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BRIEF HISTORICAL SUMMARY OF THE CAUSANT OF LISTERIOSIS AND ITS PROPERTIES

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Annotation

This article describes the sources of infection of listeriosis, the different ways of pathogen transmission, the polymorphism of clinical manifestations, and the high mortality rate in newborns and immunocompromised people. To learn enough about this, you can learn about the development and study of modern laboratory diagnostics.

Keywords: Listeriosis, infection, ways of transmission, clinical presentation, immunity, bacterial infectious disease, mortality rate, epidemiology, emergency medical care, veterinary medicine.

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

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

Listeriosis is a natural focal infectious disease of humans and animals and is an urgent medical and veterinary problem.

The beginning of the study of this disease dates back to 1892, when Luset first described the septic disease of rabbits and isolated the Gram-positive Bact.sertecimia cunniculi. In 1911, G. Nulhers isolated a gram-positive and motile bacterium from a necrotic node in the liver of a dead rabbit, which he called Vast. heratis.

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

At the same time, J. Pigue in South Africa (1927), studying the disease of wild rodents Tatera lobengullae, which bore the local name Rivere diesease (Tiger River disease), isolated a gram-positive pathogen and named it in honor of the English surgeon J. Lister - Listeria heratolutica. Later, J. Rigie established the identity of these two microorganisms, and since the name "Listerella" had already been given to one of the species of fungi, he suggested calling the pathogen Listeriamoposutogepes, which was approved by the International Classification Commission. From a person, the causative agent of listeriosis was first isolated by J. Dumont and Cotoni at the end of the First World War from the cerebrospinal fluid of a patient with meningitis. However, only after 20 years of storage of microbes in the Pasteur Institute did Paterson identify them as Listeria. In Copenhagen, A. Nyfeldt isolated Vast.moposutogepes, with monocytic angina in humans, which proceeded 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. Littele in cattle with encephalitis.

Works by E. Junghtrr on listeriosis encephalitis in sheep and J. Pategson on enzootics among chickens in southern England were published. In the USA, in the states of Illinois and Chicago, K.Ggaham, G.Dun1ar, E.Bandley found the presence of this disease among cattle and sheep. W. Roundep, D. Bell described an outbreak of acute listeriosis in cattle in Ohio. In our country, the first cultures of Listeria were obtained by T. P. Slabospitsky from piglets and gray mice, 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 Listeriamoposutogepes (Miggau E.G., Web K.A., Swapp M.V., 1926; Pirie J.I., 1927). According to the guide S. Veggey, Listeria belong to class II - Schizomycetes -Naegeli, 1957; order IV, Eubastegiales-Vichanan, 1917; in a suborder - Eubastegipae-Bgeed, Miggau, Hitcheps; family XII—Corype bastegiaceae—Lehmann et Neumann, 1896; genus II - pathogen Listeria-Rigie, 1940; mind - the causative agent of Listeriamoposutogepes, Prigie, 1940. Currently, Listerium is classified as a special kind of microorganisms, which, along with Listeriamoposutogepes, include rare species of the pathogen Listeriadenitrificans, Lisretiaggaui and Listeriamurray (SeeligerH., Welshimer, 1974; Amtsberg V., 1979),

Morphology

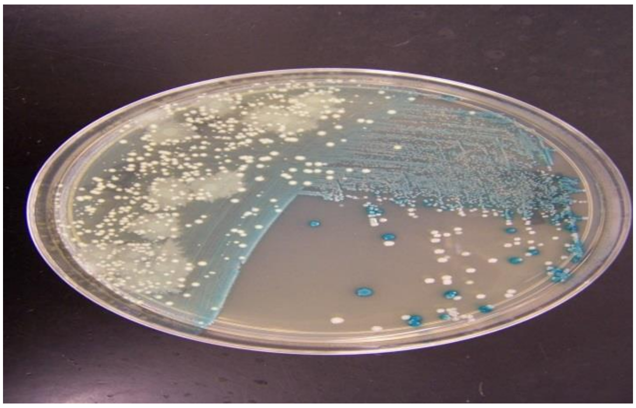
Listeriamoposutogepes is a motile, non-spore-forming, polymorphic, Gram-positive rod with rounded ends. Outside of the phenomenon of dissociation and variability, the sizes of Listeria most often vary in length from 0.3 to 0.5 μm, in width from 0.5 to 2 μm. S. A. Karpeev (1962) in an electron microscopic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

In a typical culture, the location of Listeria in smears is not characteristic. They are observed singly, in pairs, polysades of five or more sticks, or in the form of a Roman numeral V. Depending on the cultivation conditions, Listeria develop various morphological features. With bacterioscopy in pathological material and microbial cultures, you can find various forms of listeria (polymorphism). 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 there are forms with a filamentous structure up to 6-20, sometimes 275 microns. Listeria are able to take a coccoid or oval shape, diplococci can be found, as well as bipolar colored microbes.

