

**DETERMINATION OF THE SECURITY CRITERION
IN THE CONTENT OF CONVENTIONAL ANIMALS
BY THE IMPROVED HORIZONTAL METHOD
OF *LISTERIA MONOCYTOGENES* DETECTION**

N. M. Bogatko
nadiyabogatko@ukr.net

National Agrarian University,
10/1 Soborna sq., Bila Tserkva 09117, Ukraine, rectorat@btsau.net.ua

*It is particularly important for market operators — such as producers of meat from slaughtered animals, wholesale bases, agroindustrial markets, supermarkets, etc. — to determine one of the safety criteria, the presence of *Listeria monocytogenes* during the production of meat raw materials, its transportation, storage and marketing. The absence of this pathogenic microorganism indicates the proper sanitary and hygienic status of the business entities, the observance of personal hygiene for the production and circulation of beef, pork, mutton, goat meat. The conducted researches have revealed that the colonies of *Listeria monocytogenes* were detected in 24±2 hours and were small in size (1.5–2.0 mm), gray-green or olive-green, sometimes with black halo; in 46±2 hours — green with a flask center and black halo. These colonies were detected in the following samples of slaughter meat: in 2 samples of beef, 3 samples of pork and 1 sample of goat meat sold in the agroindustrial markets, in 2 samples of pork and 1 sample of beef sold in supermarkets. In beef, pork, mutton during production at facilities and storage at wholesale bases, as well as in mutton and goat meat realized on the agromarkets and in supermarkets, the characteristic colonies of *Listeria monocytogenes* were not detected. The improved horizontal detection method of *Listeria monocytogenes* is economical in terms of using nutrient media, it is simple to implement, and its results provide specific qualitative indices of the color and size of *Listeria monocytogenes* colonies, this method can be used in combination with other methods for determining the safety of slaughter animals. The reliability of the developed improved horizontal method for detecting *Listeria monocytogenes* in slaughtered animals was 99.8 %. The improved horizontal method for detecting *Listeria monocytogenes* in slaughter meat can be applied throughout the food chain for defining safety criteria for the production of safe meat, its transportation, storage and sale in production laboratories of meat processing plants, and enterprises for the implementation and storage of meat of slaughtered animals (in shops, supermarkets, wholesale bases, refrigerators, etc.), as well as in state laboratories of veterinary medicine and their departments.*

Keywords: MICROBIOLOGICAL CRITERION, SAFETY, SLAUGHTERED ANIMALS' MEAT, BEEF, PORK, MUTTON, GOAT MEAT, *LISTERIA MONOCYTOGENES*

**ВИЗНАЧЕННЯ КРИТЕРІЮ БЕЗПЕЧНОСТІ У М'ЯСІ ЗАБІЙНИХ ТВАРИН
ЗА УДОСКОНАЛЕНИМ ГОРИЗОНТАЛЬНИМ МЕТОДОМ
ВИЯВЛЕННЯ *LISTERIA MONOCYTOGENES***

Н. М. Богатко
nadiyabogatko@ukr.net

Національний аграрний університет,
пл. Соборна, 10/1, м. Біла Церква, 09117, Україна, bnau-rectorat@ukr.net

*Особливо важливо операторам ринку — потужностям з виробництва м'яса забійних тварин, оптовим базам, агропромисловим ринкам, супермаркетам тощо — за виробництва м'ясної сировини, її транспортування, зберігання та реалізації визначати один із критеріїв безпечності — наявність *Listeria monocytogenes*. Відсутність цього патогенного мікроорганізму свідчить про належний санітарно-гігієнічний стан на потужностях, дотримання особистої гігієни за виробництва та обігу яловичини, свинини, баранини, козятини. Проведеними дослідженнями встановлено, що колонії *Listeria monocytogenes* були виявлені через 24±2 год — маленьких розмірів, 1,5–2,0 мм, сіро-зеленого чи оливково-зеленого кольору, інколи з чорним ореолом; через 46±2 год — зеленого кольору із запалим центром та чорним ореолом. Колонії виявили у таких пробах м'яса забійних тварин: двох пробах яловичини, трьох пробах свинини*

