containing serum, ascites, tissue extracts in an atmosphere of 5-10% CO_2, at a temperature of 20-370C, as well as in the yolk sac of chicken embryos.

Genus Borrelia

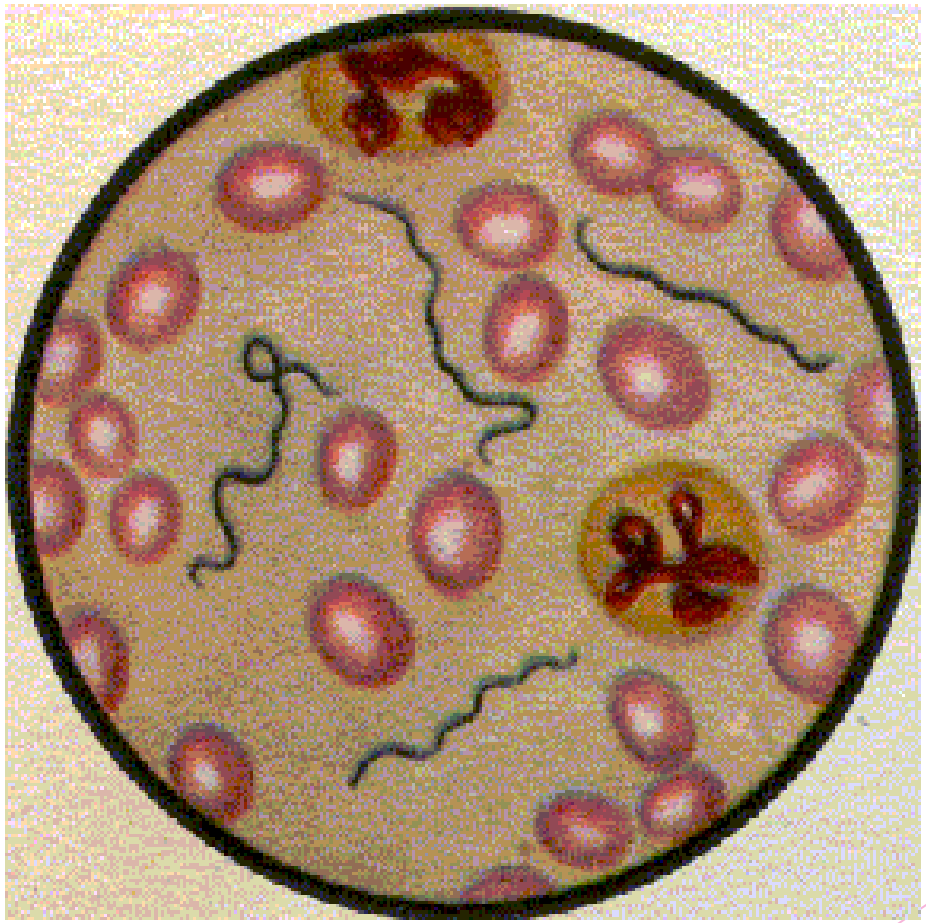
- The genus Borrelia includes more than 20 species, most of which are not pathogenic to humans.
- Some of them are detected in the normal microflora of the human body - in the oral cavity (B. buccalis), in the mucous membranes of the genitals (B. refringens).
- Species that are pathogenic to humans cause relapsing fever (typhus recurrentis) and Lyme disease.

Causative agents of relapsing fever

- Epidemic relapsing fever is caused by B. recurrentis. The source of infection is sick people. The disease is transmitted through lice. Lice become infective 1-4 weeks after sucking blood from an infected person. They become infected by rubbing the hemolymph of crushed lice into the skin when combing at the site of itching.

