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Журнал заснований 25 жовтня 2022 р. Державним науково-дослідним інститутом з лабораторної діагностики та ветеринарно-санітарної експертизи та Громадською організацією «Інститут Єдиного Здоров'я». Видання зареєстроване Міністерством юстиції України, Свідоцтво КВ № 25382-15322Р від 10.01.2023 р. Включено до Переліку наукових фахових видань України (Категорія «Б») для ветеринарних наук (наказ МОН України N 768 від 20 червня 2023 р.), біологічних наук (наказ МОН України № 1309 від 25 жовтня 2023 р.) медичних наук (теоретична медицина (наказ МОН № 220 від 21 лютого 2024 р.). Матеріали номеру схвалені до друку Редакційною колегією 03.07.2025 р.

Dear colleagues,

«One Health» is a worldwide applied multidisciplinary approach aimed at achieving optimal outcomes in the realm of health and well-being via interdisciplinary collaboration and cooperation of human health, animal health, plant health, and environmental safety domains. It grows and develops at the Global, country, region, and institutional levels.

Our multisectoral team, leaded by the State Scientific and Research Institute for Laboratory diagnostics and Veterinary and Sanitary Expertise (SSRILDVSE) – leading veterinary science and reference activities establishment of the State Food Safety and Consumer Protection Service of Ukraine and Non-Governmental Organization One Health Institute (NGO OHI), created the One Health Journal in 2022. The journal is included State register of professional journals for veterinary, biological since 2023, and for medical sciences since 2024.

Our Editorial team invites new authors for contribution of research and review papers and joint development of the One Health multidisciplinary approach implementation. We hope, that our collection will be interesting for wide auditorium of scientists and practical specialists in human medicine, veterinary medicine, biology, biotechnology and biosafety.

This issue is dedicated to different medical, veterinary and ecological problems in areas of infectious diseases, biological and food, and ecological safety.

Sincerely yours,
Prof. Dr Anton Gerilovych,
Editor-in-Chief

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RHIZOBIUM RADIOBACTER – A NEW PATHOGEN OF HEALTHCARE-ASSOCIATED INFECTIONS

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Abstract. *This paper presents the results of a study of cases of catheter-related bloodstream infections in patients with a central venous access catheter installed for long-term infusion of medications to treat the main disease. It is known that intravascular devices are a risk factor for the development of* catheter-related bloodstream infections (CRBSI). *Therefore, conducting an epidemiological study of implanted vascular catheters is an important component of infection control in healthcare facilities. During the bacteriological study of blood samples from the central catheter (using BACT/ALERT FA Plus, the BACT/ALERT 3D blood culture system and nutrient media), 19 cultures were obtained, which were identified as Rhizobium radiobacter using the automated VITEK-2 Systems (bioMerieux, Inc). In the vast majority, all isolated strains demonstrated high sensitivity to antibiotics. However, 100% resistance was found to some antibiotics. Isolation of Rhizobium radiobacter from the blood of patients encourages the expansion of the etiological structure of catheter-associated bloodstream infections and confirms the importance of epidemiological surveillance of implanted intravascular devices.*

Keywords: healthcare-associated infections, *Rhizobium radiobacter* infection control, antibiotics, antibiotic resistance.

Introduction*.* The problem of prevention and treatment of healthcare-associated infections (HAI) does not lose its relevance. According to the World Health Organization, every tenth patient is harmed while receiving medical care in a hospital setting (Roychoudhury, 2024).

A significant place among all HAI belongs to catheter-related bloodstream infections (CRBSI). This is due to the widespread use of vascular catheters for therapeutic and diagnostic procedures. However, the use of other intravascular devices can also lead to the development of bloodstream infections. According to the European Center for Disease Prevention and Control, in 2017, 44% of BSI episodes were catheter-related in the structure of the incidence of HAI in intensive care unit patients in the European Region (European Centre for Disease Prevention and Control, 2019). The results of a pilot study on the simultaneous prevalence of healthcare-associated infectious diseases, which was conducted in five regions of Ukraine in 2021, showed that 421,800 patients were infected in the hospital, the number of registered cases of bloodstream infections was 2.3% of the total number of HAI (Public health center of MoH of Ukraine, 2021). The low percentage of registered cases of CRBSI is explained by the lack of organized surveillance for the control and registration of cases of bloodstream infections, insufficient amount of performed blood culture tests, limited capabilities of medical institutions to diagnose these infectious complications, and in some cases - deliberate avoidance of documenting the presence of a case of this type of HAI.

Currently, the leaders among pathogens that cause the development of HAI are gram-negative bacteria. The World Health Organization (WHO) updated the list of priority pathogens in 2024, which included 24 pathogens representing 15 families of the prokaryotic kingdom. Among them, most microorganisms are multi-resistant to many antibacterial drugs, including reserve group antibiotics. Infections caused by such microorganisms lead to high mortality.

According to WHO, in 2019, 4.95 million people died from infections caused by gram-negative multi-resistant pathogens (WHO, 2024).

In the etiological structure of CRBSI, the most prevalent agents are gram-negative non-fermenting microorganisms. The natural habitat of the most significant bacteria in disease development from the genera *Pseudomonas* and *Acinetobacter* consists of soil and water rich in organic residues from open water bodies. In the hospital environment, these microorganisms often colonize areas that are constantly moistened (sanitary equipment, tiled floors and walls, etc.). The extraordinary plasticity of these microorganisms' genetic apparatus makes them resistant to various adverse factors in the surrounding environment and to disinfectants.

However, recent reports indicate new microorganisms responsible for the development of hospital infectious complications, especially in immunocompromised patients. Are they equally dangerous? Will they increase the list of priority pathogens in the future?

