



CAUSES AND TYPES OF LISTERIOSIS

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ABSTRACT

The article describes the sources of infection with listeriosis, various routes of transmission of the pathogen, polymorphism of clinical manifestations, high mortality in newborns and people with immunodeficiency. To learn enough about this, you can learn about the development and study of modern laboratory diagnostics.

Listeriosis is a saprozoontic bacterial infectious disease characterized by multiple sources of infection, a variety of pathogen transmission routes, polymorphism of clinical manifestations, high mortality in newborns and people with immunodeficiencies.

Only Listeriamopocytogepes causes disease in humans. Listeriosis is not a widespread infection. However, the severity of the clinical course and mortality, as well as the epidemiological dynamics (from a rare zoonotic infection of livestock farms to a saprozoontic infection common in developed countries) make this disease relevant, requiring the development of modern laboratory diagnostics to adequately study its epidemiology.

Listeriosis is a naturally occurring infectious disease of humans and animals and represents an urgent medical and veterinary problem.

The beginning of the study of this disease dates back to 1892, when Lucet first described septic disease in rabbits and isolated the gram-positive *Bact. sertecimia cuniculi*. In 1911, G. Nulphers isolated a gram-positive and motile bacterium, which he named *Bact.*, from a necrotic nodule in the liver of a dead rabbit. *heratis*.

The systematic study of listeriosis and its causative agent begins in 1924, when E. Miggau, K. Welb, M. Swapp (1926) in England during an epizootic among guinea pigs and rabbits in the nursery of the University of Cambridge isolated from the blood and mesenteric lymph nodes of dead animals a previously unknown microorganism called *Bact. mopocytogepes*. This name was given because both spontaneous and experimental disease was accompanied by mononuclear leukocytosis.

At the same time, J. Purie in South Africa (1927), while studying the disease of wild rodents *Tateria lobengullae*, which was locally called River disease (Tigray River disease), isolated a gram-positive pathogen and named it in honor of the English surgeon J. Lister -

Listeria heratolutica. Later, J. Pigie established the identity of these two microorganisms, and since the name "Listerella" had already been given to one of the types of fungi, he proposed calling the pathogen *Listeriamoposutogepes*, which was approved by the International Classification Commission. The causative agent of listeriosis was first isolated from humans by J. Dumont and Cotopi at the end of the First World War from the cerebrospinal fluid of a patient with meningitis. However, it was only after 20 years of storing the microbes at the Pasteur Institute that Paterson identified them as *Listeria*. In Copenhagen, A. Nyfeldt isolated *Bact.mopocytogepes*, with monocytic angina in humans, which occurred with a characteristic increase in monocytes in the blood up to 50%. The English scientist D.Gilli established listeriosis in New Zealand sheep, Tep Bricht - in poultry, F. Jones, R.Little - in cattle with encephalitis.

Works by E. Junghtrr on listeriosis encephalitis in sheep and J. Peterson on enzootics among chickens in Southern England were published. In the USA, in the states of Illinois and Chicago, K. Graham, G. Dun1ar, E. Bgandley established the presence of this disease among cattle and sheep. W. Rounden, D. Bell described an outbreak of acute listeriosis among cattle in Ohio. In our country, the first *Listeria* cultures were obtained by T. P. Slabospitsky from piglets and a gray mouse, and by P. P. Sakharov and I. S. Istomin from rabbits. P. M. Svintsov described listeriosis in pigs, and P. P. Sakharov and I. Gudkova published a number of works on listeriosis in animals and humans. The first works on listeriosis in cattle were published by N. G. Tregubova in 1949, and in 1955-1956. - V.I. Stolnikov and K.A. Dorofeev.

The causative agent of the disease is *Listeriamopocytogepes* (Miggau E.G., Welb K.A., Capp M.V., 1926; Pirie J.I., 1927). According to the key S. Bergey, *Listeria* belongs to: class II - Schizomycetes - Naegeli, 1957; order IV - Euasterias-Vichanan, 1917; in a row - Euasterypae-Breed, Miggau, Hitchens; family XII - Corupe Mastegiaceae - Lehmann et Neimann, 1896; genus II - the causative agent *Listeria*-Pirie, 1940; species - the causative agent *Listeriamopocytogepes*, Pigie, 1940. Currently, *Listeria* is classified as a special kind of microorganisms, which, along with *Listeriamopocytogepes*, includes rare species of the causative agent *Listeria denitrificans*, *Listeretiagray* and *Listeriamurray* (Seeliger H., Welshimer, 1974; Amtsberg V., 1979).

