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ANALYSIS OF THE RESULTS OF MONITORING STUDIES OF MUSHROOM AND BERRY SAMPLES FOR NOROVIRUS AND HEPATITIS A VIRUS BY REAL-TIME PCR METHOD FOR 2022-2024

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Abstract*. Viruses are one of the main causes of outbreaks of foodborne infections, which are accompanied by symptoms characteristic of most gastrointestinal diseases. An important direction in the prevention of norovirus infection and hepatitis A is monitoring the circulation of pathogens on an ongoing basis. The main goal of state monitoring is to prevent the consumer from consuming food products that may be contaminated with norovirus and hepatitis A virus pathogens, which pose a potential risk to human health. We conducted research on the detection of norovirus and hepatitis A virus in plant products in accordance with the Plans for State Monitoring of Food Products of Plant Origin in 2022 - 2024. The purpose of the work is to conduct molecular genetic studies of mushrooms and berries (blueberries, raspberries, bilberries) grown in Ukraine using the real-time polymerase chain reaction (Real-time PCR) method and analyze the results of studies on the presence of Norovirus and Hepatitis A virus RNA. Materials and methods. The studies were conducted in the research department of biochemical and molecular studies of food products, feed and water of the State Scientific and Research Institute for Laboratory Diagnostics and Veterinary and Sanitary Expertise (SSRILDVSE) using the real-time PCR method in accordance with ISO/TS 15216-2 Microbiology of food and animal feed – Horizontal method for determination of hepatitis A virus and norovirus in food using the real-time PCR – Part 2 and Regulation EC 178/2002 (articles 14, 31 Regulation EC №178/2002). Research results. According to the State Monitoring Plans for the detection of norovirus and hepatitis A virus, 465 samples of berries and mushrooms from different regions of Ukraine were examined in the SSRILDVSE. During the monitoring studies using the real-time PCR method, no case of contamination of food products of plant origin with pathogens of norovirus and hepatitis A virus was detected. Conclusions. According to the results of monitoring studies, no RNA of norovirus and hepatitis A wasn't detected in mushroom and berry samples.*

Keywords: norovirus, hepatitis A, viruses, real-time PCR, food pathogens, molecular genetic diagnostic methods

Introduction. Viruses are one of the causes of foodborne infections, the frequency of which has increased significantly in recent decades due to the rapid globalization of the agri-food market (Scrotska, 2014, Kalinina, 2021). They retain their infectious properties on various surfaces for a long time, remaining viable for several days on the skin, clothing and various objects, resistant to the action of disinfectants in usual concentrations (Efimochkina, 2017; Gaythorpe, 2018). The most common and highly contagious include pathogens of viral infections belonging to the *Picornaviridae* and *Caliciviridae* families (noro-, rota-, hepato-, enteroviruses, etc.) (De Wit, 2001). The main feature of viral diseases is their high contagiousness, digestive disorders, accompanied by intestinal spasms and diarrhea (norovirus) and liver damage (hepatitis A virus). The main mechanism of transmission of pathogens is fecal-oral. Routes of infection: contact-household from person to person (dominant), when consuming contaminated food and water. In 14% of cases, pathogens are transmitted through food (Haidei, 2021, Kalinina, 2021).

In July, 2013, the Codex Alimentarius Commission approved new standards for fruits, vegetables, fish and fish products, and animal feed to protect the health of consumers worldwide, which contain recommendations for producers and consumers to prevent infection and reduce the risk of spreading disease pathogens (Codex Alimentarius). Fresh berries and vegetables are part of the human diet, but they can cause foodborne diseases caused by viruses, in particular Norwalk and hepatitis A virus (Le Guader, 2004).

According to the Rapid Alert system for Food and Feed (RASFF), in 2019, 17 reports of cases of detection of noroviral infection agent – *Norwalk virus* in food products were received from EU countries (Haidei, 2021).

According to the State Statistics Service, Ukraine is one of the largest exporters of fresh and frozen berries to EU countries. One of the most common reasons for rejecting imported berries (fresh and frozen) is their possible contamination with dangerous pathogens: salmonella, E. coli, listeria, and viruses such as norovirus and hepatitis A virus. Given the large number of RASFF notifications regarding cases of norovirus and hepatitis A virus detection in berries and oysters in EU countries, and guided by the Law of Ukraine «On State Control over Compliance with Legislation on Food Products, Feed, Animal By-products, Animal Health and Welfare», in order to verify the compliance of food products, the Service of Ukraine for Food Safety and Consumer Protection approved the «Plan for State Monitoring of Food Products of Plant Origin» for laboratory studies of microbiological criteria: norovirus and hepatitis A virus in fresh mushrooms and berries in 2022, 2023, and 2024.