та одній пробі козятини, які реалізувалися на агропромислових ринках, а також у двох пробах свинини і одній пробі баранини, які реалізувалися у супермаркетах. В яловичині, свинині, баранині за виробництва на потужностях і зберігання на оптових базах, а також у баранині та козятині, які реалізувалися на агропромислових ринках і в супермаркетах, характерних колоній *Listeria monocytogenes* не було виявлено. Розроблений удосконалений горизонтальний метод виявлення *Listeria monocytogenes* є економним щодо використання поживних середовищ, простим у виконанні, його результати дають конкретні якісні показники із забарвлення та розміру колоній *Listeria monocytogenes*, може використовуватися в комплексі з іншими методами визначення безпечності м'яса забійних тварин. Вірогідність розробленого й удосконаленого горизонтального методу виявлення *Listeria monocytogenes* у м'ясі забійних тварин становила 99,8 %. Удосконалений горизонтальний метод визначення *Listeria monocytogenes* у м'ясі забійних тварин можна застосовувати на всьому харчовому ланцюзі під час виробництва безпечної м'ясної сировини, її транспортування, зберігання та реалізації у виробничих лабораторіях м'ясопереробних підприємств та підприємств з реалізації і зберігання м'яса забійних тварин (магазинах, супермаркетах, оптових базах, холодильниках тощо), а також у державних лабораторіях ветеринарної медицини та їх відділах.

Ключові слова: МІКРОБІОЛОГІЧНИЙ КРИТЕРІЙ, БЕЗПЕЧНІСТЬ, М'ЯСО ЗАБІЙНИХ ТВАРИН, ЯЛОВИЧИНА, СВИНИНА, БАРАНИНА, КОЗЛЯТИНА, *LISTERIA MONOCYTOGENES*

ОПРЕДЕЛЕНИЕ КРИТЕРИЯ БЕЗОПАСНОСТИ В МЯСЕ УБОЙНЫХ ЖИВОТНЫХ ПРИ УСОВЕРШЕНСТВОВАНИИ ГОРИЗОНТАЛЬНОГО МЕТОДА ОПРЕДЕЛЕНИЯ *LISTERIA MONOCYTOGENES*

Н. М. Богатко
nadiyabogatko@ukr.net

Национальный аграрный университет,
Соборная площадь, 10/1, г. Белая Церковь, 09117, Украина, bnau-rectorat@ukr.net

Особенно важно операторам рынка — мощностям по производству мяса убойных животных, оптовым базам, агропромышленным рынкам, супермаркетам — при производстве мясного сырья, транспортировке, хранении и реализации определять один из критериев безопасности — наличие *Listeria monocytogenes*. Отсутствие этого патогенного микроорганизма свидетельствует о надлежащем санитарно-гигиеническом состоянии на предприятиях, соблюдении личной гигиены при производстве и реализации говядины, свинины, баранины, козятины. Проведенными исследованиями колонии *Listeria monocytogenes*, обнаружены через 24 ± 2 часа, были маленьких размеров 1,5–2,0 мм серо-зеленого или оливково-зеленого цвета, иногда с черным ореолом; через 46 ± 2 часа — зеленого цвета с впавшим центром и черным ореолом. Колонии были обнаружены в следующих пробах мяса убойных животных: двух пробах говядины, трех пробах свинины и одной пробе козятины, которые реализовались на агропромышленных рынках, в двух пробах свинины и одной пробе баранины, которые реализовались в супермаркетах. В говядине, свинине, баранине при производстве на мощностях и хранении на оптовых базах, а также в баранине и козятине, которые были реализованы на агропромышленных рынках и в супермаркетах, не было обнаружено характерных колоний *Listeria monocytogenes*. Разработанный усовершенствованный горизонтальный метод выявления *Listeria monocytogenes* является экономным по использованию питательных сред, простым в исполнении, а его результаты дают конкретные качественные показатели по окраске и размерам колоний *Listeria monocytogenes*, он может использоваться в комплексе с другими методами определения безопасности мяса убойных животных. Достоверность разработанного и усовершенствованного горизонтального метода выявления *Listeria monocytogenes* в мясе убойных животных составляла 99,8 %. Усовершенствованный горизонтальный метод определения *Listeria monocytogenes* в мясе убойных животных можно применять на всей пищевой цепи при производстве безопасного мясного сырья, его транспортировке, хранении и реализации в производственных лабораториях на мясоперерабатывающих предприятиях и предприятиях по реализации и хранению мяса убойных животных (магазинах, супермаркетах, оптовых базах, холодильниках и т.п.), а также в государственных лабораториях ветеринарной медицины и их отделах.