Morphology

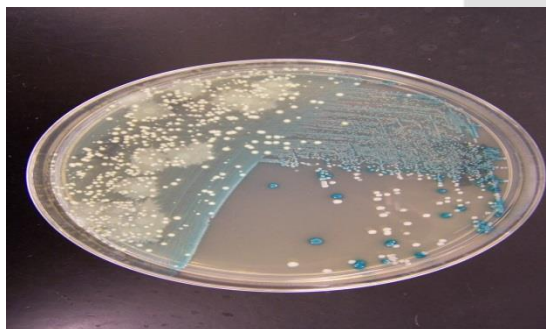
Listeriamopocytogepes is a motile, non-spore-forming, polymorphic, gram-positive rod with rounded ends. Outside of the phenomenon of dissociation and variability, the sizes of *Listeria* most often range from 0.3 to 0.5 microns in length and from 0.5 to 2 microns in width. S. A. Karpeev (1962), during an electron microscopic study, found that the size of *Listeria* varies from 0.8 to 1.8 microns in length and from 0.4 to 0.6 microns in width.

In a typical culture, the location of *Listeria* in smears is not characteristic. They are observed singly, in pairs, in polysads of five or more rods, or in the form of a Roman numeral V. Depending on the cultivation conditions, *Listeria* develops various morphological features. During bacterioscopy, various forms of *Listeria* (polymorphism) can be found in pathological material and microbial cultures. In very young cultures, up to 3-4 hours of growth at a temperature of +18... -22 °C, predominantly rod-shaped cells are observed; in a culture several days old, forms with a filamentous structure up to 6-20, sometimes 275 µm are observed. *Listeria* can take on a coccoid or oval shape; diplococci can be found, as well as bipolar-colored microbes.

D. Yu. Halla, during an electron microscopic study, established that *Listeria* sometimes has the shape of a granular ball, which breaks up into separate parts, and these, in turn, divide and bud, forming the original cells. E. I. Gudkova proved the ability of *Listeria* obtained after 15-20 passages on white mice to pass through bacterial filters of brands L and F. V. V. Slivko established the ability of *Listeria* from old cultures to pass through a Chamberlyan F2 suppository, a Seitz filter and a colloid filter No. 2. Sowing the filtrate from the brain onto meat-peptone liver agar (MPLA) and meat-peptone liver broth (MPLP) gave the growth of *Listeria* on the 11th-12th day at 37-38°C.

F. 3. Amfitheatrov, G., F. Panin, K. A. Shishkina, S. A. Karpeev (1972) also believe that *Listeria* can take the form of small coccoid formations (0.3-0.4 microns in length), which pass through bacterial filters.

In a series of works by a number of authors (Kotlyarova G. A, 1980; Bakulev I. A, 1978, 1988, 1988, etc.) materials were presented indicating the possibility of obtaining L-forms of all serotypes of *Listeria monocytogenes* under the influence of immune serum, fresh tissue suspension of mice, glycine, lysozyme and penicillin. Some of the works actively discuss the possible role of L-forms of *Listeria* in the pathogenesis, epidemiology and epizootology of listeriosis. The possibility of induction of L-forms and reversion of *Listeria* in vivo in mouse tissues was proven.



G. A. Kotlyarova, I. A. Bakulov, S. V. Prozorovsky (1969) obtained L-forms of *Listeria* on MPPA with the addition of 5% KC1, 30% normal horse serum and 100 U/ml penicillin. The crops were kept at 37 °C for 10–15 days. The change in morphology was observed under a phase contrast microscope at the border of the growth inhibition zone.

