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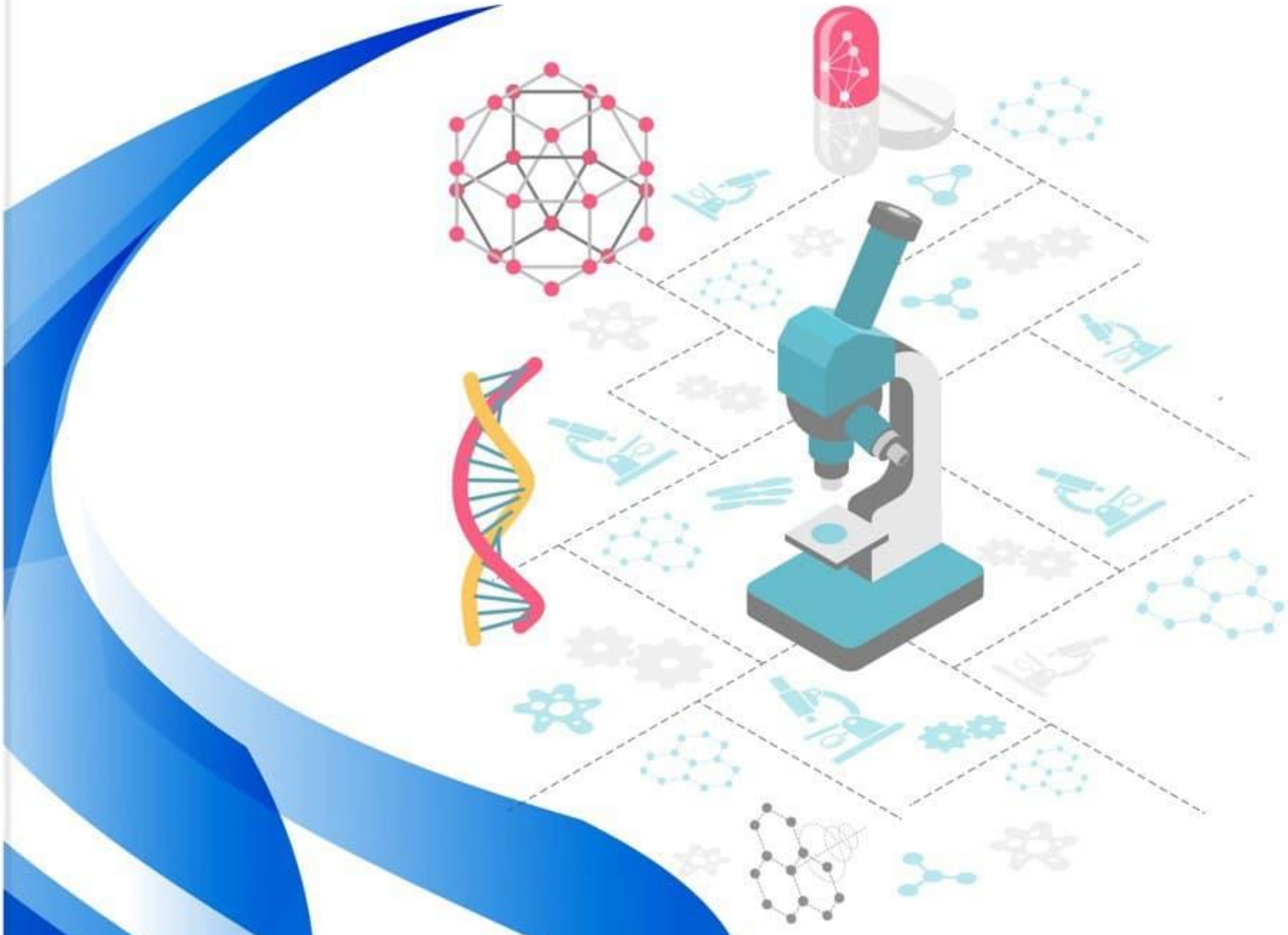

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Features of intergeneric, interspecific and serological diagnosis of listeriosis

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ABSTRACT. The review presents data on the antigenic structure of Listeria and the modern classification of epidemically significant Listeria serovars. Information is provided on the characteristic species-specific properties of Listeria serovars, which may be common to two or more species, and also have common antigens with staphylococci and typhoid-paratyphoid bacteria. It has been shown that only the antigenic scheme of Listeria monocytogenes, the only species of Listeria pathogenic for humans, is of practical interest for medicine. The importance of serotyping when conducting epidemiological analysis in order to identify the source of infections and ways of its spread has been determined. Information about the discovery of the causative agent of listeriosis is presented. Data are presented regarding differences in the designation of serovars in the diagnosis of listeriosis in domestic and foreign medical practice. The inextricable connection of Listeria serotypes with a specific host, a specific type of disease and geographic origin is shown, which is confirmed by the isolation of isolates from food. Thus, the most frequently isolated serotypes are 1 and 4. It has been shown that the high level of adaptive properties of Listeria, their ability to reproduce in an abiotic environment, including food, the increase in people with various immunodeficiencies, as well as the predominance of the food route of infection pose a significant risk increased incidence of listeriosis. The review provides information on immunochemical research methods recommended for express diagnostics, such as the immunofluorescence reaction. The review examines the current state of the problem of serological diagnosis and promising directions for serotyping of pathogenic Listeria. Serological diagnosis of Listeria has not been developed in detail, and existing serological methods are aimed at identifying specific antibodies to Listeria. The advantages of the serological method include: quick results; the ability to study any biological material. Currently available serological methods have a number of disadvantages, such as low reliability of the results and low specificity of the study.

Keywords: Listeria monocytogenes, antigenic structure, listeria typing, listeriosis, serological diagnosis, immunofluorescence.

Listeria is widespread in the environment, it is isolated from soil and water ecosystems, from food, environmental objects, circulates in the body and causes disease in animals and humans. In this regard, it is natural that close attention has been attracted to listeria infection in the last decade, both in terms of clinical and laboratory diagnostics. Particularly alarming is the increasing role of Listeria in perinatal and neonatal pathology, which is characterized by severity and high mortality [1, 2, 3]. The increase in the incidence of listeriosis is due to the unique plasticity and ability of listeria not only to persist, but also to multiply in infected products, even with strict adherence to the “cold chain”. It should be noted that a certain role is played by the increase in people suffering from various immunodeficiencies, as well as the predominance of the food route of infection. After an illness, long-term immunity is formed [4, 5, 6, 7].

In order to identify the most significant virulent strains, it is necessary to develop new approaches to Listeria typing [8, 9, 10, 11, 16, 17, 18, 19, 20]. Of the listeria species studied to date, only L. monocytogenes poses a danger to humans and animals; L. ivanovii is pathogenic for

animals [21, 22, 23]. To date, it has been established that L. monocytogenes is the etiological agent in 98% of cases of listeriosis in humans and in 85% of cases in domestic animals [4]. In 1911, the Swedish scientist G. Hulphers isolated and first described the bacterium L. monocytogenes from a purulent liver nodule of a dead rabbit [25], and an accurate and detailed description of the microbe was made later, in 1923 by E. Murray et al. [5,26]. Continuing their study, scientists determined that L. monocytogenes is a pathogen for more than 50 species of mammals, including humans, birds, ticks, fish and crustaceans. The first cases of human disease with listeriosis were registered in 1929 [6,28]. It was noted that six species of the genus Listeria have specific antigens that are characteristic of 16 serotypes: 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, 7, 5, 6a, 6b. The somatic O-antigen of Listeria is designated by numbers, the letter designation corresponds to the flagellar H-antigen, and the flagellar H-antigens are designated by the initial letters of the Latin alphabet: A, B, C, D. All serovars were found in L. monocytogenes except the last three [27, 29] . Strains of the species L. grayi have only one flagellar antigen E. L. ivanovii (serotype 5) and L. innocua (serotype 6) each have one somatic antigen.