Ключевые слова: МИКРОБІОЛОГІЧЕСКИЙ КРИТЕРІЙ, БЕЗОПАСНОСТЬ, МЯСО УБОЙНЫХ ЖИВОТНЫХ, ГОВЯДИНА, СВИНИНА, БАРАНИНА, КОЗЛЯТИНА, *LISTERIA MONOCYTOGENES*

The Scientific Committee on Food Products (SCF) and the Scientific Committee on Veterinary Measures for Public Health (SCVPH) provided recommendations on the principles of the development of food microbiological criteria in 1996 and 1997. The main principles that are highlighted in the The Codex Alimentarius are based on the fact that the microbiological criteria will be appropriate [6, 9].

Commission Regulation No. 2073/2005 establishes that food business operators should develop a sample collection program and microbiological studies for this trial. The program must be a part of the implementation procedures, developed on the basis of proper hygienic practices and HACCP system principles. The frequency of sample collection should be based on the analysis of risks, should keep in scale with the food business's character and size and also should take into account other factors such as raw materials properties, finished product, manufacturing process, etc. [1, 4].

It is particularly important for market operators — producers of meat from slaughtered animals, wholesale bases, agroindustrial markets, supermarkets, etc. — to determine one of the safety criteria, the presence of *Listeria monocytogenes* for the production of meat raw materials, its transportation, storage and marketing. The absence of this pathogenic microorganism indicates the proper sanitary and hygienic status of the business entities, the observance of personal hygiene for the production and circulation of beef, pork, lamb, goat meat [5, 10, 11].

Materials and methods

As the materials for researches samples of muscle tissue (the longest muscle of the back) of beef, pork, lamb and goat meat were used in the amount of 230, which were selected on meat production facilities, wholesale bases during storage and agromarkets and supermarkets during realization in the Kyiv region. Bacteriological studies were conducted to detect and identify *Listeria monocytogenes* with the advanced horizontal method developed using selective enriched media such as Fresher and PALKAM-agar [3].

Results and discussion

The microorganism may be in raw food, such as fresh meat — beef, pork, mutton and goat meat. EU Member States are required to apply the microbiological criteria highlighted in Commission Regulation (EC) No 2073/2005. In this document, pathogens are directly related to the type of food product. There are specific requirements for the microbiological criteria for food safety, which can be used only in domestic marketing Ukraine. However, these criteria can not be used in terms of exporting the food products to the EU market. Therefore, the purpose of our research is to develop a bacteriological research method in meat (beef, pork, mutton and meat of goat).

The European Commission and the Council (EC) No. 852/2004 on the hygiene of foodstuffs, which includes the Community legislation on microbiological criteria; The principles of the development and application of the criteria and proposals for further measures were developed in the EU countries [2, 7].

The above microbiological criteria indicate the acceptability of food products and processes for their production. The use of proper hygienic practices (GHP) and the analysis of hazardous factors and critical control points (based on the HACCP system), and the application of the structured preventive approach that provides the proper and its production process. Represented in Regulations 852/2004 on the General Sanitary Regulations and Regulations No. 853/2004 on the approval of specific hygiene rules for foodstuffs of animal origin [12].

Widespread distribution and increase in comparison with most other microorganisms, the ability to grow or survive in a cooled environment makes *Listeria monocytogenes* a significant risk factor for the production of meat from slaughtered animals, when stored at wholesale bases, as well as for marketing in agro-markets and supermarkets. Therefore, we have developed an improved horizontal method for detecting *Listeria monocytogenes* in the meat of slaughter animals.

To develop an improved horizontal method for detecting *Listeria monocytogenes* in meat of slaughtered animals, an experimental suspension was used which was prepared in a ratio of 1:5 (sam-

ples of meat and meat products in the amount of 10–11 g and 50–55 cm³ of primary selective enriched medium — half broiler Freser), followed by incubation of the resulting suspension for 21–23 hours at a temperature of 31±1 °C and subsequent secondary enrichment: after initial enrichment, the resulting culture in the amount of 0.05–0.06 cm³ is transferred to a test tube containing 5–6 cm³ of secondary enriched medium (broiler broth), then incubate the medium with crops for 46–48 hours at 37 °C, and subsequently seed from primary (5–6 cm³) and secondary (2.5–3 cm³) enriched cultures on PALKAM-agar selective medium to obtain clearly separated *Listeria monocytogenes* colonies for 24±2 hours at 37±1 °C in the form of small gray-green or olive-green colonies, with a diameter of 1.5–2 mm, sometimes with a black halo, after 48 hours — in the form of green colonies with a diameter of 1.5–2.0 mm with a burning center and black they halo around. Individual colonies were selected for confirmation of the *Listeria* genus and transplanted into MPA with 1 % glucose and tryptone soy bean agar with yeast extract (TSVEA) and cultivated at 37 °C. for 24–48 hours. The corresponding morphologically cultural and biochem-