Colonies of typical L-forms consisted of a complex of microstructures, spheres, vacuoles, granules and granular formations. Cell polymorphism, noted by many authors, is a distinctive feature of *Listeria*. However, it should be emphasized that the natural initial form for *Listeria* is rod-shaped. All other forms are temporary, intermediate, arising as a result of the influence of unfavorable factors on the cell, having become accustomed to which, *Listeria* again acquires a rod-shaped form.

It should be taken into account that in *Listeria* cultures grown at a temperature of 22°C, typical rod-shaped cells usually predominate, while in cultures incubated at 37°C, coccoid and ovoid cells are more often found. The morphology of *Listeria* grown under aerobic and anaerobic conditions does not change under the same temperature conditions.

Listeria is a non-spore-forming, non-acid-fast bacillus. Using conjugates of specific serums with ferritin, a capsule was found in *Listeria*. Histochemical methods revealed the

presence of capsules in the mucopolis capsule. The researchers note that when *Listeria* was cultivated on 1% soy agar with 10% serum and 5% glucose, the capsule was observed in all microbial cells, while on conventional nutrient media the capsule was found only in single cells.

V.P. Sukhotina (1978) also notes that the causative agent of listeriosis is capable of forming a capsule when cultivated on MPPB and MPPA with glucose and glycerol. However, there is still no definitive opinion on capsule formation in this type of microorganism.

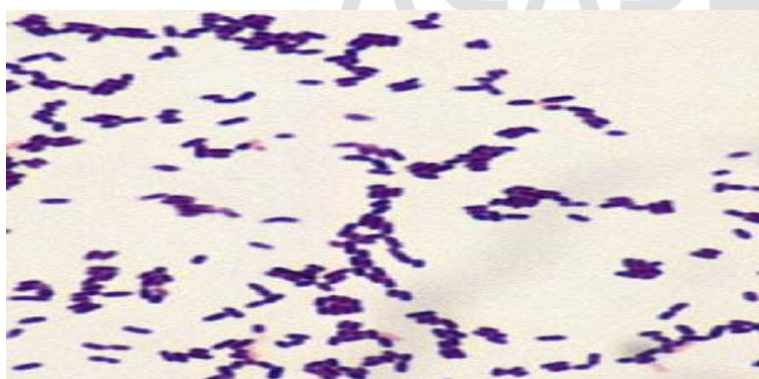


A feature of *Listeria* is its mobility. In this they differ sharply from almost all types of similar corynebacteria and the causative agent of erysipelas in pigs.

Listeria motility is clearly expressed in 4-12 hour cultures grown at room temperature (20-22°C). A liquid medium is more suitable for the process of flagella formation in the *Listeria* genus. At 20°C the formation of flagella is observed, at 37°C it is very weakly expressed, and at 38°C it is absent.

Coloring

Listeria can be painted with all aniline dyes. According to Gram, they stain positively dark purple.



In young cultures, often consisting of rod-shaped forms, Gram-positive coloring is usually observed.

In 48-hour and older cultures, some or most cells stained Gram-negative. This phenomenon is especially often observed in crops grown at 37 °C. At 20°C, Gram-positive staining is usually detected. N. Gibson (1935) indicates that from the original source it is possible to obtain gram-negative non-motile *Listeria*, which ferment sugars and glycerol with

the formation of gas. However, 4 years later, R. Web and M. Barber, having studied these cultures in detail, established the mobility of *Listeria*. This phenomenon may have been associated with temperature conditions or the composition of the nutrient medium.

According to the observations of V.V. Slivko (1984), some crops obtained from piglets in the first generation were also immobile. I. A. Bakulov (1987) notes that changes in the color of *Listeria* as cultures age are associated with the death and autolysis of bacterial cells. The older the culture, the more dead cells it contains, and therefore the more gram-negative staining it has. This explanation is confirmed by the fact that when reseeded aging cultures, microbial cells completely restore their gram-positive color.

Cultural and biochemical properties

Listeria grows under both aerobic and anaerobic conditions. The optimal growth temperature on conventional nutrient media with a pH of 7.2–7.4 (MPA and MPB) is 36–38°C. A feature of *Listeria* is its wide temperature range of growth. They can grow at temperatures from 45 to 4°C and remain viable at lower temperatures. pH range from 5 to 11.