The aim of study. Based on studies examining the etiology of a series of CRBSI cases, this research aims to assess the potential for adding to the list of HAI pathogens a new representative of gram-negative bacterial microflora, *Rhizobium radiobacter*.

Materials and methods*.* During March 2024 - December 2024, 30 cases of prolonged subfebrile temperature were observed in patients with signs of secondary immunodeficiency who were undergoing long-term outpatient treatment in a municipal medical institution. All patients were fitted with a CVC for the long-term administration of medical drugs for their primary condition. Given that intravascular devices are a risk factor for bloodstream infections, blood samples were collected from the CVC for bacteriological examination and sent to the bacteriological laboratory. The transportation of the samples was conducted in accordance with Order №354 from the Ministry of Health of Ukraine dated 02/21/2023, On the organization of prevention of nosocomial infections (Law of Ukraine, 2023).

Blood samples were cultivated in commercial vials for growing aerobic and anaerobic BACT/ALERT FA Plus and incubated in a BACT/ALERT 3D blood culture system. The growth of microorganisms in the vials was present in almost all cases for 3 days. In most cases, the aerobic vial became positive even earlier. The material from positive vials was then cultivated on BAP, CBA, Endo medium, MSA, Enterococcus Selective Agar, CHROMagar Orientation. Quality control of nutrient media was carried out in accordance with the recommendations of manufacturers, which are set out in the product certificates. In cases of positive test results, the morphological and cultural characteristics of microorganisms were studied. Species identification and antimicrobial susceptibility testing of isolated cultures was performed using the automated VITEK-2 Systems (bioMerieux, Inc).

During the study of the biochemical properties, fermentation was taken into account for 45 biochemical substrates, namely: Ala-Pne-Pro-arylamidase (APPA), adonyl (ADO), L-pyrrolidone arylamidase (PyrA), L-arabit (lARL), D-cellobiose (dCEL), beta-galactosidase (BGAL), beta-N-acetylglucosaminidase (BNAG), glutamylarylamidase (AGLTp), glucose (dGLU), gamma-glutamyltransferase (GGT), maltose (dMAL), mannitol (dMAN), mannose (dMNE), beta-glucosidase (BGLU), beta-xylosidase (BXYL), beta-alanine arylaminadase (BALap), L-proline arylamidase (ProA), as well as lipase (LIP), sucrose (SAC), palatinose (PLE), tyrosine arylamidase (TyrA), sorbitol (dSOR), trehalose (dTRE), citrate (CIT), malonate (MNT), lactate (lLATk), keto-D-gluconate (5KG), succinate (SUCT), phosphatase (PHOS), acetylgalactosaminidase (NAGA), alphagalactosidase (AGAL), ornithine decarboxylase (ODC), glucose fermentation ability (OFF) and hydrogen sulfide (H2S) production.

The assessment of the antibiotic susceptibility of the studied bacterial cultures was carried out in accordance with the EUCAST recommendation for ECOFF for Acinetobacter (EUCAST, 2025). The determination of susceptibility was carried out to the following antibiotics: imipenem, meropenem, doripenem, amikacin, gentamicin, tobramycin, levofloxacin, ciprofloxacin, trimethoprim-sulfamethoxazole.

Results. Peripheral vascular catheterization is one of the most common invasive procedures in medical practice, necessary for a number of therapeutic and diagnostic procedures. However, the use of intravascular catheters is a risk factor for the development of bloodstream infections through direct entry of microorganisms into the bloodstream. Among 30 examined blood samples from CVCs, 19 positive results were obtained, which is 63.3%. Microscopic examination of smears prepared from pure cultures of microorganisms grown on nutrient media demonstrated the presence of gram-negative non-spore-forming rods, which were located mainly singly or with a tendency to dense clusters (fig. 1).

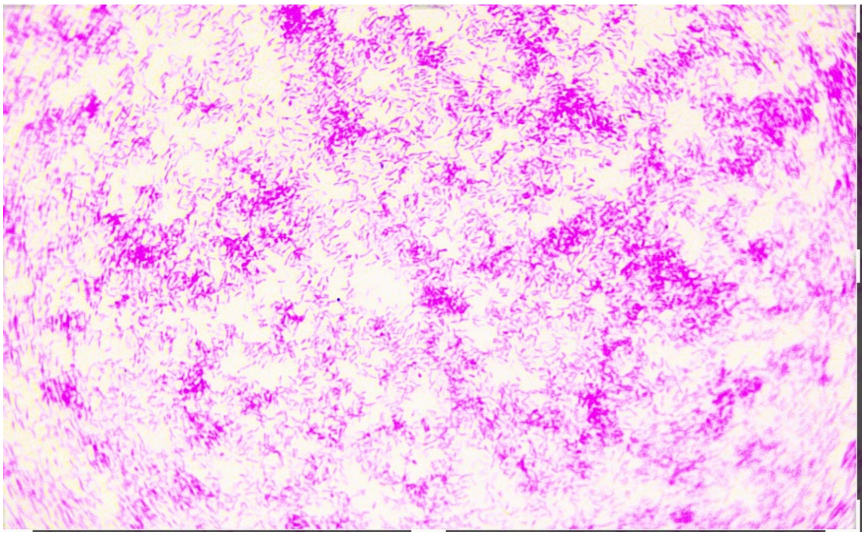

Fig. 1. *Rhizobium radiobacter* microscopy (magnification x1000)

The next day after incubation on BAP and CBA, growth of mucous colonies with a diameter of 1 mm was observed, as well as poor growth on Endo medium. After 48 hours of incubation in a thermostat at a temperature of 37°C, growth on Endo medium increased, but the colonies were small with a metallic sheen. The results of the oxidase and urease tests were positive.

Based on the obtained biochemical properties, all isolates were identified as *Rhizobium radiobacter* with a probability of 99% by the automated system VITEK-2 Systems (bioMerieux, Inc) using cards for the identification of gram-negative microorganisms (GN).