ical tests were identified and confirmed, namely: mannose fermentation, rhomniosis, β-hemolysis; hydrolysis of lecithin with coal. The results of the test of the developed advanced horizontal detection method for *Listeria monocytogenes* in the meat of slaughter animals are presented in table.

The conducted researches revealed that the colonies of *Listeria monocytogenes* detected in 24±2 hours were small in size (1.5–2.0 mm), gray-green or olive-green, sometimes with black halo; in 46±2 hours — green with a flask center and black halo in the following samples of slaughter meat: in 2 samples of beef, in 3 samples of pork and in 1 samples of goat meat sold in the agroindustrial markets and in 2 samples of pork and in 1 samples of beef sold in supermarkets. In the beef, pork, mutton for production at facilities and storage at wholesale bases, as well as in mutton and goat, realized on the agromarkets and in supermarkets, the characteristic colonies of *Listeria monocytogenes* were not found [8].

The improved horizontal detection method of *Listeria monocytogenes* is economical in terms of using nutrient media, simple to implement, and its results provide specific qualitative indices of the

Table

Indicators for the detection of *Listeria monocytogenes* in slaughtered animals according to the improved method (n=230)

No.	Kinds of meat of slaughtered animals	Identification of <i>Listeria monocytogenes</i> by color and size of colonies according to the improved method			
		Number of samples	Presence of <i>Listeria monocytogenes</i> colonies	Number of samples	Absence of <i>Listeria monocytogenes</i> colonies
<i>Facilities of meat production</i>					
1	Beef (n=26)	—	—	n=26	characteristic colonies of <i>Listeria monocytogenes</i> not found
2	Pork (n=28)	—	—	n=28	
<i>Wholesale bases</i>					
3	Beef (n=17)	—	—	n=17	characteristic colonies of <i>Listeria monocytogenes</i> not found
4	Pork (n=15)	—	—	n=15	
5	Mutton (n=12)	—	—	n=12	
<i>Agroindustrial markets</i>					
6	Beef (n=23)	n=2	in 24±2 hours. colonies are small, 1.5–2.0 mm, gray-green, sometimes with a black halo; after 46±2 hours	n=21	characteristic colonies of <i>Listeria monocytogenes</i> not found
7	Pork (n=19)	n=3	colonies are 1.5–2.0 mm, green	n=16	characteristic colonies of <i>Listeria monocytogenes</i> not found
8	Goat meat (n=11)	n=1	with a burning center and black halo	n=10	
9	Mutton (n=13)	—	—	n=13	
<i>Supermarkets</i>					
10	Beef (n=21)	—	—	n=21	characteristic colonies of <i>Listeria monocytogenes</i> not found
11	Pork (n=18)	n=2	in 24±2 hours. colonies are small, 1.5–2.0 mm, olive green; after 46±2 hours colonies are 1.5–	n=16	
12	Mutton (n=14)	n=1	2.0 mm, green with a burning center and black halo	n=13	
13	Goat meat (n=13)	—	—	n=13	

color and size of *Listeria monocytogenes* colonies and can be used in combination with other methods for determining the safety of slaughter animals.

Conclusion

The reliability of the developed improved horizontal method for detecting *Listeria monocytogenes* in slaughtered animals was 99.8 %. The improved horizontal method for detecting *Listeria monocytogenes* in slaughter meat can be applied throughout the food chain for defining safety criteria for the production of safe meat, its transportation, storage and sale in production laboratories of meat processing plants, and enterprises for the implementation and storage of meat of slaughtered animals (in shops, supermarkets, wholesale bases, refrigerators, etc.), as well as in state laboratories of veterinary medicine and their departments.

Perspectives of the future investigations.