D.Yu.Halla, during an electron microscopic examination, found that listeria sometimes have the shape of a granular ball, which breaks up into separate parts, and those, 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 L and F brands. No. 2. Sowing the filtrate from the brain on meat-peptone liver agar (MPPA) and meat-peptone liver broth (MPB) gave the growth of listeria on the 11-12th day at 37-38°C.

F. 3. Amfiteatrov, G, F. Panin, K. A. Shishkina, S. A. Karpeev (1972) also believe that listeria can look like small coccal formations (0.3-0.4 microns in length), that pass through bacteria filters.

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



G.A.Kotlyarova, I.A.Bakulov, S.V.Prozorovsky (1969) received L-forms of listeria on MPPA with the addition of 5% KC1, 30% normal horse serum and 100 IU / ml of penicillin. The crops were kept at 37°C for 10–15 days. The change in morphology was observed in 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 naturally initial form for Listeria is rod-shaped. All other forms are temporary, intermediate, resulting from the impact on the cell of adverse factors, having mastered which, Listeria again acquire a rod-shaped form.

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

Listeria is a non-spore-forming, non-acid-resistant bacterium. Using conjugates of specific sera with ferritin, a capsule was found in Listeria. Histochemical methods revealed the presence of charides in the capsule of the mucopolis. The researchers note that when listeria was cultivated on 1% soy agar with 10% whey 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 able to form a capsule when cultivated on MPPB and MPPA with glucose and glycerol. However, there is still no final opinion on the formation of capsules in this type of microorganism.

A feature of Listeria is their mobility. In this they sharply differ from almost all types of similar corynebacteria and the causative agent of swine erysipelas.

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

Coloring

Listeria are stained with all aniline dyes. According to Gram, they stain positively in a dark purple color.

In young cultures, often consisting of rod-shaped forms, a gram-positive color is usually observed.

In 48 hour and older cultures, some or most of the cells stained Gram negative. Especially often this phenomenon is observed in cultures grown at 37 °C. At 20°C, Gram-positive staining is usually detected. N.Gibson (1935) indicates that gram-negative immobile listeria can be obtained from the original source, fermenting sugars and glycerol with the formation of gas. However, 4 years later, R. Wehb and M. Barber, having studied these cultures in detail, established the mobility of Listeria. This phenomenon may have been associated with temperature characteristics or the composition of the nutrient medium.

According to the observations of VV Slivko (1984), some cultures obtained from piglets in the first generation were also immobile. IA Bakulov (1987) notes that the change in the color of Listeria as cultures age is associated with the death and autolysis of bacterial cells. The older the culture, the more dead cells in it, and consequently, the more gram-negative staining. This explanation is supported by the fact that when reseeding aging cultures, microbial cells completely restore the gram-positive color.

Cultural and biochemical properties

Listeria grow in 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 a 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 microanaerostat under vacuum conditions on blood or simple meat-peptone agar with a pH of 7.2-7.4, 24 hours after cultivation in a thermostat at a temperature of 37 ° C, the growth of Listeria in the form of dewdrop 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 led to their fairly wide distribution and significant resistance in the external environment, and, consequently, the possible variety of ways for the penetration of the causative agent of listeriosis into the body of animals and humans. Listeria grows well on hepatic media with the addition of glucose (1%) and glycerol (2-3%) and on tryptose agar.

Meat-peptone liver broth (MPB) 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 conducted by N. Ugbash, G. Schabinski (1955), F. 3. Amfiteatrov (1962), I. A. Bakulov (1967) and others, as well as our data show that Listeria multiply well in chicken embryos 6— 8 days old. Pathological changes in chicken embryos are similar to those that occur when embryos are infected with various viruses.