Analysis of the biochemical properties of the isolated cultures showed that the vast majority of strains had biochemical properties typical of the species. However, several strains differed in terms of fermentation of certain substrates (Table 2).

Table 2

Differentiation of *Rhizobium radiobacter* strains by biochemical properties

Substrate	Fermentation		No fermentation	
	№ of strains	% of strains	№ of strains	% of strains
proA	10	52,6%	9	47,4%
ADO	11	57,8%	8	42,2%
dTRE	3	15,7%	16	84,3%
BGAL	5	26,3%	14	85,7%
SAC	5	26,3%	14	85,7%
GLyA	6	31,6%	13	68,4%
PLE	4	21%	15	79%
BXYL	11	57,8%	8	42,2%

The results of determining the sensitivity of the isolated strains to antibiotics are illustrated in Figure 2.

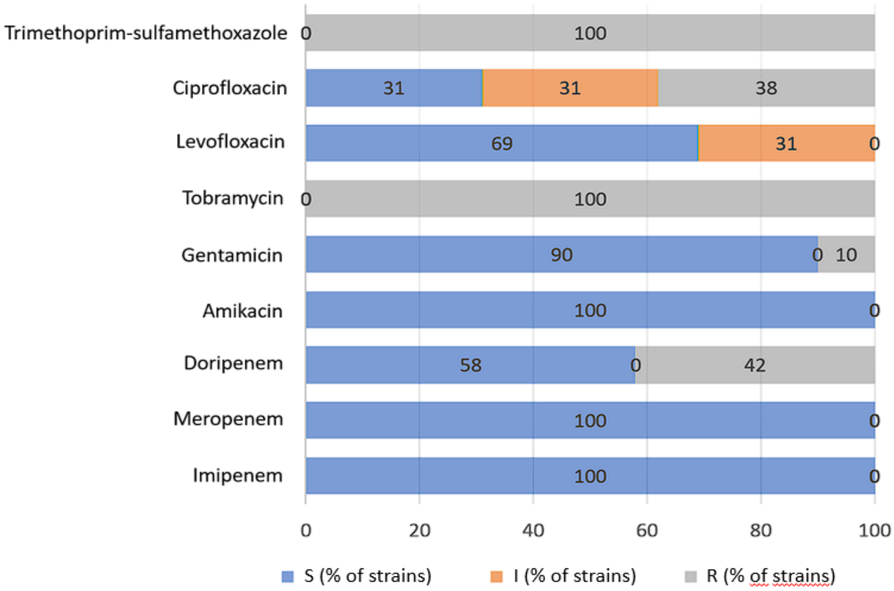

Fig. 2. Sensitivity of isolated strains of *Rhizobium radiobacter* to antibiotics

As shown in Fig. 2, most of the isolated strains of rhizobium were sensitive to carbapenems. All isolated isolates were sensitive to imipenem and meropenem. The sensitivity to doripenem (carbapenem of the 3rd generation) was 58%. It is also important that these antibiotics belong to the watch group (B) and can be prescribed only after proven ineffectiveness of antibiotics from the access group. The sensitivity of the isolated strains of *R. radiobacter* to aminoglycoside antibiotics is of considerable interest. The sensitivity to the aminoglycoside of the 2nd generation – to the gentamicin - was at the level of 90%, to amikacin all tested isolates demonstrated full sensitivity. At the same time, 100% of the isolated strains were resistant to tobramycin.

As for fluoroquinolone drugs, the sensitivity of *Rhizobium radiobacter* strains was 31% to ciprofloxacin and 69% to levofloxacin. The relatively high percentage of resistant strains to levofloxacin is noteworthy, since since 2023 this drug has been classified as a reserve drug and is prescribed only based on vital signs.

The above-mentioned sensitivity of the isolated *Rhizobium radiobacter* isolates was obtained using the automated VITEK-2 system using a card with an AST 331 reagent kit, which is used to determine antibiotic sensitivity for the group of non-fermenting gram-negative microorganisms. It is clear that the data obtained are insufficient to generalize the results regarding the resistance of rhizobia to antibiotics. Therefore, we continued to study the sensitivity to antimicrobial drugs by the disk diffusion method. The results of the study demonstrated high sensitivity of isolated strains of bacteria of the genus *Rhizobium* to inhibitor-protected and –unprotected aminopenicillins. Thus, about 96% of isolated strains were sensitive to amoxicillin and amoxicillin/clavulanate. 100% of rhizobium strains were sensitive to the third-generation cephalosporins ceftriaxone and cefoperazone/sulbactam. However, only 56% of strains were susceptible to cefoxitin. Cefepime, ceftazidime and ceftazidime/avibactam were ineffective against 95% of *R. radiobacter* strains.

Discussion. The implementation of Order No. 1614, On the Organization of Infection Prevention and Infection Control in Healthcare Institutions and Institutions Providing Social Services/Social Protection of the Population, marked a critical step in Ukraine's efforts to improve HAI registration and control. Central to this initiative is the standardized documentation of invasive procedures, including peripheral and central vascular catheterizations. Routine epidemiological surveillance of HAIs remains essential for timely detection, microbial identification, and informed antimicrobial stewardship.

Among patients receiving intravenous therapy, a subset requires long-term cyclic or course-based drug administration. For these individuals, central venous catheters (CVCs) are utilized. These devices, designed to integrate into patient tissue, are believed to reduce infection risk by creating a barrier against skin-colonizing pathogens. However, extended catheter use can predispose patients to intraluminal infections, often stemming from breaches in aseptic technique during catheter maintenance. Less commonly, hematogenous catheter contamination may occur, originating from distant infection foci such as pneumonia, urinary tract infections, or fungemia (Small, 2015; The Joint Commission, 2013; Balasoiu, A., 2022).