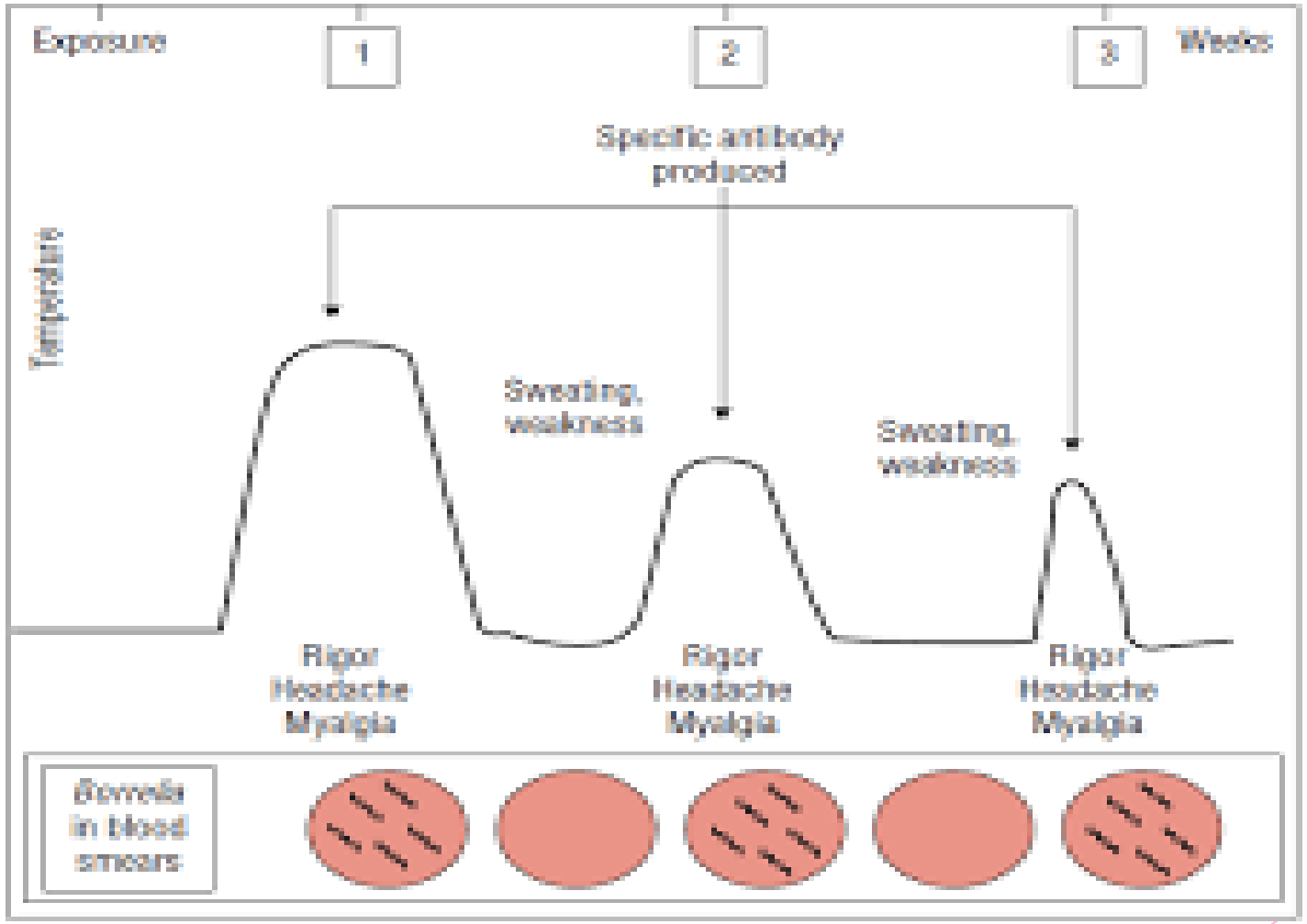
- Endemic relapsing fever, being a natural focal zoonotic disease, occurs sporadically, mainly in subtropical and tropical regions and is caused by many types of Borrelia. Among them, B. duttoni and B. persica are the most common. Borrelia, whose reservoirs in nature are rodents, are transmitted from sick animals to humans through the bites of ticks of the genus Ornithodoros. The pathogens are found in the saliva of ticks and are transmitted transovarially from generation to generation.