The morphology of colonies in Listeria does not have any features that allow them to be distinguished from the mass of colonies of other bacteria, pathogenic or banal. In particular, they bear a strong resemblance 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 (1:40,000), Listeria form round colonies with even edges, 1–3 mm in diameter. The center of the colonies is painted in bright bluish-green tones. In the usual meat-peptone broth, growth occurs slowly, only after 1-2 days a clear turbidity appears and a sour-milk smell is captured. When sowing single microbes, growth is not observed at all. Therefore, it is necessary to make abundant crops from pathological material. Cultivation of the pathogen is not always possible, however, preliminary storage of the test material in a refrigerator at 4 ° C for 4-8 weeks, according to some researchers, contributes to a positive result. When growing listeria in the refrigerator, they can be more easily isolated from mixed cultures. After 5-7 days of growth of Listeria in the BCH, a slimy sediment forms at the bottom of the test tube, which, when shaken, hardly rises up in the form of a characteristic spiral pigtail. On semi-liquid 0.3% MPA, Listeria grows in the form of cotton, which subsequently spreads throughout the medium. When sowing on meat-peptone gelatin by injection, microbial growth is observed in the form of a bayonet with lateral processes closer to the top of the injection. The most characteristic of Listeria and the most common should be considered S-shaped colonies. However, during long-term storage on artificial nutrient media, Listeria cultures undergo dissociation. Changes from smooth S-forms of colonies to rough R-forms proceed through intermediate SR and RS stages. Colonies of R-forms are difficult to remove from the agar, microbial cells from such colonies often look like filaments, when subcultured on BCH, a crumb-like sediment is observed at the bottom of the test tubes. IL Martinevsky (1962) reports that listeria from colonies of R-forms were immobile, non-pathogenic, biochemically inactive, and had the ability to produce hydrogen sulfide in a small amount. The main 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, complete clarification of the broth was observed, and at the bottom of the vessel there was a crumb-like sediment that was difficult to break when shaking. During storage and reseeding of these cultures on MPA, the growth of listeria was observed only in the S-form.

On blood agar, the colonies look like 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 was 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 α hemolysis, and subcultures caused β-hemolysis. H.Lagsen (1964), having studied 35 cultures, found that 11 strains, including Listeriagrui, 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 p-hemolysis. The authors did not find a difference in the height of the hemolysin titer depending on the serological affiliation of the strains.

A. Njoku-Obi, E.Jenkips, (1963) studied 112 strains of Listeria and found that they all have different ability to produce hemolysin.

K.Gigagd, A.Sbarga, W. Bogdwil (1963) obtained soluble Listeria hemolysin. 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% blood of cattle around the colonies, a more pronounced zone of hemolysis is formed than on MPA containing 10% blood. As a result of our studies, it was found that if on blood agar all 26 studied cultures of listeria 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 erythrocytes. To stimulate the growth of Listeria resort to the introduction of various additives into the nutrient medium.

C. 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-glycerol agar found a stimulating effect on the growth of listeria supplements of organic acids: maleic (173-259%), transaconitic (146-260%) , malonic (152-198%), pyruvic (118-159%), as well as sodium citrate (148-187%). The activating effect is observed, as a rule, in the presence of glucose.

The authors also note that the cultivation of Listeria in a casein-yeast medium (CDS) with the addition of 0.02% glucose and 1% ferroglyukin makes it possible to increase the average "yield" of bacteria to a maximum of 2 billion/ml. This indicates the stimulating effect of ferroglucin-75. KDS with these 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 of oxalinic acid to the serum-agar medium, observed inhibition of the growth of associated microbes on it. According to them, oxalinic acid retards the growth of swine erysipelas colonies 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 need the sulfur-containing amino acid cysteine (cystine). The author also points out that synthetic nutrient media with 13-18 amino acids proved to be suitable only for stationary cultivation of Listeria. Mixing and aeration in deep cultures caused the lysis of microbial cells. Further, the author notes that on the semi-synthetic medium of casein hydrolyzate (PSHC), the growth of Listeria occurs even when the cell is seeded, while in the MPB it occurs only when at least 100 cells are added to 5 ml of the medium.

A semi-synthetic casein hydrolyzate medium is prepared by diluting an enzymatic casein hydrolyzate with a synthetic medium. The synthetic medium has the following composition: trisubstituted sodium citrate -0.4; ammonium sulphate - 1.0; magnesium sulphate -0.1; ferrous iron 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 liter.

K. Girard, A. Sbarra in the USA (1992) proposed an elective nutrient medium consisting of an enrichment broth with furacin (1:100,000) and phenylethanol agar with the addition of lithium chloride (0.05%) and glycine (1%). By combining these two media, the authors have achieved an exceptionally profuse growth of Listeria from an artificial mixture of bacteria and even from feces. To detect Listeria in plant substrates, MPB with 3.75% potassium thiocyanate and MPA containing 0.0035% tryptaflavin and 0.001% nalidixic acid is used.

