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Detection of *Listeria monocytogenes* while testing food raw materials and products of animal origin for microbiological contamination

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ABSTRACT

Introduction. *Listeria*-contaminated food remains an ongoing concern. Consumption of raw or undercooked animal-derived products contaminated with pathogenic *Listeria* results in human infection. The Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing (Rospotrebnadzor) documented 100 listeriosis cases in 2023, with 18 cases resulting in death. In recent years, there has been an increase in *Listeria* contamination of both domestically produced and imported food products. Thus, detection of pathogenic *Listeria* in the products of animal origin, food raw materials, and ready-to-eat products remains a critical task.

Objective. Detecting *Listeria monocytogenes* contamination in products of animal origin (meat, fish, dairy) manufactured and marketed in the Nizhny Novgorod Oblast from 2023 to 2024.

Materials and methods. The samples were analysed and pure microbial cultures were identified in accordance with GOST 32031-2022 "Food products. Methods for detection of *Listeria monocytogenes* and other *Listeria* (*Listeria* spp.)".

Results. Analysis and synthesis of the obtained data revealed that out of 3,650 tested samples, 57 (1.6%) were contaminated with *L. monocytogenes* bacteria. The highest number of contaminated samples was found among such product categories as combined semi-finished meat products, beef products, and poultry meat products. The incidence of *L. monocytogenes* in samples of fishery products was 1.1%. The highest levels of contamination were detected in the following products: minced beef (10.7%), poultry meat products wrapped in dough (9.3%), mechanically deboned poultry meat (7.1%), large-cut semi-finished products (4.6%), beef offal (4.3%), and chopped semi-finished poultry meat products (4.2%).

Conclusion. The test results show that the number of contaminated samples among combined semi-finished meat products was 4.3%, non-compliance with the safety requirements of beef products was detected in 3.7%; 2.8% of poultry product samples were contaminated with *L. monocytogenes* bacteria. The number and percentage of contaminated samples among frozen and refrigerated products did not significantly differ and amounted to 0.7 and 0.8%, respectively. *L. monocytogenes* were not detected in samples of dairy and ready-to-eat meat products that do not require heat treatment.

Keywords: listeriosis, *Listeria monocytogenes*, contamination, meat, milk, food products

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Индикация бактерий *Listeria monocytogenes* при оценке микробиологической контаминации сырья и продуктов животного происхождения

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РЕЗЮМЕ

Введение. Проблема контаминации пищевых продуктов листериями не теряет своей актуальности. Употребление в пищу обсемененных патогенными бактериями рода *Listeria* продуктов животного происхождения в сыром или недостаточно термически обработанном виде приводит к заражению человека. По данным Государственного доклада Роспотребнадзора, в 2023 г. в нашей стране было зарегистрировано 100 случаев листериоза, из которых

18 – с летальным исходом. В последние годы наблюдается рост контаминации листериями пищевых продуктов как отечественного, так и зарубежного производства. Таким образом, выявление патогенных видов *Listeria* в продуктах животного происхождения, пищевом сырье и готовых пищевых продуктах является актуальной задачей.

Цель исследования. Определение контаминации бактериями *Listeria monocytogenes* продуктов животного происхождения (мясных, рыбных, молочных), произведенных и реализуемых в Нижегородской области в период с 2023 по 2024 г.

Материалы и методы. Исследование проб, а также идентификацию чистой культуры микроорганизмов проводили в соответствии с ГОСТ 32031-2022 «Продукты пищевые. Методы выявления бактерий *Listeria monocytogenes* и других видов *Listeria* (*Listeria* spp.)».

Результаты. При анализе и обобщении полученных данных было показано, что из 3650 исследованных проб бактериями *L. monocytogenes* были контаминированы 57 образцов (1,6%). В таких категориях продуктов, как полуфабрикаты мясные смешанного состава, продукты из говядины и мяса птицы, было определено наибольшее количество контаминированных проб. При исследовании проб рыбных продуктов инцидентность *L. monocytogenes* составила 1,1%. Наибольший уровень контаминации отмечен в таких видах продуктов, как фарш говяжий (10,7%), полуфабрикаты из мяса птицы в тестовой оболочке (9,3%), мясо птицы механической обвалки (7,1%), полуфабрикаты крупнокусковые (4,6%) и субпродукты (4,3%) из говядины, полуфабрикаты из мяса птицы рубленые (4,2%).

Заключение. В результате испытаний было установлено, что количество контаминированных проб полуфабрикатов мясных смешанного состава достигло 4,3%, несоответствия требованиям безопасности продуктов из говядины выявлены в 3,7% случаев, 2,8% проб продуктов птицеводства были обсеменены бактериями *L. monocytogenes.* Количество и процентное соотношение контаминированных проб замороженных и охлажденных продуктов достоверно не различались и составили 0,7 и 0,8% соответственно. Бактерии *L. monocytogenes* не были выявлены в пробах молочных и готовых мясных продуктов, не требующих термической обработки.

Ключевые слова: листериоз, *Listeria monocytogenes*, контаминация, мясо, молоко, пищевые продукты

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INTRODUCTION

Listeriosis is an infectious disease affecting most farm and domestic species (pigs, horses, cattle, sheep, goats, rabbits, chickens, and ducks) as well as humans. The disease-related economic damage includes reduced animal productivity, expenses for medical treatment and prevention, and quarantine control measures. In Russia and other countries worldwide, the incidence of foodborne diseases, including listeriosis, shows no signs of decline [1, 2, 3]. Listeriosis ranks fifth among the most frequently reported zoonoses in humans in the European Union and is one of the most significant foodborne diseases [4]. Infection caused by *Listeria monocytogenes* is highly dangerous for pregnant women (causing miscarriages), infants (resulting in newborn fatalities) and immunocompromised individuals [5]. Listeriosis in pregnant women and their newborns have been reported in the Republic of Dagestan [6]. In 2021, a *Listeria* meningoencephalitis case was diagnosed in a COVID-19 patient in the Voronezh Oblast [7]. In 2022, a case of neonatal listeriosis was registered in the Tula Oblast [8].

In the Russian Federation, animal listeriosis has been documented since 1956, while human listeriosis was officially recognized as a distinct nosological phenomenon and included in health records by the Russian Ministry of Health in 1992 [9, 10]. Pursuant to the State Report of the Rospotrebnadzor, 100 cases of listeriosis (18 deaths) were registered in the Rusian Federation in 2023. Most cases were reported: in Moscow (32 cases) and St. Petersburg (19 cases) [11].

A key feature of *Listeria* is its wide growth temperature range, i.e. from 4 to 45 °C (optimum 36–38 °C) and pH range of 5–11 [12]. It has been established that *L. monocytogenes* is killed when heated to 100 °C for 3–5 minutes or to 75–90 °C for 20 minutes [13]. *Listeria* demonstrates environmental persistence [14, 15], grows in high concentrations of sodium chloride and carbon dioxide, and can survive freezing and drying. They can survive in an oxygen-free environment and are capable of intracellular parasitism [16].

Listeria infection usually occurs when eating contaminated products of animal origin, including fish and seafood that have not been properly heat-treated, as well as vegetables and fruit [17]. A large number of listeriosis cases (15–20%) are associated with consumption of contaminated meat from domestic animals and poultry (15–80%) [18]. In Ethiopia, according to X. Wei et al., *L. monocytogenes* was detected in raw and pasteurized milk [19]. As the US Centers for Disease Control and Prevention (CDC) reports, there was a listeriosis human case in 2022 caused by cheese produced by Old Europe Cheese, Inc. In 2022, Big Olaf ice cream was the reason behind listeriosis outbreaks in the States of Florida and Ohio. In 2023, CDC reported listeriosis outbreaks associated with leafy greens, as well as peaches, nectarines,

and plums [20]. In 2024, in multiple U. S. States, deli meats and liver sausage products under the Boar's Head brand were contaminated with *Listeria*, resulting in human disease and death cases [21, 22]. According to several studies, the infection may develop even at relatively low bacterial concentrations in food products (10^2 CFU/g) [23].

According to Food Safety News, in 2022 Federal Office of Public Health and Federal Office for Food Safety and Veterinary Affairs of Switzerland reported a listeriosis outbreak associated with smoked trout [24].

Thus, detection of pathogenic *Listeria* in the products of animal origin, food raw materials, and ready-to-eat products remains a critical task.

The objective of this research is to detect *L. monocytogenes* contamination in products of animal origin (meat, fish, dairy) manufactured and marketed in the Nizhny Novgorod Oblast from 2023 to 2024.

MATERIALS AND METHODS

Samples of animal products, including food raw materials and ready-to-eat food products were submitted to the testing laboratory of the Nizhny Novgorod Branch of Federal Centre for Animal Health for the required tests.

Test materials. Totally, 3,650 samples from livestock and aquaculture were analyzed. The samples were collected between 2023 and 2024 from commercial poultry farms and retail chains in the Nizhny Novgorod Oblast of the Russian Federation.

Sampling. Product samples were collected at different storage time points (within the shelf-life period) in accordance with established sampling requirements for microbiological testing. They were delivered to the laboratory in a cooler bag. The delivery time did not exceed an hour.

Culture media: Fraser broth (State Research Center for Applied Biotechnology and Microbiology, Russia); ALOA – Agar *Listeria* according to Ottaviani and Agosti (Merck, Germany); *Listeria* Identification Agar Base, PALCAM agar (HiMedia Laboratories Pvt Ltd., India); blood agar (Sredoff, Russia).

Methods. The samples were analyzed and the pure microbial culture was identified based on a set of morphological and biochemical characteristics confirming they belong to *L. monocytogenes*, in accordance with GOST 32031-2022 "Food products. Methods for detection of *Listeria monocytogenes* and other *Listeria (Listeria* spp.)"[1].

Preparations from pure microbial cultures were fixed, Gram-stained, examined microscopically, and identified based on their ability to grow at 25 °C, *β*-hemolysis, catalase production, Voges – Proskauer test, fermentation of xylose and rhamnose, and lecithinase activity. API *Listeria* identification system (bioMérieux, France), which includes 10 biochemical tests, was used for identification.

Automatic miniVidas analyzer and a Vidas *Listeria* test kit (bioMérieux, France) were used to detect *L. monocytogenes* in food samples.

[1] https://docs.cntd.ru/document/1200193714

For statistical data processing and graph construction, Microsoft Excel and standard statistical data analysis methods were used.

RESULTS AND DISCUSSION

Between 2023 and 2024, the testing laboratory of the Nizhny Novgorod Branch of Federal Centre for Animal Health tested 3,650 samples of animal-derived products for *L. monocytogenes,* i.e. 680 samples of dairy products, 615 samples of fishery products, and samples of 2,355 meat products. Non-heat treated meat products included 323 beef samples, 834 poultry meat samples, 326 combined semi-finished meat products, and 288 pork samples. Moreover 584 samples of finished meat products were also tested, including 187 samples of poultry meat and 397 samples of ready-to-eat meat products, except for poultry meat products. Ready-to-eat meat products included jellied products, sausages, pâtés, as well as heat-treated meat and meat-based convenience products, excluding sausages.

The conducted tests revealed that the bacterial isolates exhibited cell morphology characteristic of *Listeria*, stained Gram-positive, were catalase-positive, were Voges – Proskauer positive, motile at (25 ± 1) °C, fermented rhamnose but did not ferment xylose, showed lecithinase activity on charcoal agar, and formed a zone of *β*-hemolysis on blood agar. Thus, identification of pure bacterial cultures isolated from the contaminated samples of the tested products demonstrated that all cultures belonged to *L. monocytogenes* species.

The test results for animal products produced and marketed in the Nizhny Novgorod Oblast from 2023 to 2024 are given in Table 1.

In 2023, tests revealed 41 *L. monocytogenes*-contaminated samples, which accounted for 2.1% of all the tested samples (N = 1,970). The proportion of *Listeria* detected in frozen and chilled products did not differ statistically and amounted to 1.1 and 1.0%, respectively.

Table 1
Detection of *L. monocytogenes* in products of animal origin in the Nizhny Novgorod Oblast (from 2023 to 2024)

Food product	% of positive samples	
	2023	2024
Meat products requiring heat treatment, including:	4.0 (N = 910)	1.6 (N = 861)
beef products	5.1 (N = 175)	2.0 (N = 148)
poultry meat products	3.8 (N = 424)	1.7 (N = 410)
pork products	0 (N = 135)	0.6 (N = 153)
combined semi-finished meat products	6.3 (N = 176)	2.0 (N = 150)
Dairy products	0 (N = 316)	0 (N = 364)
Fish and fishery products	1.0 (N = 479)	1.5 (N = 136)
Ready-to-eat meat products	0 (N = 265)	0 (N = 319)
Total	2.1 (N = 1,970)	1.0 (N = 1,680)

N – number of samples tested.

Testing of food products produced in 2024 revealed 16 *L. monocytogenes*-contaminated samples, which accounted for 1.0% of all the samples tested (*N* = 1,680). In frozen and chilled products, this type of *Listeria* was detected in 0.4 and 0.6% of cases, respectively.

Figure 1 shows results for food samples tested in 2023. The maximum detection rate of *Listeria* was recorded in combined semi-finished meat products – 6.3%, beef products – 5.1% and poultry meat – 3.8%. The bacteria rate detected in fishery products was 1.0% of the total number of the tested samples in this category.

Figure 2 shows results for products of animal origin tested in 2024. It was established, that 2.0% of positive samples accounted for combined semi-finished meat products and beef products. *Listeria* detections in fishery products was 1.5% of the total number of such products tested; in poultry products *Listeria* was detected in 1.7%; the lowest percentage of detections was noted in pork products – 0.6%.

No *L. monocytogenes* were detected in dairy products and ready-to-eat meat products that we tested in 2023 and 2024. In 2023, no pathogenic microorganisms were detected in pork products either.

Table 2 gives data on *Listeria* detected in poultry meat products produced from 2023 to 2024. The maximum *L. monocytogenes* detection rate was reported in poultry products (*N* = 834), i.e. in chopped semi-finished poultry meat products (including minced meat) – 1.2%, and the minimum detection rate was reported in mechanically deboned poultry meat – 0.4%.

When testing poultry products, namely poultry meat (carcasses, half-carcasses, wing, leg, thigh), *L. monocytogenes* were detected in 1.6% of the tested samples of this product category (total number *N* = 385), and in frozen and chilled products the detection number was equal – 0.8% for each category.

Listeria contamination in the tested chopped semi-finished poultry meat products, such as cutlets, kupati (i.e. spicy Georgian sausages), minced meat, etc., amounted to 4.2% of the tested samples belonging to this product group (*N* = 236), while 0.4% of detections were reported in frozen products and 3.8% of detections were reported in chilled products.

Listeria was recorded in 9.3% of samples of dough-wrapped semi-finished poultry meat products (frozen dumplings, *N* = 43), and in 7.1% of tested samples of mechanically deboned poultry meat (only in frozen products, *N* = 42).

No *L. monocytogenes* were detected in samples of poultry offal (heart, stomach, liver, raw fat), skin, uncooked convenience semi-finished poultry meat products, and cut-style poultry meat semi-finished products (including the ones soaked in marinade).

Percentage of contaminated samples from all the beef products tested in 2023–2024 (*N* = 323) (by product type): large-cut semi-finished products – 1.9% (0.6% – frozen; 1.2% – chilled products), minced beef – 0.9% (chilled products), offal, small-cut semi-finished products and semi-finished products wrapped in dough – 0.3% each. No *L. monocytogenes* were detected in chopped semi-finished meat products (cutlets, meatballs and etc.). The relevant tests results are given in Table 3.

From 2023 to 2024 *L. monocytogenes* was found in the following types of combined semi-finished meat products (*N* = 326): in chopped semi-finished products – 2.76% (1.23% – in frozen and 1.53% – in chilled products), in minced meat (chilled only) –

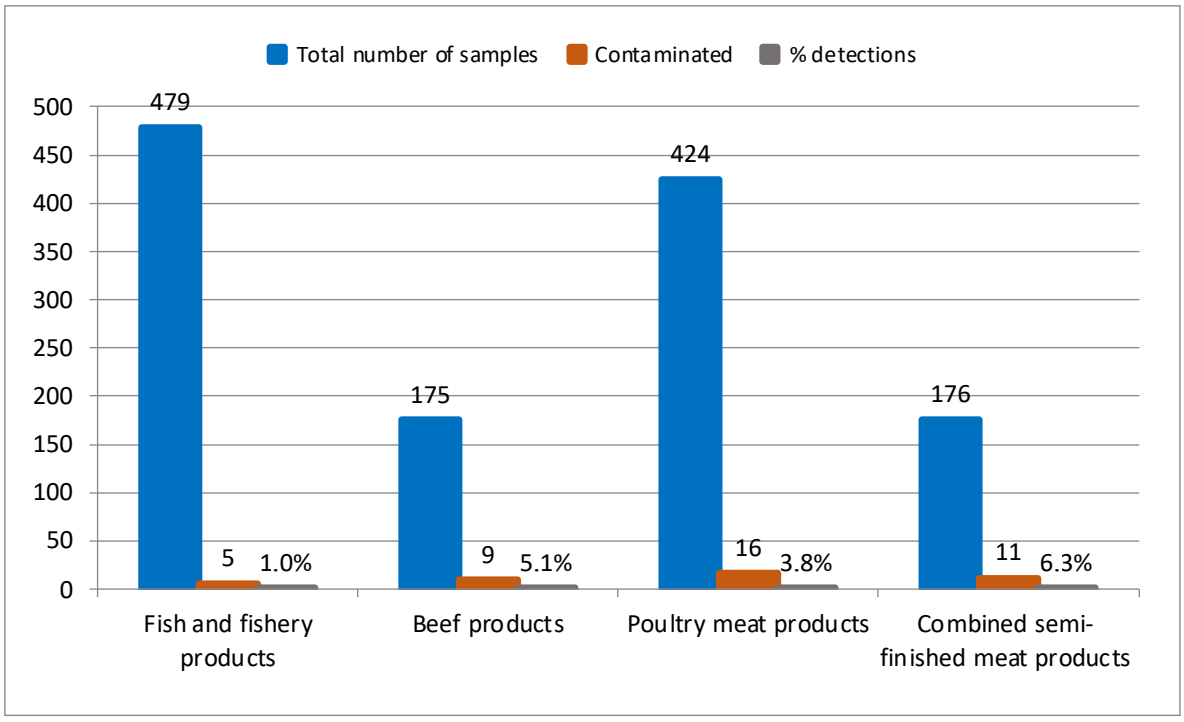

Fig. 1. Detection of L. monocytogenes in products of animal origin in the Nizhny Novgorod Oblast in 2023

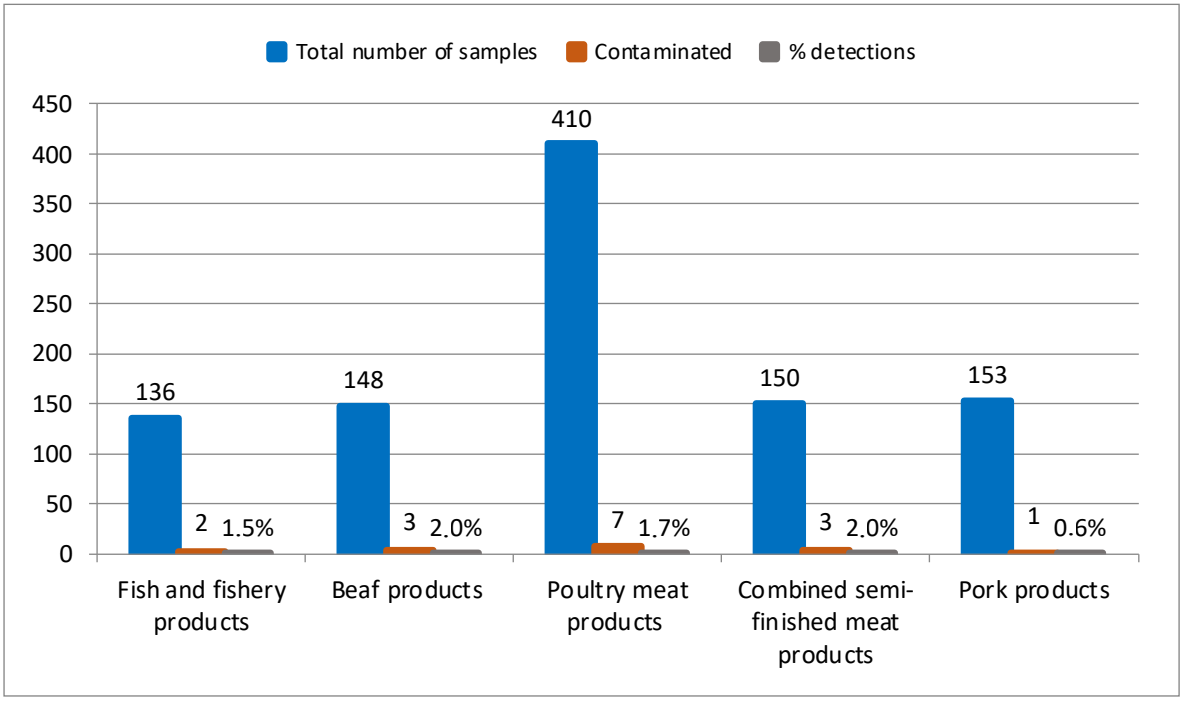

Fig. 2. Detection of L. monocytogenes in products of animal origin in the Nizhny Novgorod Oblast in 2024

in 0.6% of samples, in dough-wrapped semi-finished products – 0.9% (in frozen).

Listeria contamination was also detected in fishery products, namely in freshly frozen shrimps and semi-finished shrimp products containing a flour component (breaded cutlets).

Contamination with *L. monocytogenes* was found in one of the samples of frozen minced pork, which accounted for 0.3% of the total number of pork products samples ($N = 288$). The remaining pork processed products (large and small-cut semi-finished products, chopped products, products wrapped in dough, offal) met safety requirements and did not contain *Listeria*.

The work conducted between 2023 and 2024 resulted in detection of 57 *L. monocytogenes* contaminated samples, which accounted for 1.6% of the tested animal products. The percentage of *Listeria* detected in frozen and chilled meat products did not differ statistically – 0.7 and 0.8% of the total number of the samples tested, respectively. *Listeria* contamination in various food products ranged from 0.6% (samples of pork products) to 6.3% (samples of combined semi-finished meat products). *L. monocytogenes* detection in fishery products accounted for 1.1% of the total number of tested samples of these products ($N = 615$), in pork products – 0.3%.

Table 2
Contamination of poultry products with *L. monocytogenes*

Types of products	Number of samples	Contaminated	*Listeria* detected (%) in this type of products	*Listeria* detected (%) in the total number of poultry products
Poultry meat (carcasses, parts of carcasses)	385	6	1.6	0.7
Chopped semi-finished poultry meat products (including minced meat)	236	10	4.2	1.2
Semi-finished poultry meat products wrapped in dough	43	4	9.3	0.5
Mechanically deboned poultry meat	42	3	7.1	0.4
Offal	74	0	0	0
Skin	11	0	0	0
Convenience semi-finished poultry meat products, partially cooked	19	0	0	0
Cut-style semi-finished poultry meat products (soaked in marinade)	24	0	0	0

Table 3
***L. monocytogenes* contamination of beef products**

Types of products	Number of samples	Contaminated	*Listeria* detected (%) in this type of products	*Listeria* detected (%) in the total number of beef products
Ground beef	28	3	10.7	0.9
Large-cut semi-finished products	130	6	4.6	1.9
Chopped semi-finished meat products	26	0	0	0
Offal	23	1	4.3	0.3
Small-cut semi-finished meat products	74	1	1.4	0.3
Dough-wrapped semi-finished products	42	1	2.4	0.3

The results we obtained align with the published scientific data from other researchers. Thus, according to the results of food monitoring in 14 EU countries, the total number of *L. monocytogenes* detections in beef products intended for human consumption in 2019 was 4.2%, in 2020 the number increased to 7.4%, and in 2021, decreased to 3.9% [4]. According to the foreign data, in 2023, number of samples contaminated with *L. monocytogenes* in category "fish" was 1.1% [3], which correlates with the results of our tests.

As foreign sources report, *L. monocytogenes* has been detected in milk, different types of cheese, butter, cream, and ice cream [4, 20, 25, 26], as well as in ready-to-eat meat products [21, 22, 27]. Absence of *L. monocytogenes* in ready-to-eat meat and dairy products, as confirmed in tests conducted in the Nizhny Novgorod Branch of Federal Centre for Animal Health, may suggest that the establishments where these food products have been produced comply with technological and sanitary standards, the products are properly heat-treated, production hygiene is maintained, quality control of raw materials is well-organized, an effective quality control system is in place, product storage and transportation of conditions meet relevant regulatory requirements. However, regardless of the good current results, continuous monitoring of products shall be in place.

The data obtained indicate that contamination of meat products with *L. monocytogenes* does not depend on their storage temperature (frozen or chilled). This confirms the cold stress adaptation mechanisms of this pathogen described by foreign researchers [28, 29, 30]

CONCLUSION

Tests of products of animal origin manufactured and sold in the Nizhny Novgorod Oblast conducted in 2023–2024 show that 1.6% of samples were *L. monocytogenes*-contaminated. The highest level of contamination was found in combined semi-finished meat products (6.3% in 2023 and 2.0% in 2024), as well as in beef products (5.1 and 2.0%, respectively) and poultry meat (3.8 and 1.7%). At the same time, no *L. monocytogenes* was detected in dairy products and ready-to-eat meat products, which may indicate compliance of production with technological and sanitary standards.

As for poultry meat products, the highest level of contamination was detected in semi-finished poultry meat products wrapped in dough (9.3%), in mechanically deboned poultry meat samples (7.1%) and chopped products (4.2%), while in whole poultry carcasses the level was lower (1.6%). Among beef products were minced beef (10.7%) and large-cut semi-finished beef products (4.6%). As for fishery products, *L. monocytogenes* was found in 1.1% (in shrimps and fish cutlets).

Storage temperature (refrigeration/freezing) did not have a significant impact on the level of *L. monocytogenes* contamination, indicating the cold tolerance of this pathogenic microorganism.

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