Table 1.

Intergeneric differentiation of Listeria

Genus	**Test or sign**					
	mobility, t °C	**Requirements About 2**	**Growth at 37°C**	**Catalase**	**Production H_2S**	**Acid from glucose**
Brochothrix	-	Optional	–	+	-	+
Erysipelotrix	-	Optional	+	-	+	+
Listeria	20 – 25^{0}C	Optional	+	+	-	+
Lactobacillus	-	Optional	+	-	-	+
Kurtia	+	Strict aerobes	+	+	-	-

According to the tinctorial properties of representatives of the genus*Listeria*more often can be mistaken for bacteria of the genera: *Brochothrix*, *Erysipelothrix*, *Lactobacillus* and *Kurthia*. Table 1.1. the properties most often used by bacteriologists in differentiating microorganisms of the genus are given *Listeria*from bacteria of other genera, also gram-positive, not forming spores, having the shape of rods. Coccobacillary forms of the genus *Listeria* similar to streptococci, but this problem is solved using a catalase reaction. Listeria is easily distinguished from members of the genus *Kurthia* on fermentation of various sugars – types *Kurthia*in fermentation reactions, they either form a small amount of acid or do not form it at all.

Listeria culture is differentiated from bacteria *Erysipelothrix* by reaction to catalase, good growth on regular nutrient media, the formation of colonies with a noticeable blue-green sheen on tryptose agar when examined under a microscope using oblique illumination, motility, stronger saccharolytic activity, hydrolysis of esculin and sodium hippurate. There are differences *Listeria* and *Erysipelothrix* according to antigenic composition.

Listeria can be distinguished from most lactobacilli by the presence of a positive reaction to catalase in the former. There are, however, catalase-positive lactobacilli and some listeria strains that are catalase-negative. Bacteria genus *Listeria* grow very slowly or not at all on MRS medium

(de Mann et al. 1960), on which lactobacilli grow very well. Research has shown that strains *Listeria*, cultivated at room temperature are motile, but most lactobacilli are not motile. When examined microscopically using oblique illumination, colonies of lactobacilli on tryptose agar do not have a blue-green tint. The difference between the two genera also lies in fermentation.

Serologically genus *Listeria* and gender *Lactobacillus* differ from each other.

Table 1.2.

Intraspecific differentiation of the genus ***Listeria***(D. A. Vasiliev, 1991)

№	Test or sign	Species of the genus Listeria					
1	β-hemolysis (*S. aureus*)	+	+	_	+	_	_
2	CAMP test*S. aureus*)	+	_	_	+	_	_
3	3 CAMP test (*Rh.equi*	+ (±)	+	-	-	_	_
4	Acid formation:						
	Dextrin	-	-	-	-	-	+
	galactose	-	-	-	-	-	+
	D-lactose	-	-	-	-	-	+
	beckons	-	-	-	-	-	+
	mannose	+	-	+	-	+	-
	rhamnose	+	-	-	-	-	-
	Starch	-	-	-	-	-	+
	D-xylose	-	+	-	+	+	-
5	Voges-Proskaur test	+	+	+	+	+	+
6	Hydrolysis of hippurate	+	+	+	-	-	-
7	Lecithin hydrolysis	-	+	-	-	-	-
8	Pathogenicity for white miceth	+	+	-	-	-	-

Currently, only two species of Listeria are believed to be *L.monocytogenes* and *L.ivanovii* are pathogenic and1 pose a danger to the macroorganism. For bacteriological identification, the characteristics of each type of Listeria are given (Table 1.2)

*L.monocytogenes***.**These are non-spore-forming, short rods of regular shape, 0.4-0.5 μm in diameter and 0.5-2.0 μm in length with rounded ends. When listing Listeria microscopy in smears, you can see their group arrangement, parallel, along the long axis. In smears from infected pathological material or from a liquid medium, listeria of the coccal form is found with a diameter of 0.4-0.5 microns and a length of 0.4-0.6 microns. They may be mistaken for streptococci. In more mature or unprocessed crops, forms in the form of fibers up to 6-20 μm long can be found. Gram stain is positive, but in old cultures cells lose the ability to retain Gram stain. Non-acid resistant.

The listeria bacillus is always mobile during cultivation at room temperature. At a temperature of 37 °C, flagella, as a rule, are not formed and listeria are immobile or weakly mobile.

The listeria culture of this species is aerobic and facultatively anaerobic. Temperature range for growth is from 1 to 44 °C. However, the culture remains viable at higher temperatures (up to 70-71 °C). Temperature optimum 25-37 °C. Listeria of this species are able to grow and reproduce in the pH range from 6.0 to 9.0, but retain their viability at a pH value of 5.0-11.0. Optimal pH values for growth are 7.0-7.4. Under aerobic conditions, on nutrient agar at the optimal temperature range, 24-48-hour cultures grow in the form of dew-colored colonies with a diameter of 0.5-1.5 mm, round in shape, translucent droplets, convex with a clearly defined surface and a solid edge. Under normal lighting, colonies have a gray-bluish color. The size of 3-7 day old colonies reaches a diameter of 3.0-5.5 mm with an opaque center and irregular shape. In a semi-liquid medium of 0.25% agar, daily growth occurs along the prick. In MPB, growth is slow, but after 28-48 hours a delicate, opalescent cloudiness appears. The bacteria have hemolytic activity. Weak or barely detectable hemolytic activity can be enhanced by the use of a synergist bacterium (*S. aureus*) in the CAMP test. Listeria culture in most cases is catalase-positive and oxidase-negative, but it must be taken into account that if the culture media contains low concentrations of meat or yeast extract, a negative catalase reaction is possible. Catalase activity is also suppressed in media containing a high (more than 10%) concentration of glucose. Listeria of this species give positive reactions with methyl-rot and Voges-Proskaur. They do not liquefy gelatin or hydrolyze casein and molonate. They do not produce indole and do not form hydrogen sulfide on ordinary nutrient media. Sugar metabolism

The ditch is accompanied by the formation of acid, but not gas. Listeria can grow in media of complex composition containing up to 20% NaCl, with 16% sodium chloride in the medium (pH 6.0) and remain viable for a year.

It should be noted that although the above properties of Listeria are quite typical, deviations are possible due to the composition of the nutrient media and the isolation of new strains of Listeria.

Strains of the species differ in antigenic composition and include the following serovars: 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4av, 4c, 4d, 4e and serovar 7.

Experimentally pathogenic in mice and guinea pigs. This species is widespread in nature. It is isolated from wastewater, soil, silage, representatives of aquafauna, and from the feces of healthy animals and people. In humans and animals it is isolated during clinical signs of disease. Causes meningitis, encephalitis, septicemia, endocarditis, abortions, abscesses and local purulent lesions.

L.ivanovii, named in 1985 in honor of Ivan Ivanov, a Bulgarian microbiologist.

The morphological feature is the absence of flagella, as a result of which the strains of the species are immobile. Pronounced hemolysis with double or triple zones is observed if Listeria of this species is grown on agar containing defibrinated blood of sheep or horses (5% volume/volume). A positive CAMP reaction is observed when using *R.equi* and negative co *S. aureus*. Acid is not formed from D-mannitol, L-rhamnose or X-methyl-D-mannoside. All studied strains belong to serovar 5. This antigenic composition in strains of other species of the genus *Listeria* not found. *L.ivanovii* pathogenic for mice, the LD50 value for some bacteria of this species ranges between 1x105-3x106CFU. Pathogenic for animals, especially pregnant sheep. Listeria of this species was also isolated from the body of a healthy animal, a human carrier, from the environment, and very rarely from the human body in the clinical manifestation of the disease.