Borrelia recurrentis



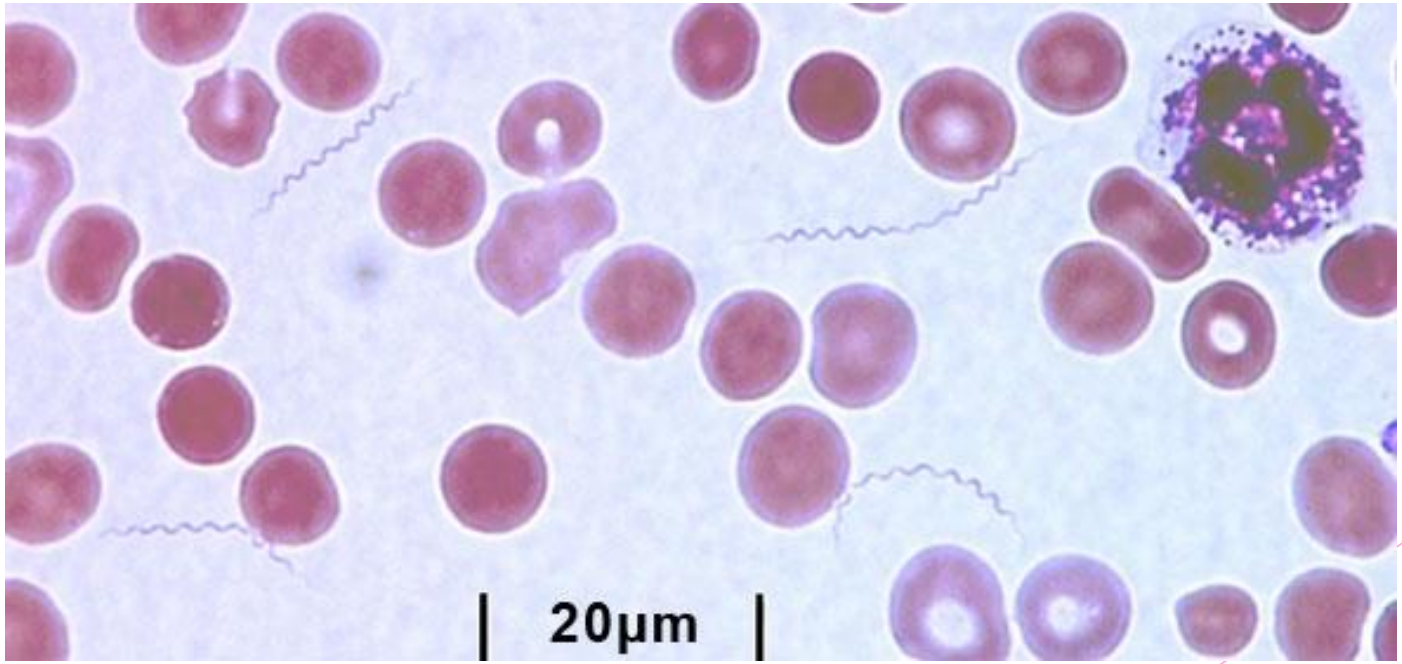
Relapsing fever vectors

The pathogenesis of relapsing fever



Microbiological diagnostics

Based on the study of blood samples taken during the febrile period. The causative agent can be detected microscopically in thick and thin blood smears stained by the Giemsa method.



Microbiological diagnostics

- Differentiation of causative agents of epidemic and endemic relapsing fever can be carried out by means of a biological sample. Blood taken from patients is injected into the abdominal cavity of laboratory animals.
- Guinea pigs are susceptible to endemic relapsing fever, and white mice are susceptible to B. recurrentis.
- After 2-4 days in the blood of infected animals, pathogens can be detected microscopically.

Treatment and prevention

- Treatment. Tetracycline, erythromycin and penicillin are used.
- Prevention. Non-specific prevention of epidemic relapsing fever is based on the fight against lice, and endemic relapsing fever - on the fight against ticks and rodents in their natural habitat.
- Specific prophylaxis has not been developed.

The causative agents of Lyme disease

- Lyme disease, chronic erythema migrans or lymoborreliosis, is a chronic infection accompanied by damage to the skin, cardiovascular and nervous systems, arthralgia and arthritis.
- The disease in America is caused by Borrelia burgdorferi, and on the Eurasian continent by B. garini and B. afzelii.

Borrelia burgdorferi

- Morpho-biological features. B.burgdorferi is the largest borrelia, the average size of which is 20-30x0.2-0.3 microns. In their morphological and tinctorial properties, they are similar to other Borrelia, have 7-11 pairs of whorls and are very mobile. Easily detected by aniline dyes and silver impregnation.
- B.burgdorferi develop rapidly in complex liquid media (Barbour-Stoenner-Kelli medium), are easily isolated from erythema on the skin, and are rarely isolated from other materials.

Borrelia burgdorferi

- ❖ Antigenic structure and pathogenicity factors.
- ❖ Osp-proteins (English outer surface protein) - proteins of the outer membrane, consisting of lipoproteins, have protective activity.
- ❖ In the development cycle, the antigenic composition of Borrelia undergoes changes.

- ❖ During cultivation on nutrient media and in the late stages of the disease, the OspA antigen predominates in humans, while in ticks and in the early stages of the disease, the OspC antigen predominates in humans.
- Osp proteins provide the adhesive ability of Borrelia to host cells.
- As a result of the interaction of Borrelia with macrophages, the secretion of cytokines (IL-1, etc.) induces inflammatory processes.
- The OspA protein is involved in the development of immunopathological reactions leading to the development of arthritis.

Borrelia burgdorferi

Ecology, sources of infection and ways of infection. Under natural conditions, pathogens are mainly found in small rodents, especially wood mice. Ticks of the genus Ixodes infect humans through saliva when they bite or regurgitate the contents of the midgut.

Pathogenesis and clinical manifestations of Lyme disease

During the incubation period, Borrelia mites that enter the body with saliva migrate to the surrounding tissues, which leads to the appearance of a characteristic migratory erythema (erythema migrans) on the skin.

Pathogenesis and clinical manifestations of Lyme disease

- Like other spirochetosis, Lyme disease also has a staged course.
- The first stage is characterized by flu-like symptoms, lymphadenitis, myalgia, and the formation of rapidly growing erythema annulare - erythema migrans at the site of the tick bite.
- The second stage is accompanied by arthralgia and arthritis, meningitis, facial paralysis, radiculopathy, and myopericarditis 1-4 weeks after the onset of the disease. The third stage is manifested by the development of chronic processes on the skin, in the nervous system and joints a few months after the onset of the disease.

Lyme disease (erythema migrans)

Microbiological diagnostics

- Symptomatically, the disease can be recognized by erythema on the skin. In the absence of skin symptoms, as well as in the later stages of the disease, microbiological diagnostic methods are used.
- Skin biopsies taken from the area of erythema, synovial fluid, cerebrospinal fluid (CSF), blood serum are taken as material for research. Various methods are used depending on the stage of the disease. In these materials, B. burdorferi can be detected by PCR.