Despite state-level surveillance efforts, CRBSI control remains suboptimal. To some extent, this is due to the fact that patients with installed intravascular devices for long-term drug administration spend most of their time outside the hospital and there is no certainty about proper care of the CVC site. Accordingly, early detection of symptoms of local inflammation (redness, swelling, etc.) is difficult.

R. radiobacter (formerly *Agrobacterium tumefaciens*) is a gram-negative, non-spore-forming, motile aerobic rod, usually a phytopathogen, causing the development of plant tumors. Its natural habitat is soil, along with other non-fermenting bacteria. Human disease caused by representatives of the genus *Rhizobium* is an infrequent phenomenon. There are five known species of bacteria of the genus *Rhizobium*, namely: *Rhizobium radiobacter, Rhizobium rhizogenes, Rhizobium rubi, Rhizobium undicola, Rhizobium vitis*. Interestingly, *R. radiobacter*, with its low virulence, is the only species involved in the development of diseases in humans (Aujoulat, 2015).

The earliest report of *R. radiobacter* causing endocarditis following aortic valve replacement dates back to 1980 (Plotkin, 1980). Since the early 2000s, increasing numbers of infections have been reported, including endocarditis post-heart valve replacement, bacteremia in cancer patients, neonatal sepsis, and, more rarely, UTIs in dialysis patients, nosocomial pneumonia, and brain abscesses (Tiwari, 2015; Fatma, 2024; Sood, 2010). In addition, episodes of the participation of these microorganisms in the development of urinary tract infections (in patients requiring hemodialysis), nosocomial pneumonia, brain abscesses, etc. have been confirmed. The placement and presence of plastic devices in the human body is a major factor in the development of infections caused by this pathogen. This is explained by the high adhesion properties of rhizobia to plastic/silicone surfaces due to the production of extracellular mucus (Lai, 2004).

The detection of *R. radiobacter* in blood samples of 19 patients led to the assumption of the presence of an intrahospital reservoir of infection and prompted an epidemiological investigation. However, a thorough bacteriological examination of the hospital environment where intravascular devices were installed did not yield positive results. According to the literature, one of the possible sources of rhizobia is the gastrointestinal tract, from where they can subsequently spread into the bloodstream. In addition, the outpatient nature of the treatment of the examined patients suggests the presence of many sources of infection during the patients' stay outside medical institutions (Wang, 2019).

Whether these cases constitute a hospital outbreak remains inconclusive. Nonetheless, the frequent detection of *R. radiobacter* in CVC blood cultures underscores its potential role in expanding the known etiological spectrum of CRBSI. Rhizobium spp. should be regarded as opportunistic pathogens capable of causing bloodstream infections in immunocompromised individuals—those with cancer, organ transplants, HIV, or undergoing corticosteroid or cytostatic therapy.

The general susceptibility of *R. radiobacter* to antibiotics suggests these infections are currently manageable. However, the notable aminoglycoside resistance patterns—especially to tobramycin—warrant further investigation, not only for treatment purposes but also for use as diagnostic markers.

Currently, the list of antibiotics for susceptibility testing of *R. radiobacter* is undefined. EUCAST guidelines, while authoritative, do not include this organism in their 2025 update, creating ambiguity around appropriate testing standards. This gap requires further discussion within the microbiological and infectious disease community.

Conclusion. The results of the study confirm the need and importance of epidemiological surveillance of the use of intravascular catheters and other devices that are incorporated into vessels or other cavities of the human body for a long time for therapeutic and diagnostic purposes. At the same time, the possibility of isolating emergent species of bacteria should be considered, especially in patients with primary or secondary immunodeficiencies, and comprehensively study each positive bacteriological culture. The repeated isolation of *R. radiobacter* strains from the blood of CVCs emphasizes the need for further research into its virulence factors, adhesive capabilities, and biofilm-forming properties. Advanced diagnostic systems—including automated blood culture systems and microbial analyzers—should be employed to ensure precise identification and resistance profiling in each CRBSI case.

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RHIZOBIUM RADIOBACTER – НОВИЙ ЗБУДНИК ІНФЕКЦІЙ, ПОВ'ЯЗАНИХ З НАДАННЯМ МЕДИЧНОЇ ДОПОМОГИ

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Анотація. *У статті представлені результати проведеного дослідження випадків катетер-асоційованих інфекцій кровотоку у пацієнтів з встановленим центральним портом для проведення довготривалої інфузії медичних препаратів з метою лікування основного захворювання. Відомо, що встановлені внутрішньо-судинні девайси є фактором ризику розвитку КАІК, тому проведення епідеміологічного дослідження встановлених судинних катетерів є важливим компонентом інфекційного контролю закладів охорони здоров'я. При проведенні бактеріологічного дослідження крові з центрального порту (із використанням BACT/ALERT FA Plus та гемокультиватора BACT/ALERT 3D та поживних середовищ) було отримано 19 позитивних культур, які, за допомогою автоматизованої системи VITEK-2 Systems (bioMerieux, Inc) були ідентифіковані як Rhizobium radiobacter. В переважній більшості всі виділені штами продемонстрували високу чутливість до антибіотиків, проте, до деяких антибіотиків виявили 100% резистентність.*

Виділення Rhizobium radiobacter із крові пацієнтів спонукає до розширення етіологічної структури катетер-асоційованих інфекцій кровотоку та підтверджує важливість епідемічного нагляду за встановленими внутрішньосудинними девайсами.

Ключові слова: інфекції, пов'язані з наданням медичної допомоги, *Rhizobium radiobacter,* інфекційний контроль, антибіотики, антибіотикорезистентність.

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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.