In the literature, one can find recipes for various elective media, developed mainly by foreign authors. The possibility of using such media in industrial laboratories is limited due to the scarcity of their components. According to V. D. Timakov, G. Ya. Kagan, I. A. Bakulov, G. A. Kotlyarov (1999) and others, semi-liquid 0.3% agar turned out to be highly effective for the induction of Listeria L-forms. They also note that there is no universal medium for obtaining and passage of L-forms, but an indispensable condition for the induction of L-variants is the inclusion of normal mammalian serum in the medium, normal horse serum is especially effective.

Listeria have limited biochemical activity. They do not dilute nutritious gelatin, do not change milk, discolor litmus milk, but do not coagulate it. Listeria cultures are catalase-positive, reduce methylene blue, do not form indole. After 24 hours at 37°C, Listeria constantly ferment levulose, trehalose, and salicin with the formation of acid. On the 3-10th day, fermentation can be observed with the formation of acid without gas, galactose, lactose, maltose, rhamnose, sucrose, dextrin, sorbitol, exulin and melecytose. Listeria does not alter media containing raffinose, inulin, inositol, dulcitol, adonite, and mannitol.

In 1986 I. Larsen. I. Seeliger isolated a listeria culture from rabbit feces, which fermented 1% mannitol for 24 hours. In honor of M. Ogau, an expert on listeriosis, this strain was named Listeriagraiy. Listeria when grown under normal conditions do not form hydrogen sulfide. However, dissociated cultures of Listeria can produce it. L. A. Timofeeva, V. Ya. Golovacheva, I. Z. Trofimenko (1967) found that 20 Listeria strains they tested produced hydrogen sulfide on the first day of cultivation in Hottinger broth with a deep breakdown of the protein molecule and a content of 1500 mg of amine nitrogen.

O. A. Kotylev, A. M. Alimov (1972) indicate that the Listeria strains studied by them also had the ability to produce hydrogen sulfide when cultivated in Hottinger, Marten broths and a semi-synthetic medium of casein hydrolyzate prepared from fresh digests. The formation of hydrogen sulfide by Listeria depends on the presence of sulfur-containing amino acids cysteine and cystine in nutrient media. Characterizing the causative agent of listeriosis, it is necessary to provide some data on the chemical composition of listeria microbial cells. They contain from 19.2 to 53.5% protein, 4.0-5.1% hexosamine and a small amount of nucleic acids. Cytoplasmic fractions contain 2.25-2.55% protein and 19.2-20.7% nucleic acids. Listeria, due to their lack of proteolytic enzymes, use low-molecular nitrogenous compounds - amino acids - for the synthesis of bacterial cell proteins.

The identity of the qualitative amino acid composition of the causative agent of listeriosis of different serological types was established, the following amino acids were identified in it: alanine, aspartic acid, lysine, methionine, leucine, arginine, valine, threolin, glycine, isoleucine, proline, serine, phenylalanine, histidine, cystin, tyrosine , glutamic acid.

According to A. M. Alimov (1974), in Listeria, glutamic acid (22.2–26.6%) occupies the largest share of the total number of amino acids. The author's studies provide new data on the content of hexosamine in various lister strains. The content of hexosamine in virulent strains is 16–24% higher than in weakly virulent strains. A constant feature of Listeria is their ability to secrete the enzyme catalase. Some strains do not release lecithinase to the same extent. Listeria do not form fibrinolysin, hyaluronidase, plasma coagulase and DNase, but they have a distribution factor.

When environmental conditions change, Listeria are able to rebuild their metabolism and, therefore, adapt to existence in various conditions. In the process of long-term storage, listeria does not lose their enzymatic features or acquire any properties unusual for them.

Other cultural properties of note include the ability of Listeria to grow in BCH with the addition of 10% common salt, and the release of bacteriocin-like substances by some non-lysogenic Listeria strains after exposure to ultraviolet light. These substances, called monocins, were studied in more detail by Y. Namop and Y. Perop (1959, 1961, 1962, 1963). Monocins are active not only against strains of Listeria, but also against some strains of staphylococci, Bac. megatirium. You. subtilis, Bac. cereus. They are destroyed at 48-50 °C. Precipitated with ammonium sulfate at 50% saturation. A characteristic feature of monocins is their resistance to trypsin and a-chymotrypsin. According to thermolability t U. Natop, V. Perop divided monocins into 10 types (from A1 to A9 and B).

In 26 studied strains of Listeria isolated from cattle, bacteriocins could not be detected. In the experiments, the method of R. Frederica was used. By exposing Listeria to ultraviolet rays, a listeriosis bacteriophage was obtained, which is used for strain typing.

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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