- Serological tests are carried out starting from the second stage of the disease. Antibodies (IgM and IgG) to the pathogen in the blood serum can be determined using IGF, ELISA and immunoblotting.
- Antibodies to the pathogen in the body are formed gradually. The acute phase of the disease is seropositive only in 30-40% of cases, after 2-4 weeks serological reactions are positive in 60-70% of patients. After 4-6 weeks, IgG antibodies to B. burdorferi are detected in 90% of patients. Although the antibody titer gradually decreases after treatment, it remains in the serum of patients for years.

Lyme disease

- Treatment. Etiotropic treatment with doxycycline or amoxicillin is carried out for 20-30 days. Doxycycline is more effective. Good results in arthritis gives the use of large doses of penicillin.
- Prevention. Non-specific prophylaxis consists of measures to control and protect against ticks.
- Specific prophylaxis has not been developed.

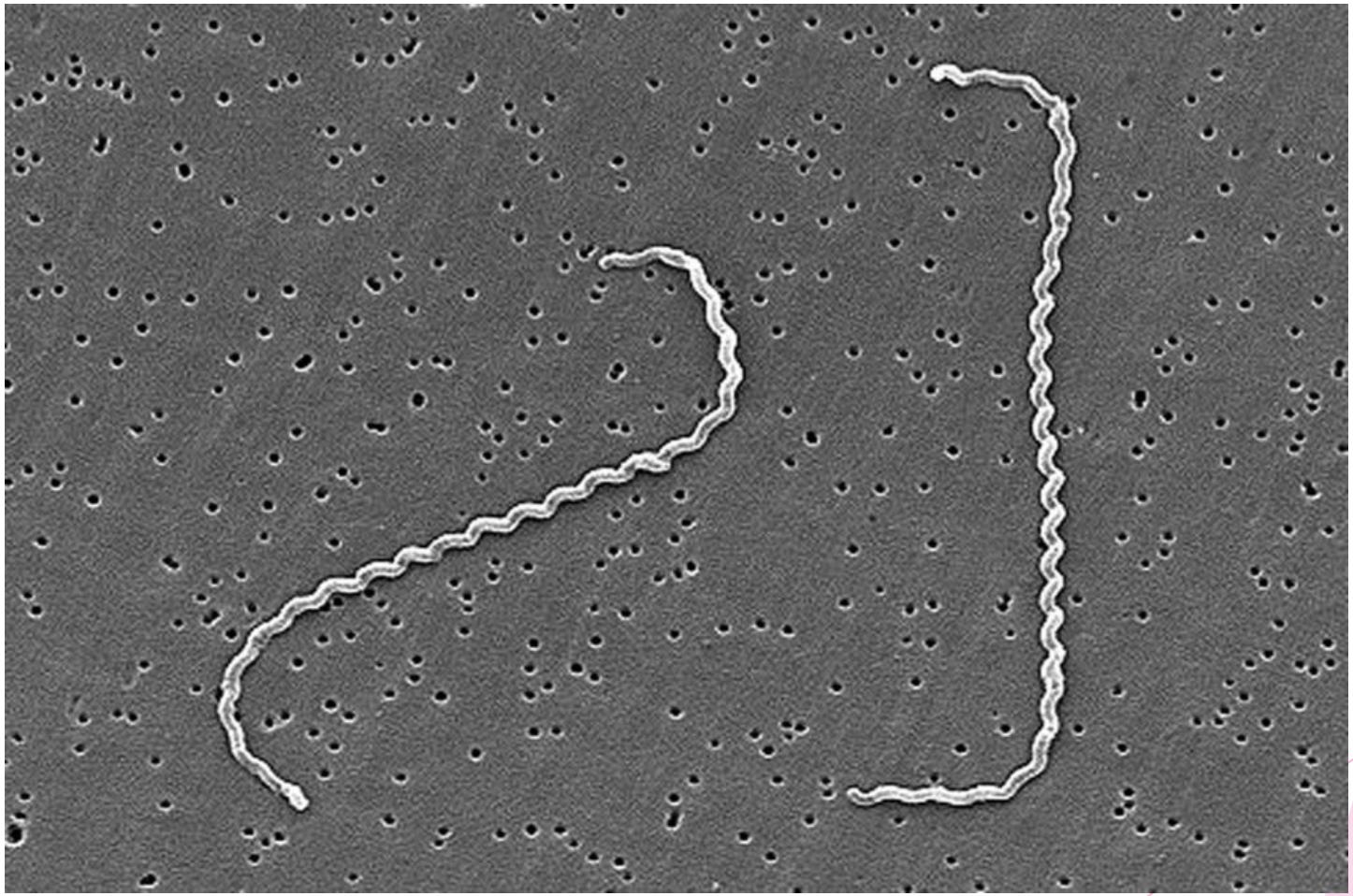
Genus Leptospira

- Taxonomy. Leptospira belong to the Leptospiraceae family, genus Leptospira. Their traditional classification is based on their biochemical and serological features.
- The genus Leptospira includes the pathogenic species L.interrhogans and the non-pathogenic species L.biflexa.
- L.interrhogans has more than 200 serotypes, and L.biflexa has more than 60 serotypes.
- Unlike other microorganisms, Leptospira serotypes have specific names. For example, L.bovis, L.grippotyphosa, L.hebdomadis, L.icterohaemorrhagiae, L.mitis, L.pamona and L.interrhogans are among the most common serotypes.