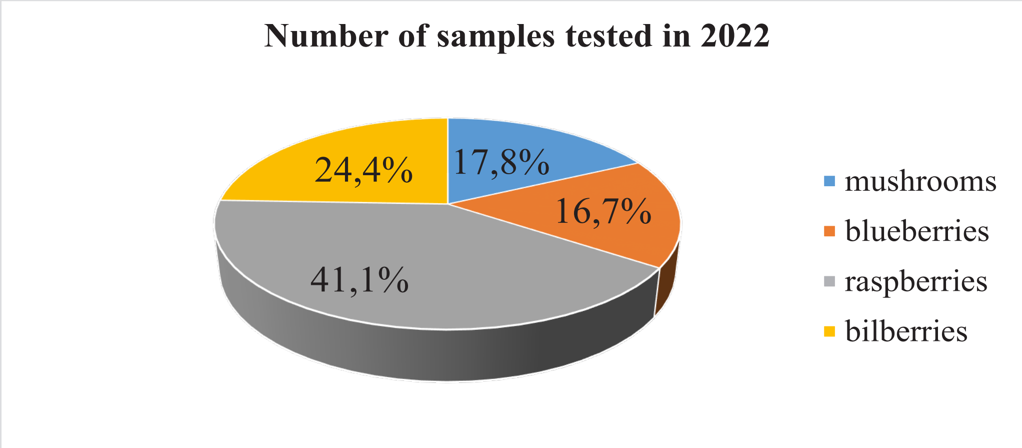

Fig. 1. Number of monitoring studies of mushrooms and berries for *Norovirus* and *Hepatitis A virus* in 2022

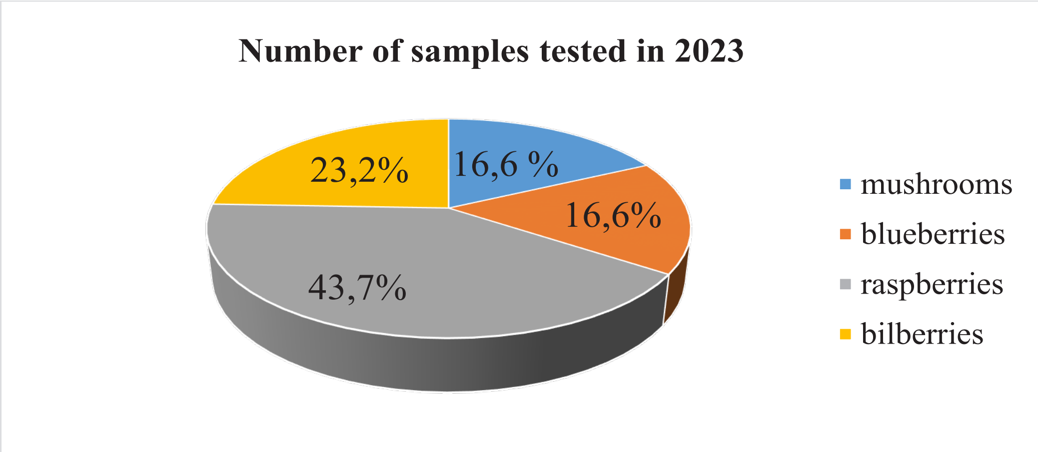

Fig. 2. Number of monitoring studies of mushrooms and berries for *Norovirus* and *Hepatitis A virus* in 2023

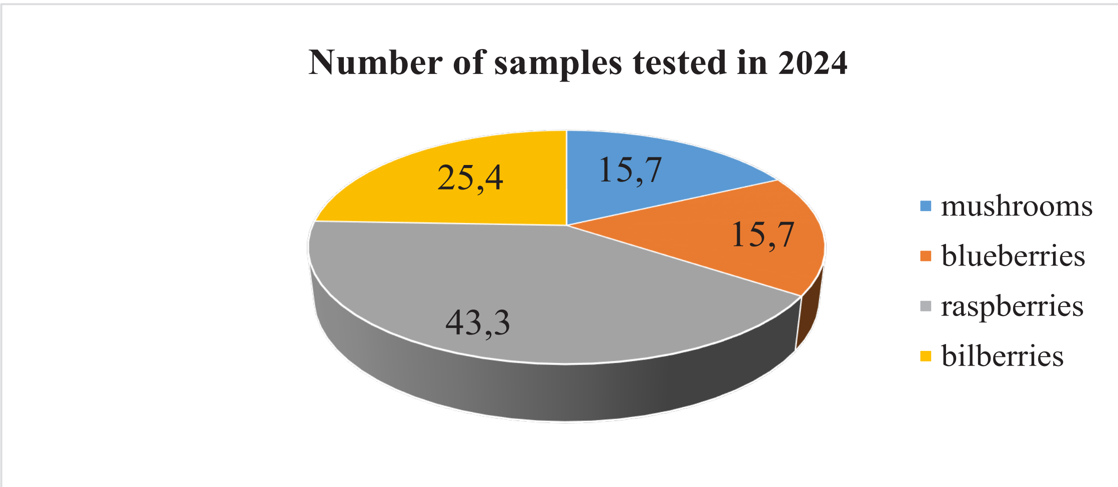

Fig. 3. Number of monitoring studies of mushrooms and berries for *Norovirus* and *Hepatitis A virus* in 2024

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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РОЗДІЛ 2. Благополуччя довкілля та екологічна безпека
SECTION 2. Environmental well-being and safety

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ASSESSMENT OF THE SUITABILITY OF THE SPECTROMETRIC METHOD OF MEASURING THE SPECIFIC ACTIVITY OF CESIUM-137 IN WATER SAMPLES USING SCINTILLATION GAMMA SPECTROMETER AND SOFTWARE "PROGRESS"