In a microaerostat under vacuum conditions on blood or simple meat-peptone agar with a pH of 7.2–7.4, 24 hours after growing in a thermostat at a temperature of 37 °C, the growth of *Listeria* in the form of dew-colored colonies is clearly visible. The virulent properties of microorganisms grown under aerobic or anaerobic conditions remain the same. The ability of *Listeria* to grow both in the presence of oxygen and without it determined their fairly wide distribution and significant stability in the external environment, and, consequently, the possible variety of ways in which the causative agent of listeriosis enters the body of animals and humans. *Listeria* grows well on liver media with the addition of glucose (1%) and glycerol (2–3%) and on tryptose agar.

Meat-peptone liver broth (MPLP) with 0.05% potassium tellurite or 0.01–0.02% potassium tellurite in an aqueous solution of glycerin and a solution of florimycin or polymyxin (500 thousand units in 10 ml of isotonic sodium chloride solution). Studies carried out by N. Urbash, G. Shabinski (1955), F. Z. Amfitheatrov (1962), I. A. Bakulov (1967), and others, as well as our data show that *Listeria* reproduces well in chicken embryos 6–8 days of age. Pathological changes in chicken embryos are similar to the changes that occur when embryos are infected with various viruses.

The morphology of *Listeria* colonies does not have any features that make it possible to distinguish them from the mass of colonies of other bacteria, pathogenic or banal. In particular, they are very similar to colonies of enterococci.

On MPA, round, convex, transparent colonies are formed, ranging in size from 0.2–0.4 to 2 mm in diameter, acquiring a bluish tint in transmitted light. On MPA with the addition of methylene blue (1:40000), *Listeria* forms round colonies with smooth edges with a diameter of 1–3 mm. The center of the colonies is painted in bright bluish-green tones.

In ordinary meat-peptone broth, growth occurs slowly, only after 1–2 days does obvious turbidity appear and a sour-milk smell is detected. When inoculating single microbes, no growth is observed at all. Therefore, it is necessary to make abundant cultures from pathological material. Cultivation of the pathogen is not always successful, but preliminary keeping of the test material in a refrigerator at 4°C for 4–8 weeks, according to some researchers, helps to obtain a positive result. When *Listeria* is grown in a refrigerator, it can be more easily isolated from mixed cultures. After 5–7 days of *Listeria* growth in the MPB, a

mucous sediment forms at the bottom of the test tube, which, when shaken, rises with difficulty in the form of a characteristic spiral-shaped pigtail. On semi-liquid 0.3% MPA, listeria grows in the form of cotton, which subsequently spreads throughout the medium. When sown on meat-peptone gelatin by injection, microbial growth is observed in the form of a bayonet with lateral shoots closer to the top of the injection. S-form colonies should be considered the most characteristic of *Listeria* and the most common. However, during long-term storage on artificial nutrient media, *Listeria* cultures undergo dissociation. Changes from smooth S-forms of colonies to rough R-forms occur through intermediate SR and RS stages.

Colonies of R-forms are difficult to remove from agar; microbial cells from such colonies often have the appearance of threads; when subcultured on MPB, a crumbly sediment is observed at the bottom of the tubes. I. L. Martinevsky (1962) reports that listeria from colonies of R-forms were immotile, non-pathogenic, biochemically inactive, and had the ability to produce hydrogen sulfide in small quantities. The basic biochemical properties of R-cultures do not change compared to S-cultures; only virulence decreases and agglutinability increases. In our experiments, when R-cultures were sown on MPB, after 72 hours, the broth became completely clear and at the bottom of the vessel there was a crumbly sediment that was difficult to break when shaken. During storage and reseeded of these cultures on MPA, the growth of *Listeria* only in the S-form was observed.

On blood agar, colonies appear as dewdrops and are surrounded by a small colorless zone of hemolysis. With weak hemolysis, this zone is present only under the colonies.