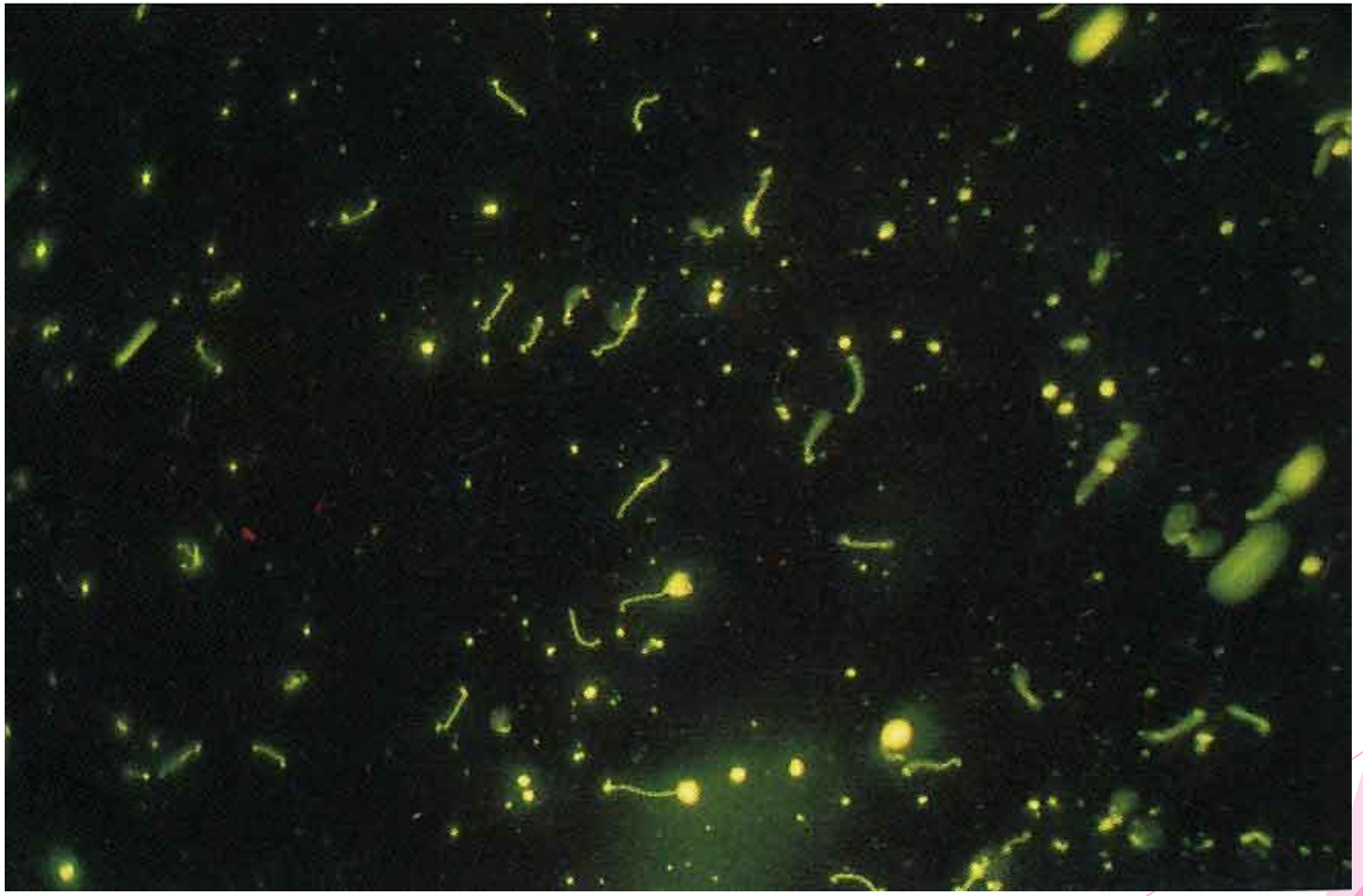
Genus Leptospira

- Morpho-biological features. Leptospira are thin spirochetes 5-15 µm long and 0.1-0.2 µm thick with 20-40 whorls. One of the ends is often bent, forming a hook. The motor apparatus consists of flagella located at both poles of the cell.
- In the preparations are difficult to distinguish, tk. weakly stained pink by the Giemsa method. They stain well when impregnated with silver. The active mobility of Leptospira can be observed in the dark field and phase-contrast microscopy of native preparations.