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***Abstract**. Cesium-137 is one of the primary radionuclides used as an indicator of environmental radiological contamination. Due to its relatively long half-life (30 years), it poses a significant radiological hazard, as it can penetrate and accumulate in various tissues and organs, causing internal exposure.*
To prevent the accumulation of radionuclides in the human body and reduce associated health risks, as well as to ensure the safety of food products in Ukraine and the European Union, maximum permissible levels of cesium-137 in food are regulated at the legislative level. Radiological monitoring of foodstuffs is routinely conducted using gamma-spectrometric analysis, which allows for rapid identification and quantification of radionuclide content.
According to DSTU EN ISO/IEC 17025:2019 General Requirements for the Competence of Testing and Calibration Laboratories, all analytical methods used by state laboratories must be validated and accredited.
This article describes the procedure for conducting abbreviated validation of the method for measuring the specific activity of cesium-137 in control water samples artificially enriched with the analyte, using a scintillation gamma spectrometer. The study was conducted at the State Research Institute for Laboratory Diagnosis and Veterinary and Sanitary Expertise. The suitability of the method for its intended purpose was evaluated based on key parameters such as measurement accuracy and intermediate precision.
The validation results demonstrated that the measurements of cesium-137 specific activity did not exceed the normative limits specified by the measurement method. Therefore, this method can be considered suitable for specific applications, providing accurate and reliable results.
Keywords: cesium-137, control samples, gamma spectrometry, accuracy, precision, bias, method suitability

Radioactive contamination of the environment is considered one of the greatest dangers of the modern world, as radioactivity can negatively affect both the environment and its inhabitants for many years (Gupta, 2018; IAEA, 2011; Kashparov, 2019; Russell, 2015).

According to literature data, the radionuclides cesium-137 and strontium-90 play the most significant role in the radiological contamination of the biosphere. Due to their relatively long half-lives—30 years for cesium-137 and 29 years for strontium-90—they can accumulate significant doses of radiation (Jeskovsky, 2019; Kotelevych, 2019; Malimon, 2023). The main sources of these radionuclides entering the air environment are nuclear tests and accidents at nuclear power plants (Fesenko, 2013; IAEA, 2019).

One of the main radionuclide contaminants in food consumed by humans is cesium-137 (Zdrojewicz, 2016; Romanchuk, 2019). Long-term consumption of products contaminated with radiocesium can cause inflammatory processes in various tissues and organs of the human body, with the most pronounced inflammation observed in the gastrointestinal tract, urinary tract, and reproductive system (Dutov, 2015; Gudkov, 2012; Godyn, 2016; Konoplev, 2020). To prevent the accumulation of radionuclides in the human body and reduce the negative impact of radiation on health, as well as to ensure the population has safe food, Ukraine and the European Union have legally established maximum permissible levels of cesium-137 in food products. Additionally, in accordance with current regulations, systematic monitoring of food products for radiological indicators, including the measurement of cesium-137 and strontium-90 content using spectrometric analysis methods, is conducted. These methods allow for rapid identification and quantitative determination of radionuclide content in the matrix (Zdrojewicz, 2016).

According to DSTU EN ISO/IEC 17025:2019 General requirements for the competence of testing and calibration laboratories, all methods used by state laboratories of the State Consumer Service of Ukraine must be validated and accredited. Validation provides an understanding of the capabilities and limitations of the method during routine use and ensures the reliability of the obtained results (Initial validation and transfer, 2010; Eurachem guide, 2016; ISO17025:2019; ISO 5725).

The aim of study. To determine the suitability of the spectrometric method for measuring the specific activity of cesium-137 in water samples using a scintillation gamma spectrometer and Progress software.

Materials and methods. The research was conducted at the State Scientific and Research Institute for Laboratory Diagnostics and Veterinary and Sanitary Expertise, Kyiv, Ukraine. The test material consisted of control water samples enriched at a level of 1.80 Bq/kg with a standard solution of cesium-137 radionuclide type RR^{137}Cs No. 08-030-24, with a nominal volumetric activity of cesium-137 of 100 Bq/kg ±20%.

Measurements of the specific activity of cesium-137 in the control samples were performed using the Gamma Plus universal spectrometric complex with an NaI detection block and Progress software.

Results and discussion. This article describes the parameters used for the abbreviated validation of the scintillation gamma spectrometry method using control samples enriched with cesium-137 analyte. The research included the specifics of the measurement method, taking into account all critical stages of the process. The suitability of the method for the intended purpose was confirmed based on key parameters such as the accuracy and intermediate precision of the measurement results (Initial validation and transfer, 2010; ISO17025:2019; ISO 5725).

Before starting the measurements, preliminary preparation of the control samples was performed using the physical concentration method. The specific activity of cesium-137 was measured according to the methodology for measuring radionuclide activity using a scintillation gamma spectrometer with software "Progress".

The accuracy of the measurements was assessed under conditions of intermediate convergence. For this purpose, one operator conducted ten consecutive studies of the control samples using the same measurement method, the same instrument, while changing one factor (day of measurement).

The obtained measurement results were processed using generally accepted statistical methods and are presented in Table 1.

The accuracy of the measurement expresses how close the average value of an infinite number of measurement results is to the reference value. Since it is impossible to perform an infinite number of measurements, the accuracy was evaluated through «bias». For this purpose, the average value of the ten measurements, which was 1.89 Bq/kg, was compared with the reference value of 1.80 Bq/kg. According to the obtained data, the "bias" value can be considered insignificant, as it was only 0.09 Bq/kg.

Figure 1 shows a histogram evaluating the accuracy of the measurements by determining the percentage recovery of the added analyte (cesium-137) to the matrix (water).