Outbreaks of infection often occur as a result of the use of infected water, its use in irrigation systems for watering plantations, consumption of infected food products that are contaminated through contact with personnel of the enterprise where the products are processed and packaged, and imperfection of the technology for decontamination of products that are subject to freezing in fresh form (Rodríguez-Lázaro, 2012; L'vov, 2013; Verhoef, 2013; Efimochkina, 2017, Maunula, 2009, Bozkurt, 2021).

The main method for detecting cases of contamination by food pathogens is the real-time PCR method, which is based on working with nucleic acids, is characterized by high sensitivity, specificity and informativeness, which allows detecting and identifying viral RNA in fresh products and those that have undergone technological processing.

The aim of study: Conducting monitoring studies of mushrooms and berries (blueberries, raspberries, bilberries) using the real-time PCR method and analyzing the results of studies for 2022-2024 on the detection of RNA of *Norovirus* and *Hepatitis A virus*.

Materials and methods. The studies were conducted on the basis of the Research Department of Biochemical and Molecular Research of Food, Feed and Water of the SSRILDVSE by the real-time PCR method in accordance with ISO/TS 15216-2 Microbiology of food and animal feed – Horizontal method for determination of hepatitis A virus and norovirus in food using the real-time PCR – Part 2 and Regulation EC 178/2002 (articles 14, 31 Regulation EC №178/2002). For RNA extraction, the SureFast ® PREP DNA/RNA Virus kit, Congen (Germany) was used. PCR the real-time was performed on an Applied Biosystems QuantStudio5 amplifier using the SureFast Norovirus/Hepatitis A 3Plex test system, Congen (Germany). The main stages of the research: sample preparation of the studied samples, RNA extraction, preparation of the PCR mixture, amplification and interpretation of the research results.

Accounting for the amplification results was carried out using the Applied Biosystems QuantStudio5 amplifier software.

Results. According to the Plans for State Monitoring of Food Products of Plant Origin for 2022, 2023 and 2024 for the detection of norovirus and hepatitis A virus, 465 samples of berries and mushrooms from different regions of Ukraine were examined by the the real-time PCR method in the Scientific and Research Department of Biochemical and Molecular Research of Food Products, Feed and Water of the SSRILDVSE. In 2022 - 180 samples, of which 32 - mushrooms, 148 - berries (30 - blueberries, 74 - raspberries, 44 - bilberries). In 2023 - 151 samples, of which 25 - mushrooms, 126 - berries (25 - blueberries, 66 - raspberries, 35 - bilberries). In 2024 - 134 samples, of which 21 - mushrooms, 113 - berries (21 - blueberries, 58 - raspberries, 34 - bilberries). Figures 1, 2 and 3 show the number of samples tested in percentages by product type.

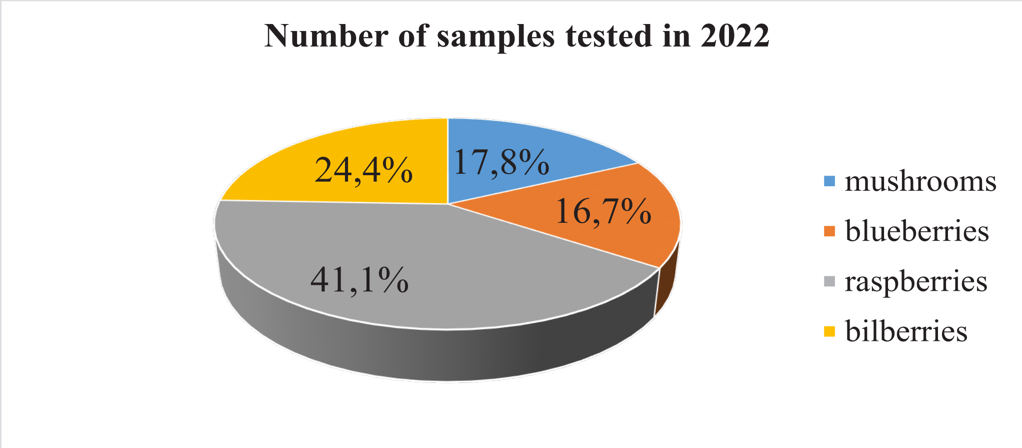

Fig. 1. Number of monitoring studies of mushrooms and berries for *Norovirus* and *Hepatitis A virus* in 2022