L.innocua, named in 1981 due to its harmlessness (innocus – safe). The morphology of bacteria is similar *L.monocytogenes*. Does not cause hemolysis on media containing 5 or 10% (v/v) blood from sheep, rabbits, horses or humans. Most of the strains studied produce acid from rhamnose. All strains studied so far have the antigenic composition of serogroup 6 (serovars 6a and 6b). In animal experiments, this species was not pathogenic for mice and guinea pigs when administered intraperitoneally with a microbial suspension containing 1010b.w./ml.

Isolated from soil, plants, human and animal feces, and bird droppings. Never isolated from pathological material from humans or animals. *L.welshimeri* named in 1983 in honor of the American bacteriologist HJ Welshimer.

Morphology similar to bacteria of the species *L.monocytogenes*. No hemolysis is detected on blood agar, CAMP reactions with *S. aureus*, *R.equi*–negative. The type strain does not form acid from L-rhamnose and D-mannitol. The strains currently assigned to this species belong to serovar 6a or 6b. The antigenic composition of the type strain is the same as that of serovar 6c. Serovars 6a and 6b are also found in *L.innocua*, and some strains *L.seeligeri* have the antigenic composition of serovar 6b. This species is not experimentally pathogenic for mice. It is isolated from soil samples and rotting vegetation in the United States; it is probably widespread in nature.

*L.seeligeri***,** named in 1983 in honor of HPR Seeliger, a German bacteriologist.

Morphology, same as species *L.monocytogenes*. Hemolytic activity is weakly expressed. CAMP reaction is positive when used *S. Aureus* and negative with *R.equi*. The acid is formed from D-mannitol and L-rhamnose. The strains currently classified as this species have the antigenic composition of serovars 1/2b, 4c, 4d (which are also found in *L.monocytogenes*) and 6b (which is found in some strains *L.ivanovii*, *L.welshimeri*). The type strain belongs to serovar 1/2b. In experiments it was not pathogenic for mice. It is isolated from plants, soil and animal feces in Europe; probably widespread in nature.

L. grayi sub. grayi named in 1966 in honor of M. Gray, an American bacteriologist known for his work on listeriosis. Cultural and morphological properties are similar *L.monocytogenes*. When studying small colonies (0.2 mm) on media (tryptone soy agar) in indirect light they show the same blue-green tint as *L.monocytogenes*. After longer incubation, the colonies become orange-red, especially at the edges. Hemolysis does not cause. Optimal growth temperature is 30-37 °C. Temperature limits for growth are 1-45 °C. They die when heated at 60 degrees for 30 minutes. Catalase positive, oxidase negative. Requires organic growth factors. They grow at pH 5.0-9.0, but at pH 9.6 no growth is observed. Strains of the species are sensitive to penicillin, streptomycin, chloramphenicol, erythromycin, novobiocin, neomycin; resistant to sulfanilamide, polymyxin, colostin sulfate and nalidixic acid. Culture does not grow on the environment Lardner (1966). Slow growth can occur on MPS medium (Mann et al., 1960).

Enzymatic metabolism of glucose leads to the formation of lactic acid and several other products. Esculin, amygdalin, cyllobiose, dextrin, galactose, glucose, lactose, fructose, maltose, mannitol, mannose, salicin, starch, trehalose produce acid, not gas. Acid is not usually formed from adonite, arabinose, dulcitol, erythritol, glycogen, inositol, uniline, melibiose, melecitose, raffinose, rhamnose, sorbitol, sucrose and xylose. Litmus milk contains a weak acid and a slight reductase. The reaction between methyl red and starch is not constant. Tween 20 hydrolyzes slowly (14 days). Tween 80 does not hydrolyze. Slowly forms deoxyribonucleases, ribonucleases and phosphatases. Sulfatase is not formed. Xenatin, tyrosine and chitin are not broken down. Cellulose, gelatin, casein and milk do not hydrolyze. Indole does not form. Urea does not hydrolyze. Serologically distinct from members of the genus *Listeria*. Antigenic composition identical*L. grayi*sub. *murrayi.* Not pathogenic for mice after intraperitoneal or intracerebral injection, but cells at a concentration of 5x108may be toxic to mice. Non-pathogenic for pregnant rabbits after intravenous administration.

Anton's test is negative. Isolated from chinchilla feces.

L. grayi sub.*murrayi***.** Considering that in many publications and reference books this subspecies is considered as an independent species and differentiated by phenotypic properties, we provide its characteristics. This subspecies was named in 1971 in honor of E. Murray, one of the listeria researchers. Cultural, morphological and biochemical properties are similar *L. grayi*sub.*grayi*. When microscopying cultures grown on the surface of tryptose agar, small colonies (0.2 mm) have the same green-blue hue as *L.monocytogenes*. After 2 days of incubation, larger colonies show an orange-red hue (H. Welshimer et al. 1971). At 22-25 °C on nutrient agar

containing glucose, the colonies appear yellow pigment. In the absence of glucose, no pigment is detected. They do not have hemolytic activity. The optimal growth temperature is 30-37 °C. Serologically distinct from species *L.monocytogenes*. The antigenic composition is identical to the antigenic composition *L. grayi* sub.*grayi*.

Anton's test is negative. Non-pathogenic either when administered intravenously to rabbits or when administered intraperitoneally 108cells to mice, weighing 18-20 g. Isolated from rotting leaves of cereals (maize); the natural habitat is probably soil and plants.

It should be noted that domestic serological diagnostics has its own characteristics: thus, L. monocytogenes serovars, designated in accordance with the international classification 1/2a 1/2b, 1/2c, 3a, 3b 3c, are combined into the first serological group, and the remaining serovars are to the second. The wide range of host organisms in which the pathogen can multiply has determined the antigenic heterogeneity of the outer shell of L. monocytogenes [27, 28, 29, 30]. RH Orsi et al. [31] demonstrated that using molecular typing methods it is possible to divide L. monocytogenes into three evolutionary lineages characterized by different pathogenic potentials: the first lineage is strains associated with epidemic outbreaks of listeriosis (serotypes 1/2b, 3b, 4b, 4d and 4e); second line - strains isolated during sporadic cases of listeriosis (serotypes 1/2a, 1/2c, 3a and 3c); the third lineage is strains rarely associated with cases of listeriosis (serotypes 4a and 4c). At the same time, no patterns were found between the serovars of the isolated strains and the biological type of the host, as well as the severity of the disease. Host specificity and the course of the pathological process are determined by Listeria pathogenicity factors: listeriolysin, internalins A and B [13, 19,22].

According to foreign authors, sequences encoding pathogenicity factors were found much more often in strains of serovar 4b [23]. The serological features of the isolated cultures are not limited to the described scheme. In the USA, a culture of Listeria serovariant 4b was isolated, which contained genetic sequences characteristic of other serovars [14]. In addition to intraspecific cross-reactions, serological cross-reactions with staphylococci and typhoid-paratyphoid bacteria are observed in Listeria [9]. When conducting epidemiological analysis in order to identify the source of infections and the routes of its spread, it is of practical interest for medical microbiology to study the antigenic structure of L. monocytogenes [12, 13]. In particular, when studying the serological landscape of strains isolated from patients with listeriosis, it was found that most cases of disease are associated with serotypes 4b, 1/2a, 1/2b. An analysis of the incidence of listeriosis has demonstrated that about 50% of all cases of listeriosis in the world are caused by strains of serovar 4b, although among the strains isolated from contaminated products, L. monocytogenes serovars 1/2a, 1/2b, 1/2c dominate.

Outbreaks of enteric diseases 1998–1999 in the USA after eating sausages were caused by the serovar 4b strain, which has been the etiological agent of listeriosis in the UK for 30 years. It was found that out of 2232 isolates isolated from sick people, 60% of cases were serovar 4b, and in 17%, 11% and 4% of cases the diseases were caused by serovars 1a, 1/ab and 1c, respectively. Serovar 1/2a has been most frequently reported in Eastern Europe, Eastern Africa, Central Germany, Finland and Switzerland. while the co-isolation of serovariants 1/2a and 4b in approximately equal proportions was noted in France and the Netherlands [10,12]. It is difficult to determine the diagnosis of “listeriosis” only based on clinical and epidemiological information due to the polymorphism of clinical manifestations and the inability to identify the source of infection - for this reason, laboratory diagnosis is of primary importance. A final diagnosis can be made only after bacteriological examination [20]. Despite the fact that bacteriological isolation of the pathogen culture is recognized as the “gold standard” in the diagnosis of listeriosis, serological methods,

being auxiliary, still play an important role in the diagnosis of this infection. The advantages of serological methods include: rapid results, relative simplicity of reactions, as well as the ability to study a variety of biological material [7,22]. One of the methods of serological diagnosis is the determination of antibodies to the secreted pathogenicity factor of Listeria - listeriolysin O.

There are a number of serological methods that are used in clinical laboratory diagnostics and are aimed at identifying specific antibodies to Listeria. Their use is advisable from the second week of the disease. Antibodies against Listeria persist for several years after the disease. Serological tests used to diagnose listeriosis include: enzyme-linked immunosorbent assay (ELISA), agglutination test (RA), complement fixation test (FFR), and indirect hemagglutination test (IRHA). The material for the study is blood and cerebrospinal fluid (CSF). The result is considered positive by the presence of antibodies in a titer from 1:250 to 1:5000 [25]. It is well known that serovars and serotypes of Listeria are not species specific. They may be common to different Listeria species, regardless of their pathogenicity to humans. Analysis of the serological structure of Listeria showed that it is extremely inconvenient for diagnosis. L. monocytogenes shares one or more antigenic determinants with Listeria species other than L. welshimeri.

Therefore, the determination of serovar alone without the use of other methods does not allow establishing the diagnosis of infection caused by L. monocytogenes [8,23]. Serological methods that are currently used have a number of disadvantages: the study has low specificity (listeria antigens are very similar in structure to the antigens of other microorganisms, therefore false positive or false negative results are often obtained; the method itself does not detect the pathogen, but detects antibodies; the results are low reliability, on their basis one can only suspect listeriosis; in severe immunodeficiency states, the body loses the ability to form antibodies, ELISA will be negative even with the most severe course of listeriosis; analysis is possible only in the later stages of the disease, starting from the second week from the first symptoms. The diagnosis of “listeriosis” can be suspected or made when there is a significant difference in antibody titers in paired sera of patients with a characteristic clinical picture (RA with a color diagnostic, RSC, indirect immunofluorescence reaction (IDIF), RNAG), during the study of CSF (IRIF, PCR, ELISA, microscopy) and bacteriological examination by enrichment method with carbon immunoglobulin sorbent [2, 14, 26,].

Conclusion. Nevertheless, in the practice of domestic bacteriologists, serological methods for laboratory diagnosis of listeriosis remain the main ones and make it possible to establish a presumptive diagnosis of listeriosis infection with further confirmation by a bacteriological method. Of course, the results of a serological examination provide certain information about the contact of various population groups or risk groups with the pathogen, but do not allow diagnosing listeriosis with a high degree of accuracy, even when using several serological methods. The slide agglutination method remains simple and reliable, the implementation of which requires agglutinating listeria sera. The main factor limiting the diagnostic capabilities of bacteriological laboratories is the lack of commercial, registered drugs for typing L. monocytogenes cultures. In this regard, improvement of methods for obtaining listeria sera, allowing for early identification of L. monocytogenes

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