Table 1

Results of measurements of control samples enriched with cesium-137

Research days	Added standard at the level, Bq/kg	Parameters of the state of the premises			Measurement results, Bq/kg	Standard deviation, (S), Bq/kg	Relative standard deviation (RSD), %	Bias (b), Bq/kg
		Temperature, °C	Humidity, %	Atmospheric pressure, kPa				
1	1.80	20	58	100.0	1.71	0.12	6.50	0.09
2		20	63	100.3	1.96			
3		20	63	100.3	1.97			
4		20	67	100.6	1.98			
5		20	63	100.1	1.83			
6		20	67	100.3	1.98			
7		20	64	100.8	2.05			
8		20	55	100.3	1.68			
9		20	58	100.0	1.90			
10		20	63	100.3	1.84			

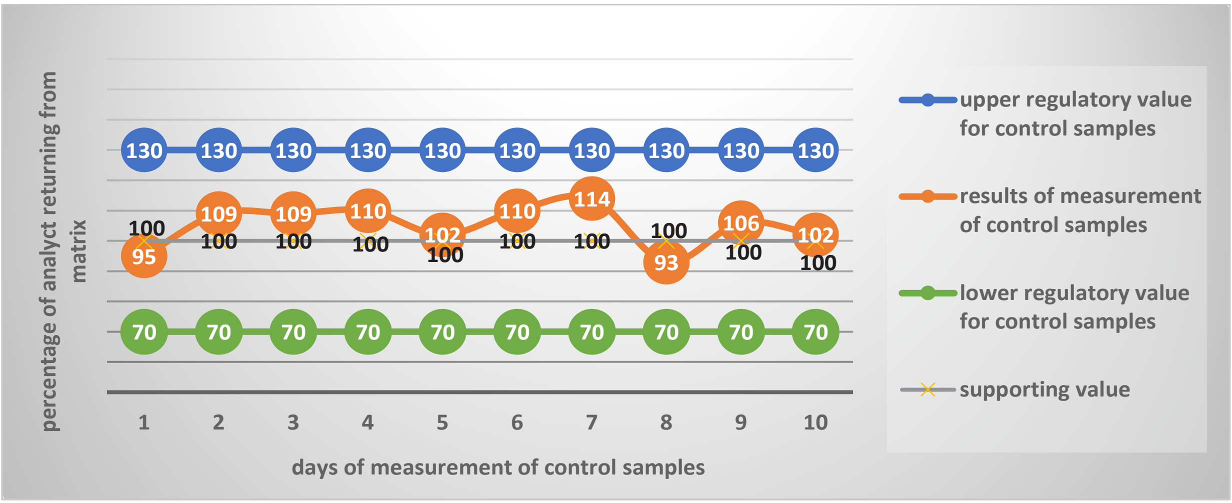

Fig.1. Percentage recovery of the added cesium-137 analyte in the control water samples

Analyzing the histogram data, it is noted that the percentage recovery of the analyte in the measured control samples enriched with cesium-137 relative to the reference value of 1.80 Bq/kg (100%) ranged from 93% to 114%. Therefore, the measurement results can be considered acceptable and reliable, as they fall within the normative value (100±30%) specified by this measurement method.

The second important indicator for evaluating the suitability of the method is the intermediate precision of the measurements, usually expressed by statistical parameters characterizing the dispersion of measurement results, such as convergence and intra-laboratory reproducibility.

In this work, we used the method of simultaneously determining convergence and intra-laboratory reproducibility of measurement results described in ISO 5725-3:2005 Accuracy (trueness and precision) of measurement methods and results. For this purpose, three series of measurements were conducted, with three measurements in each series. Convergence was assessed within one series (measurements were performed by one operator on the same instrument within one day). Intra-laboratory reproducibility of the measurement results of the control samples was performed under maximally different conditions (series), with tests conducted by different operators on different instruments on different days. The results of the measurements are presented below in Table 2.

Table 2

Results of measurements of the specific activity of cesium-137 in control samples under conditions of convergence and intra-laboratory reproducibility

Research days	Parameters of the state of the premises			Measuring series	Measurement results, Bq/kg		
	Temperature, ºC	Humidity %	Atmospheric pressure, kPa		X_1	X_2	X_3
1	20	63	100.2	A	1.87	1.67	1.62
2	21	55	100.0	B	1.79	1.96	1.83
3	21	59	99.6	C	1.86	2.13	1.82
Relative standard deviation of convergence, %						7.07	
Relative standard deviation of convergence of internally laboratory reproduction, %						8.16	

According to the data in the table, the smallest spread of measurement results was observed when conducting studies under convergence conditions. In contrast, conducting studies under intra-laboratory reproducibility conditions was accompanied by an increase in the variability of measurement results and a greater spread between them.

Since precision depends on the concentration of the analyte in the control sample, it is advisable to express it through the relative standard deviation. The calculation of the relative standard deviation was performed in accordance with the Guideline Fitness for Purpose of Analytical Methods. A Laboratory Guide to Method Validation and Related Topics (Eurachem guide, 2016). The relative standard deviation of convergence was evaluated as intra-group precision and amounted to 7.07%, while the relative standard deviation of intra-laboratory reproducibility—as the square root of the sum of the squares of intra-group and inter-group precision—was 8.16%.

In summary, the obtained data indicate that this method is suitable for use, as the obtained values of the relative standard deviation of convergence and the relative standard deviation of intra-laboratory reproducibility did not exceed the relative standard deviation established by the measurement method (no more than 30%).

Conclusions.

Based on the obtained and analyzed results of the abbreviated validation of the methodology for determining the specific activity of cesium-137 using a scintillation gamma spectrometer and the Progress software, the method was found to meet key validation criteria. In particular, the accuracy of the measurements evaluated by bias and the percentage recovery of the analyte added to the matrix as well as the precision of the measurements, including convergence and intra-laboratory reproducibility, were assessed using control water samples.