The ability of *Listeria* to lyse erythrocytes has been observed by many authors. Most described type β hemolysis, but Smith (1960) et al reported that original cultures isolated from cows and aborted fetuses caused type α hemolysis, and subcultures caused β -hemolysis. N. Lahrsen (1964), having studied 35 cultures, found that 11 strains, including *Listeria garyan*, had a fairly high titer of hemolysin and at the same time did not give a conjunctival reaction. On blood agar, 98 out of 100 strains caused β -hemolysis. The authors did not find a difference in the height of hemolysin titer depending on the serological affiliation of the strains.

A. Njoku-Obi, E. Jenkips (1963) studied 112 listeria strains and found that they all had different abilities to produce hemolysin.

K. Gigard, A. Sjarga, W. Bogdwil (1963) obtained soluble hemolysin from *Listeria*. It had a protein nature, was thermostable, sensitive to proteolysis when treated with trypsin, and had antigenic properties. Using electrophoresis on paper, they established the proximity of hemolysin to the gamma-globulin fraction of the protein.

The hemolytic properties of *Listeria* are better expressed on solid nutrient media. On MPA containing 5% cattle blood, a more clearly defined hemolysis zone is formed around the colonies than on MPA containing 10% blood. As a result of our studies, it was established that if on blood agar all 26 studied listeria cultures isolated from cattle had hemolytic properties, then on blood broth only three of them caused hemolysis of erythrocytes with good growth of microorganisms in the medium.

Hemolysin is also formed on media that do not contain blood. The filtrate of such media causes hemolysis of red blood cells. To stimulate the growth of *Listeria*, they resort to introducing various additives into the nutrient medium.

S. Sword (1906) notes that the addition of iron compounds to the nutrient medium at a concentration of 0.1-100 mg/ml stimulates the growth of *Listeria*. L. Ya. Telishevskaya and L.

I. Trusova (1975) on meat-peptone-liver-sugar-glycerin agar discovered a stimulating effect on the growth of *Listeria* of organic acid additives: maleic (173-259%), transaconitic (146-260%), malonic acid (152-198%), pyruvic acid (118-159%), and sodium citrate (148-187%). The activating effect is observed, as a rule, in the presence of glucose.

The authors also note that cultivating *Listeria* in casein-yeast medium (YYM) with the addition of 0.02% glucose and 1% ferroglucin can increase the average "yield" of bacteria to a maximum of 2 billion/ml. This indicates the stimulating effect of ferroglucin-75. KDS with the indicated additives is not inferior to Hottinger broth, but has advantages in terms of standardization and economic efficiency. A. Foggau, T. Angyal (1998), adding 40 mg/ml oxalinic acid to the whey-agar medium, observed inhibition of the growth of accompanying microbes on it. Oxalinic acid, according to their data, retards the growth of colonies of swine erysipelas, similar to *Listeria*.

A.M. Alimov (1994), studying the effect of individual amino acids and their various combinations on the growth of *Listeria*, found that the studied strains are natural auxotrophs that require the sulfur-containing amino acid - cysteine (cystine). The author also points out that synthetic nutrient media with 13-18 amino acids turned out to be suitable only for stationary cultivation of *Listeria*. Stirring and aeration in deep cultures caused the lysis of microbial cells. The author further notes that on the semi-synthetic medium of casein hydrolyzate (PSHC), *Listeria* growth occurs even when 1 cell is inoculated, while in MPB it occurs only when at least 100 cells are added to 5 ml of medium.

Semi-synthetic casein hydrolyzate medium is prepared by diluting the enzymatic casein hydrolyzate with synthetic medium. The synthetic medium has the following composition: sodium citrate trisubstituted -0.4; ammonium sulfate - 1.0; magnesium sulfate - 0.1; Ferrous citrate - 0.05; thiamine - 2 mg; biotin - 2 mg; riboflavin - 5 mg; L-cysteine - 100 mg; L-cystine - 50 mg; glucose - 2 g and phosphate buffer (1/15 M, pH 7.2-7.4) - up to 1 l.

In 26 studied strains of *Listeria* isolated from cattle, bacteriocins could not be detected. The experiments used the method of R. Frederica. By exposing *Listeria* to ultraviolet rays, a *Listeria* bacteriophage was obtained, which is used for typing strains. In conclusion, it should be noted that the morphological cultural and biochemical properties of *Listeria* are fixed quite firmly and do not change outside the conditions of special experiments.

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