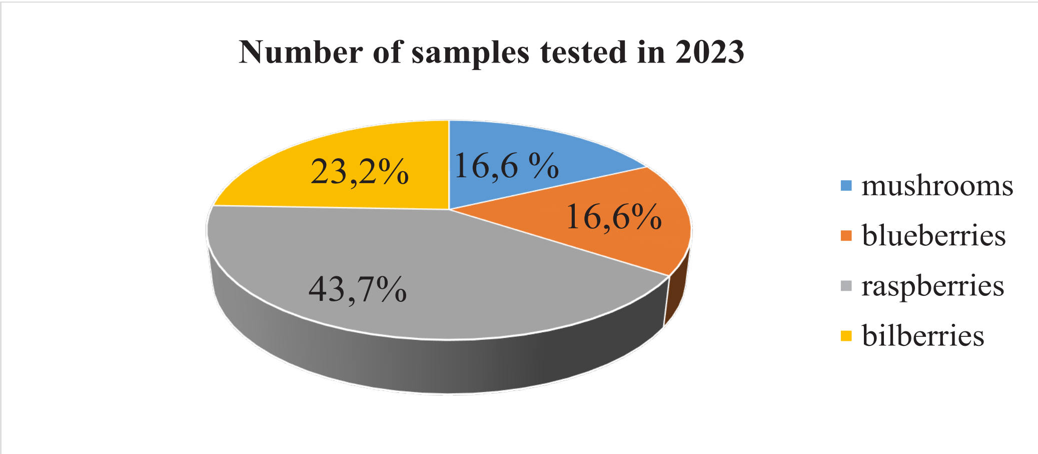

Fig. 2. Number of monitoring studies of mushrooms and berries for *Norovirus* and *Hepatitis A virus* in 2023

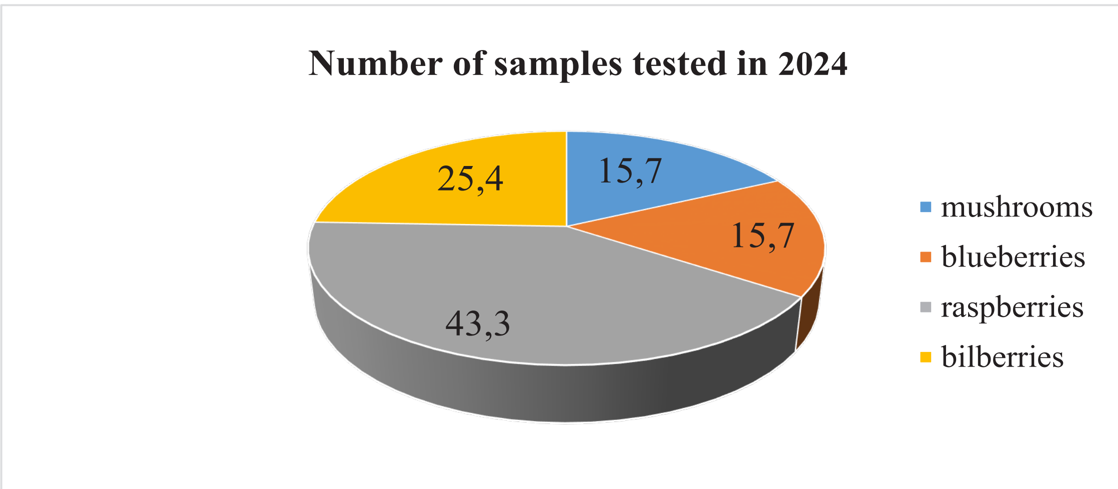

Fig. 3. Number of monitoring studies of mushrooms and berries for *Norovirus* and *Hepatitis A virus* in 2024

Analyzing the results of monitoring studies of plant products grown in Ukraine, PCR the real-time method did not reveal a single case of contamination of mushrooms and berries with food pathogens: *Norovirus* and *Hepatitis A virus*.

Discussion. *Norovirus* and *Hepatitis A virus* are the most common pathogens that are a frequent cause of outbreaks of acute gastroenteritis and infectious hepatitis (Fraisse, 2017, Cook, 2019). The Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) Expert Committee published a report recognizing viruses as a major cause of foodborne illness, and classified berries as the second priority microbiological hazard group (FAO) among fresh fruits and vegetables. These viruses are persistent in the environment and can enter food through contamination with wastewater, water used for irrigation during outdoor and indoor growing, or by food handlers during harvesting and packaging, as well as through contaminated equipment. (Verhoef, 2013, Polkowska, 2021, Li, 2018, Miotti, 2024).

In Europe, 4.4% of zoonotic outbreaks are directly linked to food of non-animal origin. According to EFSA, berries rank fourth and sixth among human diseases caused by norovirus, as berries are consumed fresh, with little or no prior processing, and therefore pose a threat (EFSA, 2014). In September-October 2012, an outbreak of norovirus gastroenteritis was recorded in Germany, which affected 11,000 people (Made, 2013). Epidemiological studies have shown that frozen strawberries and raspberries are the cause of infection. By 2018, fresh and frozen berries were responsible for 94 outbreaks, resulting in 23,433 cases worldwide. (Bozkurt, 2021, Raymond, 2022, Steele, 2022). According to data (Cook, 2018), in England, a study of 310 fresh and 274 frozen raspberry samples revealed a positive result for norovirus RNA in seven fresh raspberry samples (2.3%) and 10 frozen raspberry samples (3.6%). In China, a study of 900 samples of frozen and 900 samples of fresh berries in 2016-2017 found noroviral RNA in 81 samples of frozen berries (9.0%) and 109 samples of fresh berries (12.1%) (Gao, 2019).

Conclusions. According to the results of the research, no RNA of *Norovirus* and *Hepatitis A virus* was detected in the samples of berries and mushrooms. The prospect of our further research is to continue the implementation of the State Monitoring Plan for Plant Products for the presence of *Norovirus* and *Hepatitis A virus* on an ongoing basis in order to prevent contamination of food products with pathogens in the territory of Ukraine.

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АНАЛІЗ РЕЗУЛЬТАТІВ МОНІТОРИНГОВИХ ДОСЛІДЖЕНЬ ЗРАЗКІВ ГРИБІВ ТА ЯГІД ЩОДО НОРОВІРУСУ ТА ВІРУСУ ГЕПАТИТУ А МЕТОДОМ ПЛР-РЧ ЗА 2022 - 2024 РОКИ

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Резюме. *Віруси є однією з основних причиною спалахів харчових інфекцій, які супроводжуються симптомами, характерними для більшості захворювань шлунково-кишкового тракту. Важливим напрямком профілактики норовірусної інфекції та гепатиту А є моніторинг циркуляції збудників на постійній основі. Основною метою державного моніторингу є недопущення до споживача харчових продуктів, які можуть бути контаміновані збудниками норовірусу та вірусу гепатиту А, що становлять потенційний ризик для здоров'я людей. Нами проведено дослідження щодо виявлення норовірусу та вірусу гепатиту А в рослинній продукції згідно Планів державного моніторингу харчових продуктів рослинного походження у 2022 - 2024 роках. Мета роботи – провести молекулярно-генетичні дослідження грибів та ягід (лохини, малини, чорниці), вирощених в Україні, методом полімеразної ланцюгової реакції в режимі реального часу (ПЛР-РЧ) та проаналізувати результати досліджень щодо наявності РНК норовірусу та вірусу гепатиту А. Матеріали і методи. Дослідження проводились в науково-дослідному відділі біохімічних та молекулярних досліджень харчових продуктів, кормів та води державного науково-дослідного інституту з лабораторної діагностики та ветеринарно-санітарної експертизи (ДНДІЛДВСЕ) методом ПЛР-РЧ згідно ISO/TS 15216-2 Microbiology of food and animal feed – Horizontal method for determination of hepatitis A virus and norovirus in food using real-time RT-PCR – Part 2 та Регламенту ЄК 178/2002 (articles 14, 31 Regulation EC №178/2002). Результати досліджень. Згідно Планів державного моніторингу, щодо виявлення норовірусу та вірусу гепатиту А, в ДНДІЛДВСЕ досліджено 465 проб ягід та грибів з різних областей України. В ході виконання моніторингових досліджень методом ПЛР-РЧ не виявлено жодного випадку контамінації харчових продуктів рослинного походження збудниками норовірусу та гепатиту А. Висновки. За результатами проведених моніторингових досліджень в зразках грибів та ягід не виявлено РНК норовірусу та вірусу гепатиту А.*

Ключові слова: норовірус, гепатит А, віруси, ПЛР-РЧ, харчові патогени, молекулярно-генетичні методи діагностики.

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