In further scientific studies it is necessary to establish the content of the total number of microorganisms in the meat of slaughter animals for their treatment; also, to study qualitative indices of beef, pork, mutton, goat meat which *Listeria monocytogenes* was detected in; to establish a traceability system for inspection of the sanitary condition and the quality of disinfection of veterinary supervision facilities at meat production facilities for slaughtered animals, wholesale bases, agroindustrial markets, supermarkets.

1. Bogatko D. L., Bogatko N. M. Features of introduction of the HACCP system at meat processing enterprises of Ukraine. *Collection of scientific works of the Kharkiv State Veterinary Academy. Problems of zoinengineering and veterinary medicine*, Kharkiv, 2014, 28, part 2 "Veterinary science", pp. 49–55. (in Ukrainian)
2. Bogatko N. M. Application of new express methods of quality control of slaughter animals in their veterinary and sanitary assessment. *Visnyk of Sumy National Agrarian University: collection of scientific works, Series "Veterinary Medicine"*, Sumy, 2010, issue 3 (26), pp. 13–17. (in Ukrainian)
3. Bogatko N. M., Bukalova N. V., Bogatko A. F. A method for improving the horizontal detection method of *Listeria monocytogenes* in slaughtered animals, poultry and meat products. Patent 109383, 2016. (in Ukrainian)
4. Bogatko N. M., Bukalova N. V., Sakhnuk V. V., Jmil V. I. *Peculiarities of introduction of HACCP system at meat, milk and fish processing enterprises of Ukraine. A textbook*. Bila Tserkva, 2016, 283 p. (in Ukrainian)
5. Bogatko N. M., Sachnyuk N. I. Application of microbiological criteria in Ukraine for establishing the safety of food products. *Collection of scientific works of the Kharkiv State Veterinary Academy. Problems of zoinengineering and veterinary medicine*, Kharkiv, 2013, 26, part 2 "Veterinary science", pp. 254–259. (in Ukrainian)
6. Bogatko N. M., Salata V. Z., Semanuk V. I., Vlasenko V. V., Shakh V. V., Murza I. G. Food safety, food chain tracking, and the application of the Rapid Reaction System. *Scientific Messenger of Lviv National University of Veterinary Medicine and Biotechnology named after S. Z. Gzhytsky, Series "Food Technologies"*, Lviv, 2011, vol. 13, no. 2 (48), P. 2, pp. 322–329. (in Ukrainian)
7. Bogatko N. M., Semanuk V. I., Salata V. Z., Konstantinov P. D., Sachnyuk N. I., Bogatko L. M. Influence of sanitary and hygienic condition of objects of meat processing enterprise on indicators of safety of produced beef and pork. *Scientific Messenger of Lviv National University of Veterinary Medicine and Biotechnology named after S. Z. Gzhytsky, Series "Food Technologies", Series "Veterinary Science"*, Lviv, 2012, vol. 14, no. 2 (52), P. 3, pp. 13–20. (in Ukrainian)
8. Bogatko N. M., Shcherevich G. P., Bukalova N. V., Bogatko L. M., Salata V. Z., Serdyukov Ya. K. Application of microbiological criteria in food processing enterprises of Ukraine, adopted in the EU. *Scientific Messenger of Lviv National University of Veterinary Medicine and Biotechnology named after S. Z. Gzhytsky, Series "Veterinary Science"*, Lviv, 2016, vol. 18, no. 1 (65), pp. 172–176. (in Ukrainian)
9. Microbiology of food and animal feed. Horizontal method Liquid monocytogenes detection and calculation. Part 1. Detection method (ISO 11290-1:1996, ITD): DSTU ISO 11290-1:2003: National Standard of Ukraine, 2005.
10. Navratilova P., Schlegelova J., Sustackova A., Napravnikova E., Lukasova J., Klimova E. Prevalence of *Listeria monocytogenes* in milk, meat and foodstuff of animal origin and phenotype of antibiotic resistance of isolated strains. *Veterinarni Medicina*, 2004, vol. 49, issue 7, pp. 243–252. DOI: 10.17221/5701-VETMED. (in Ukrainian)
11. Oluwafemi R. A., Edugbo O. M., Solanke E. O., Akinyeye A. J. Meat quality, nutrition security and public health: a review of beef processing practices in Nigeria. *African Journal of Food Science and Technology*, 2013, vol. 4 (5), pp. 96–99. Available at: <https://www.interestjournals.org/articles/meat-quality-nutrition-security-and-public-health-are-view-of-beef-processing-practices-in-nigeria.pdf>
12. Regulation (EC) No. 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety.