

## STUDY OF LISTERIA RESISTANCE IN FOOD PRODUCTS

**G.U.Sagdullayeva**

Department of the Medical Biology

Bukhara state medical institute,

Uzbekistan, Bukhara,

[sagdullaevagulandon@gmailcom.com](mailto:sagdullaevagulandon@gmailcom.com)

### ANNOTATION

Listeriosis is a zoonotic bacterial infectious disease characterized by multiple sources of infection, a variety of pathways of transmission of the pathogen, polymorphism of clinical manifestations, and high mortality in newborns and people with immunodeficiency. The preservation of the viability of pathogenic microorganisms has a certain theoretical and practical significance in the hygienic assessment of environmental objects. The survival of microorganisms under various physical and chemical interactions may be important in the study of anthropogenic pollution of water bodies.

**Keywords:** listeria, sapronosis, pasteurization, meat-peptone broth, catalase test, indicator nutrient media, differentiation, indication.

### Introduction

Listeriosis is a natural focal infectious disease of humans and animals and is an urgent medical and veterinary problem. Diseases in humans are caused only by *Listeria monocytogenes*. 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 saprozoonotic infection common in

developed countries) make this disease relevant, requiring the development of modern laboratory diagnostics for an adequate study of its epidemiology.

**The purpose** of this study was to study the dynamics of death of pathogenic listeria in order to identify the possibility of using melon methods for disinfection of the environment.

Resistance to environmental factors. The pathogen's resistance to various environmental factors is high: in soil, manure, water, and plants, they remain viable for up to 600 days, and on polluted surfaces of agricultural premises in summer (9... 22 ° C) listeria remain viable up to 25 days, and in winter (-2 ... -23 ° C) up to 130 days. Reservoirs contaminated with listeria are dangerous epizootically and epidemiologically.

The duration of listeria survival in the external environment depends on the temperature, pH of the medium, and the specific and quantitative composition of organic or inorganic substances in which the bacteria are found. Listeria have the ability to reproduce even at low temperatures (4-6 ° C), and they can persist in ice for 5.5 months to 2.5 years.

Resistance of the pathogen to the physico-chemical factors of meat production. Listeria has a high viability to the effects of various factors and techniques used in the production technology of meat and meat products.

Cooling of meat for up to 17 days. (shelf life of chilled meat) It reduces the viability of the causative agent of listeriosis by 4 orders of magnitude compared to their original content, but during this period listeria does not completely die off.

The process of storing frozen mutton at -10 ... -28 °C for 20 days, and are safe at -10 ... -20 °C for 14 months, does not inactivate listeria. When storing frozen beef meat (-16 ... -18 ° C) for 9 months, the number of listeria actively decreases in the first 3 months, after which, by the end of the shelf life of 9 months, their number decreases

by 4 orders of magnitude compared to the initial one, and there is no complete loss of viability of the pathogen.

Listeria is highly thermally stable within the range of pasteurization and cooking temperatures of sausage products. The thermal stability of listeria decreases with an increase in the content of connective tissue in meat. Thus, compared with premium grade beef ( $D_{70} c = 10.89$  min;  $D_{72} c = 7.93$  min), the value of DT for Grade I beef (with 6% connective tissue content) is  $D_{70} c = 9.93$  min;  $D_{72} c = 7.6$  min, and in Grade II beef (with 20% connective tissue content fabrics) -  $D_{70} s = 9.78$  min;  $D_{72} s = 6.89$  min.

Fat has a protective effect on the thermal stability of listeria. Value the DT in fat pork (with 20% fat content) is  $D_{70} c = 10.94$  min;  $D_{72} c = 9.26$  min, in fat pork (with 50% fat content) -  $D_{70} c = 1-39$  min;  $D_{72} c = 10.89$  min.

Cooking tea sausage (heating medium temperature 75-80 ° C) with a diameter of 35-50 mm inactivates listeria within 75 minutes, and with a diameter of 65 mm - after 90 minutes. When cooking pieces of mutton weighing 1-2.5 kg and 8-10 cm thick, the causative agent of listeriosis dies within 1 hour.

pH values in the range of 7.2; 6.5; 5.5 do not significantly affect the viability of listeria at 4-6 ° C for 5 days.

Listeria remains viable in a meat-peptone broth (MBB) containing 6% NaCl for more than a year, in the organs of infected animals with the same concentration of table salt - up to 2 months, in MBB with 24% NaCl - up to 22 days. In meat stored in 24% brine, listeria remains viable for up to 400 days. The NaCl content in the range of 2.5; 4.5; 10% for 5 days at 4-6 ° C leads to a decrease in the amount of *L. monocytogenes* by 4 orders of magnitude. The NaCl concentration of 14% reduces the listeria population by 5 orders of magnitude after 5 days. In canned hides with salt, the causative agent of listeriosis remains viable for up to 62 days. Exposure to food phosphate (Polyphan A, 0.3%), sodium nitrite (0.005%) at 4-6 ° C for 5 days reduces

the number of listeria by 1 order of magnitude after 2 days. Application of emulsions (0.005%) of black, red, fragrant pepper, Coriander, nutmeg, cardamom, cumin did not cause a significant decrease in the viability of listeria for 15 days at 4-6 ° C. The content of garlic emulsion (0.005%) reduces the number of this type of microorganisms by 2 orders of magnitude after 5 days, and by 4 orders of magnitude after 15 days.

The process of storing sausages at low plus temperatures although and it reduces the viability of listeria, but it does not completely suppress them. When infected meat from pigs, sheep and rabbits matures, listeria remains pathogenic. at 37 ° C with daily viewing for the first 3-4 days. In the absence of growth, the crops are monitored for 2 weeks.

On MPA, listeria colonies grow in the form of small, round, transparent acolonies when viewed in passing light (they look like colonies of the erysipelas pathogen); after a few days, the colonies turn cloudy. In the smear from the agar culture, listeria are straight short (0.3-0.5 x 1.2 microns) ovoid rods, sometimes almost cocci, arranged singly or in clusters. By mistake, the culture of listeria can be attributed to another type of bacteria. On MPA with 1% glucose and 2% glycerol, as well as on liver media, growth resembles colonies of E. coli bacteria. On blood agar, listeria causes alpha-hemolysis. On BCH, listeria causes uniform turbidity of the medium, moire waves are observed when shaken, but rougher than with the growth of erysipelatos bacteria, a precipitate forms on 8-10 days, which rises upward in the form of a pigtail when shaken.

In young crops (6-24 hours), listeria are mobile; their mobility is better visible after cultivation at room temperature; they ferment salicin, glucose, lactose and glycerin to form acid without gas; they do not ferment mannitol, dulcete; they do not dilute gelatin; they do not change milk; they reduce methylene blue.

To differentiate listeria from the causative agent of porcine erysipelas, a catalase test is performed. 1 ml of 10% hydrogen peroxide is added to the test tube with the



studied 12-24-hour culture on BCH: in the presence of catalase, the liquid foams, and porcine erysipelas bacteria do not form catalase.

A reliable test for distinguishing listeria from erysipelas is an eye test: 1-2 drops of saline solution of a daily culture with MPA are injected into the conjunctival sac of a guinea pig and carefully rubbed into the mucous membrane of the eyelid. Usually, after 24 hours, eyelid edema, hyperemia, and lacrimation appear; after 36-72 hours, the eyelids swell and purulent exudate is released from the eye.

For accelerated differentiation of the causative agent of listeriosis from the causative agent of erysipelas

Indicator media are used in pigs: with litmus, neutralrote mixed with methylene blue, methylrote, congorote and amido-black. After 3-6 hours, listeria is discolored with litmus medium and neutralrotum medium mixed with methylene blue to the color of BCH, only a colored rim remains at the surface at the air boundary. When shaken, the color is partially restored, so the crops are viewed without shaking the test tubes. The medium with methylrote discolours after 3-6 hours, but the color of the medium is not restored. Discoloration of media with conguration and with amido-black occurs at a later date - after 6-48 hours, after discoloration of the medium, the original color is not restored. The causative agent of porcine erysipelas does not discolor any of the above-mentioned media with indicators.

Preparation of indicator media:

- Litmus medium: 1 ml of tincture of litmus is added to 100 ml of BCH or Hottinger broth

- . Wednesday's color is lilac;

- Neutralroot medium mixed with methylene blue: 1 ml of 0.1% solutions of neutralroot and methylene blue are added to 100 ml of BCH and Hottinger broth. The color of the medium is greenish-bluish or green. Media with litmus and neutral alcohol

mixed with methylene blue are poured into tubes with cotton plugs and sterilized at 0.1 Мра 30min;

- Methylroth medium: 0.3 ml of sterile 0.1% aqueous methylroth solution is added to a test tube with 10 ml of sterile BCH or Hottinger broth, the color of the medium is lemon yellow;

- Congested medium: 0.3 ml of sterile 0.1% aqueous congested solution is added to a test tube with 10 liters of sterile BCH or Hottinger broth, the color of the medium is red;

- Medium with amido-black: 0.3 ml of sterile 0.1% aqueous solution of amido-black is added to 10 ml of sterile BCH or Hottinger broth, the color of the medium is black with a purple tinge.

- Blood tellurite agar: 10 ml of defibrinated horse or bovine blood and 2 ml of a 2% solution of potassium tellurite are added to 100 ml of nutrient agar melted and cooled to 45-50 ° C (pH 7.2-7.4) and 2 ml of a 2% solution of potassium tellurite (the medium can also be prepared with dry blood: 15 mg of dry blood per 100 ml of agar). The medium is thoroughly stirred and poured into petri dishes. On this medium, listeria colonies turn black or have a black center (reduction of metallic tellurium). In the study of a low content of listeria, 0.3-0.5 ml of a solution of florimycin or polymyxin (500 thousand mg) is added to the medium to suppress the growth of extraneous microflora. Units of the drug in 10 ml of saline solution). For serological identification of isolated listeria, RA is used with multivalent and group listeriosis sera. The culture under study is recognized as listeria when receiving positive RA with listeria serum and negative RA in the control with saline solution. Listeriosis bacteriophage (2A, ZA and 4A) is also used to identify the causative agent of listeriosis.

Personal prevention measures

When working with animals, slaughtering and butchering carcasses of sick or suspected listeriosis animals, workers and veterinary staff must strictly observe the rules of personal hygiene and prevention.

Employees are provided with sanitary clothing, rubber gloves, shoes and other protective equipment. If workers have abrasions, cuts or other skin injuries on their hands, they are allowed to work in rubber gloves, having previously treated the wound site with iodine tincture and bandaged or coated it with BF-6 glue.

Persons under the age of 18, pregnant and lactating women are strictly prohibited from caring for, slaughtering, and processing carcasses and raw materials obtained from them. All employees are introduced to the rules of prevention against listeriosis infection before being allowed to care for and slaughter animals that respond positively to listeriosis and processing carcasses and raw materials from them. Before starting work, workers wash their hands thoroughly, put on sanitary and work clothes, shoes and other protective equipment.

It is forbidden to leave the workshop in sanitary clothes. Workers hand over sanitary and special clothes and shoes for disinfection at the end of the work shift, disinfect their hands and take a shower.

The procedure for sanitary treatment of premises, equipment and other facilities at meat processing plants is disinfected in cases of detection of sick animals during pre-slaughter and detection of lesions in slaughter products characteristic of listeriosis.

If a sick animal with listeriosis is found at a slaughterhouse, then after its placement in an isolation unit, disinfection is performed only in an appropriate room or pen with a solution of bleach containing 2% active chlorine at the rate of 1 liter of solution per 1 m<sup>2</sup> of area (exposure 4 hours). Previously, in order to prevent spraying of the causative agent of listeriosis during cleaning, the area to be disinfected is irrigated with a solution of bleach containing 2% active chlorine.

The premises of the quarantine department, isolation ward and sanitary slaughterhouse, after irrigation with disinfectant solutions, are cleaned of impurities, washed with hot water and disinfected with a 2% hot solution of caustic soda (exposure 3 hours) or a solution of bleach containing 2% active chlorine, or 2% formaldehyde solution (4 hours). After disinfection, the rooms are ventilated and, if necessary, rinsed with hot water.

When listeriosis is detected in a slaughterhouse, sanitation is carried out primarily in pre-slaughter facilities, stun boxes where animals with listeriosis were located, and other production facilities where infected slaughter products got into, and all technological equipment and inventory located in the premises. Disinfection is carried out in the same way as in a sanitary slaughterhouse. To destroy the causative agent of listeriosis, the premises and equipment of the slaughterhouse are abundantly irrigated with a 2% solution of caustic soda heated to 70 °-80 ° C, then thoroughly washed with hot water and re-irrigated with either a 4% hot solution of caustic soda (exposure 3 hours), or a 16% solution of soda ash at a temperature of 70-80 ° C (exposure 4 hours), or a solution of bleach containing 2% active chlorine (exposure of 4 hours). After such de-treatment, the rooms are washed with hot water.

For disinfection, equipment, animal care items, and tools are boiled for 30 minutes or immersed for 1 hour in a solution (15-20 °C) bleach containing 2% active chlorine, or for 2 hours in a 10% solution (15-20%) of soda ash.

Sanitary clothing is disinfected by boiling in a 1% solution of soda ash, hands are treated in a solution of chloramine containing 0.2% active chlorine. Sanitary treatment is performed by workers who have no medical contraindications to this work, who have been trained and instructed on the safety of working with disinfectants and detergents.

Wastewater from the quarantine department, isolation unit and sanitary slaughterhouse, as well as water after flushing the adjacent territory before being released into the external sewer network, is disinfected using chlorine, which is dosed



using chlorinator devices at the rate of 35 mg /l of chlorine for at least 1 hour. The sediment is mixed with bleach in a ratio of 5:1 and taken to a specially designated place and buried in the ground.

Manure is disinfected biothermically. Chemical and steam jet methods are most suitable for meat industry enterprises.

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