Leptospira interrhogans



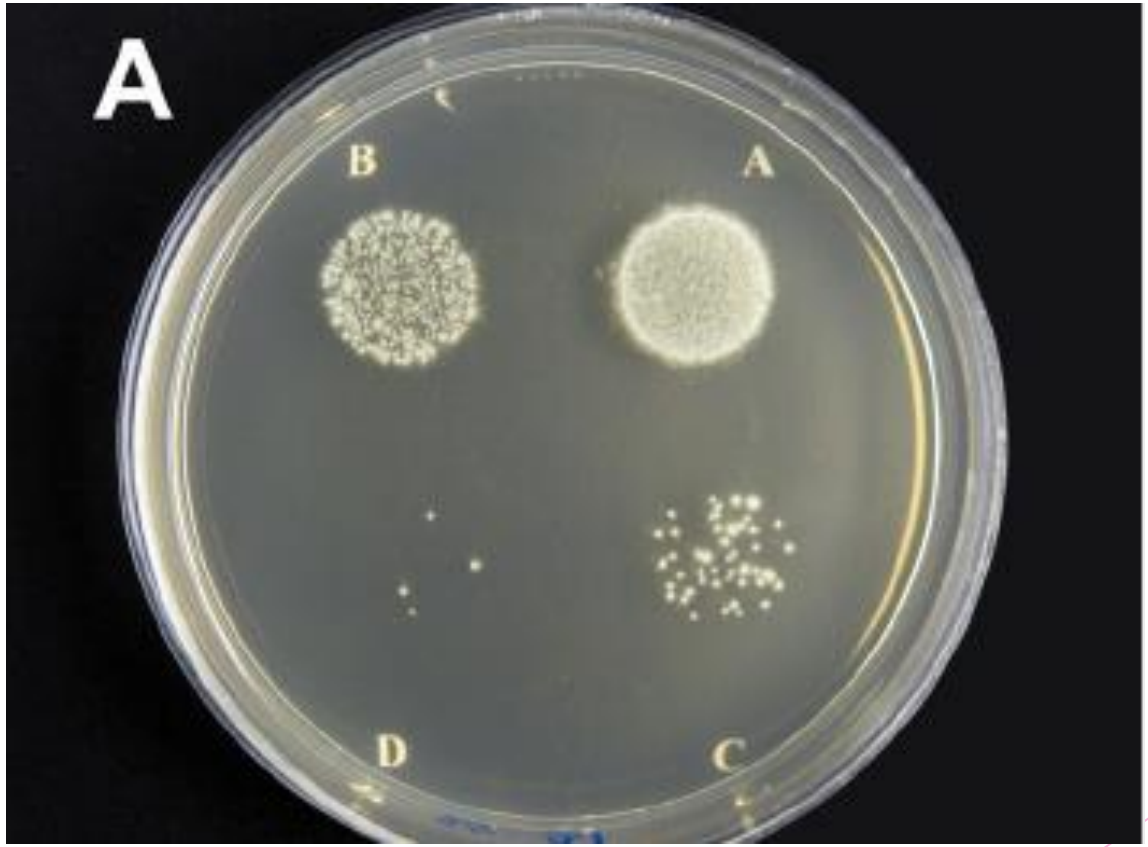
Leptospira interrhogans



Leptospira interrhogans

- Leptospira are cultivated under aerobic conditions in liquid and semi-liquid media (Fletcher, Stuart and other media) with the addition of serum at 28-30 C.
- They do not form turbidity when grown (growth) on liquid nutrient media. After 1-2 weeks, a diffuse growth zone is formed on semi-liquid media closer to the surface of the nutrient medium, and then in areas of the medium optimally supplied with oxygen, growth in the form of a ring is observed.

Leptospira interrhogans



Leptospira interrhogans

- Antigenic structure. The outer membrane of L. interrhogans is rich in lipopolysaccharides (LPS).
- Antigenic diversity of LPS is observed in different strains. This diversity of LPS underlies the serological classification of L. interrhogans and determines their division into numerous (more than 200) serovars.
- L. interrhogans serovars similar in antigenic structure cross-react in serological tests.

Ecology, source of infection and routes of infection

L.interrhogans are widespread in nature, causing leptospirosis in humans and animals. Leptospirosis is a zoonotic infection. The source of infection are mainly rodents (common and wild rodents), as well as domestic animals (cattle, pigs, dogs, etc.).

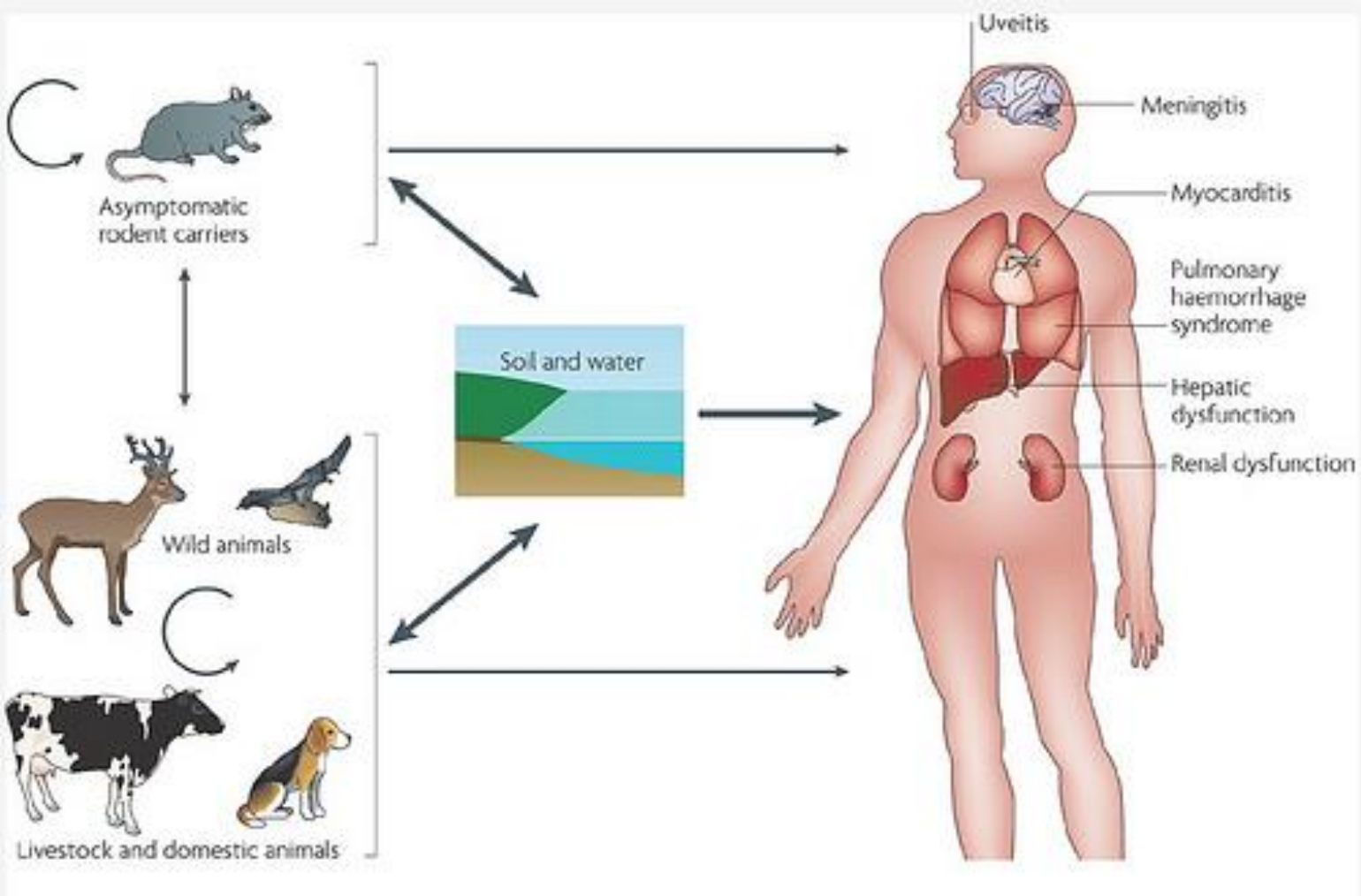
In animals, the infection occurs in the form of nephritis, has a chronic course, mostly without clinical manifestations; excreting pathogenic microorganisms in the urine, they contaminate water bodies, food and soil. People become infected mainly by contact with water contaminated with the excrement of sick animals (the previous name of the disease "water fever" was associated with this). Gates for infection are damage to the skin and mucous membranes (mucous membranes of the mouth and nasal cavity, conjunctiva). The use of water containing leptospira is insignificant for infection.

Pathogenesis and clinical manifestations of leptospirosis

- Leptospirosis is an acute infectious disease characterized by fever, intoxication, damage to the capillaries of the liver, kidneys and central nervous system.
- Pathogenic microorganisms penetrating the body through the skin and mucous membranes, after a 1-2-week incubation period, enter the bloodstream, causing bacteremia (spirochetemia), accompanied by various febrile reactions. The causative agent enters the parenchymal organs abundantly supplied with blood (especially the kidneys and liver), where it causes hemorrhages and necrosis, which leads to hepatitis, manifested by nephritis and jaundice.

- The disease often proceeds in two phases, an increase in the titer of IgM antibodies, which occurs after improvement after the first phase, leads to the development of "aseptic meningitis", manifested by intense headaches, meningeal syndromes, and pleocytosis in the cerebrospinal fluid. At the same time, damage to the skin, muscles and eyes can be observed.
- In the clinic of the disease, hepatitis is more common, which is accompanied by an increase in the enzyme creatine phosphokinase in the blood serum (with viral hepatitis, the amount of this enzyme remains normal!).

Pathogenesis and clinical manifestations of leptospirosis



Leptospirosis

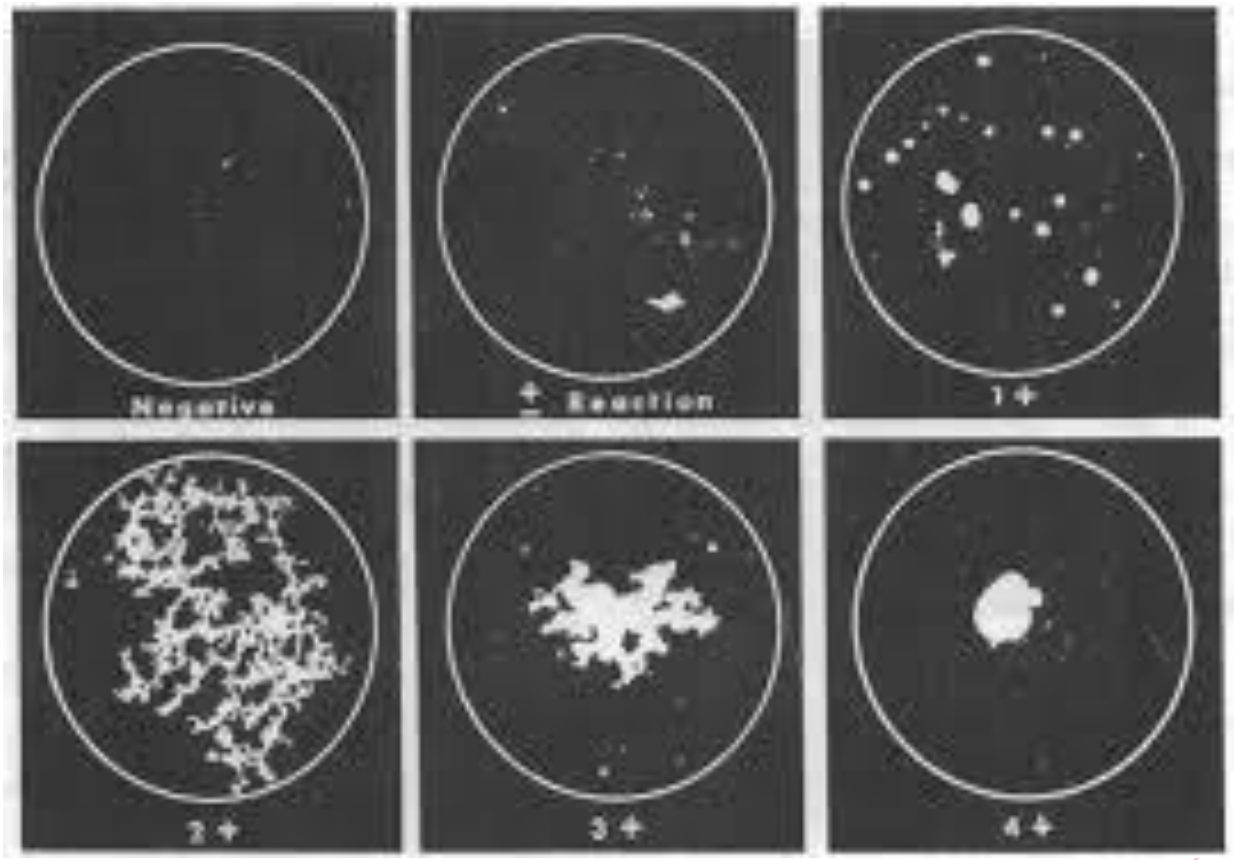
Microbiological diagnostics

- For research, blood, cerebrospinal fluid, urine and blood serum are taken.
- Microscopic method. In the early stages of the disease, leptospira can sometimes be detected in a thin blood smear stained by the Giemsa method and also in a dark field microscope. Dark-field microscopy of urine sediment also reveals the pathogen.

- Bacteriological method. The culture of the pathogen can be obtained and identified by culturing on a semi-liquid medium (Fletcher, Stewart, etc.) samples of fresh blood, urine, and cerebrospinal fluid of patients. Leptospira develop gradually, culture can be obtained only after 1-2 weeks. The microagglutination reaction is used for identification.

- the reaction is an agglutination test on glass in the presence of specific antibodies to L. interrhogans serotypes, the result of which is assessed microscopically. A positive reaction is accompanied by leptospira agglutination - the formation of conglomerates in the form of "spiders".

Microagglutination reaction



Microbiological diagnostics

- The biological sample is considered a sensitive method in the diagnosis of leptospirosis. The patient's serum or urine is injected intraperitoneally into mice and guinea pigs. After a few days, leptospira can be detected in the peritoneal fluid. After 1-2 weeks, hemorrhagic lesions are detected in the internal organs of dead animals.

- Serological method. With leptospirosis, the titers of agglutinating antibodies reach a maximum at 5-8 weeks of illness (1: 10,000 and above). To detect these antibodies, a microagglutination reaction is used using control (reference) Leptospira strains. The response is very sensitive and, when used with the appropriate serotypes, it is also highly specific.

- Indirect hemagglutination and ELISA are also used to detect antibodies.

Leptospirosis

- Treatment. Mild forms of the disease are treated with oral doxycycline, ampicillin, or amoxicillin. In the treatment of severe forms, penicillin or ampicillin is prescribed intravenously.
- Prevention:

- Non-specific prophylaxis includes rodent control, vaccination of agricultural and domestic animals and veterinary measures.
- For epidemiological indications, specific prophylaxis is carried out with a heat-treated inactivated corpuscular vaccine, consisting of various serotypes of the pathogen.