The validation data confirmed that under highly variable measurement conditions, the method provides accurate and statistically reliable results. Therefore, the analytical method can be considered suitable for further application in the radiological monitoring of food products and water.

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ОЦІНКА ПРИДАТНОСТІ СПЕКТРОМЕТРИЧНОГО МЕТОДУ ВИМІРЮВАННЯ ПИТОМОЇ АКТИВНОСТІ ЦЕЗІЮ-137 В ЗРАЗКАХ ВОДИ З ВИКОРИСТАННЯМ ГАММА-СПЕКТРОМЕТРА ТА ПРОГРАМНОГО ЗАБЕЗПЕЧЕННЯ «ПРОГРЕС»

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Резюме. *Цезій-137 є одним із головних радіонуклідів, який служить індикатором радіологічного забруднення навколишнього середовища. Завдяки своєму відносно довгому періоду напіврозпаду (30 років) радіоцезій вважається найбільш небезпечним джерелом гамма опромінення, оскільки здатний проникати та накопичуватися у різних тканинах та органах організму, спричиняючи його внутрішнє опромінення.*

З метою запобігання накопичуванню радіонуклідів в організмі людини та зменшення впливу негативних факторів на її здоров'я, а також з метою забезпечення населення безпечними харчовими продуктами в Україні та в країнах Європейського Союзу на законодавчому рівні регламентуються максимально допустимі рівні цезію-137 в харчових продуктах. Також на постійній основі проводиться моніторинг харчових продуктів за радіологічними показниками методом гамма-спектрометричного аналізу, який дозволяє швидко ідентифікувати та кількісно визначати вміст радіонуклідів.

Виходячи з того, що відповідно до ДСТУ EN ISO/IEC 17025:2019 «Загальні вимоги до компетентності випробувальних та калібрувальних лабораторій» усі методи якими володіють державні лабораторії для визначення показників безпеки та якості харчових продуктів повинні бути валідованими (верифікованими) та акредитованими.

У даній статті описано процедуру проведення верифікації методики вимірювання питомої активності цезію-137 в контрольних зразках води штучно збагачених аналітом цезію-137 з використанням сцинтиляційного гамма-спектрометра. Дослідження проводилися на базі Науково-дослідного інституту з лабораторної діагностики та ветеринарно-санітарної експертизи. Підтвердження придатності даного методу поставленим цілям оцінювали за такими ключовими параметрами, як: правильність та проміжна прецизійність результатів вимірювань.

Оскільки, по усім аналізуючим параметрам, результати вимірювань питомої активності цезію-137 не перевищували нормативні значення зазначенні методикою вимірювань, можемо стверджувати що даний метод придатний для конкретного застосування, а отримані результати вимірювань є правильними та достовірними.

Ключові слова: цезій-137, контрольні зразки, гамма-спектрометрія, правильність, прецизійність, «зсув», придатність методу

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РОЗДІЛ 3. Здоров'я тварин
SECTION 3. Animal health

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THE IMPACT OF NON-IONIZING RADIATION ON THE QUANTITATIVE AND QUALITATIVE INDICATORS OF CHICKEN MEAT PRODUCTS

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Abstract. *The growing interest in non-ionizing radiation and its potential biological effects has stimulated research into its application in animal husbandry. This article examines the impact of non-ionizing radiation, specifically extremely low-frequency pulsed electromagnetic fields (ELF-PEMF), on the productive indicators of dual-purpose chickens of the Dominant D959 crossbreed. Changes in the mass of eviscerated carcasses and selected internal organs were analyzed under the influence of different levels of extremely low-frequency non-ionizing radiation. Particular attention was paid to the qualitative characteristics of the meat – the content of crude protein in hydrolysates of breast muscle proteins in the experimental chickens. The study revealed no pathological changes in the internal organs of chickens after exposure to ELF-PEMF, confirming the biological safety of the applied irradiation modes regardless of the protein content in the diet. The results indicate that statistically significant changes in the examined parameters occur under certain exposure conditions. In particular, a reliable increase in the mass of the eviscerated carcass was observed under a 30-minute daily irradiation regimen over 150 days, with the chickens being fed diets either 15% higher or lower in protein content. The research suggests a potentially positive role of periodic ELF-PEMF exposure as an adaptogenic factor that helps maintain or even increase protein content in muscle tissue, especially under conditions of moderate stress and adaptive protein deficiency. At the same time, constant electromagnetic exposure under nutrient-deficient conditions was found to suppress protein metabolism. The data obtained support the prospect of using ELF-PEMF as a growth-promoting factor for chicken muscle mass, with constant exposure appearing more effective than periodic exposure. The mode of electromagnetic field exposure should be considered a significant regulator of metabolic processes capable of modulating protein metabolism efficiency depending on the organism's nutritional status. Further research should aim to explore the underlying mechanisms of this influence and to optimize the parameters for applying ELF-PEMF in poultry farming to achieve maximum productivity.*
Keywords: non-ionizing radiation, ELF-PEMF, Dominant D959 chickens, productivity, carcass weight, internal organs, breast muscles, crude protein

In the current context of poultry farming development, increasing the productivity of agricultural poultry and improving meat quality have become particularly important (Balamatsia, 2006; Asmarani, 2024). The growing demand for environmentally safe and high-quality meat encourages the search for new technologies that achieve high performance without the use of chemical growth stimulants (Alugwu, 2022). One promising direction is the use of physical factors, particularly non-ionizing radiation, which includes electromagnetic radiation of various ranges (ultraviolet, infrared, microwave, etc.) (Lin, 2011; Prosyanyi, 2020; You, 2024).

Among environmental factors that cause significant changes in the functional state of biological organisms, electromagnetic fields (EMFs) play a special role (Zymantienė, 2020; Tong, 2024).

Studies on the biological effects of the hypogeomagnetic field indicate that this factor induces a wide range of physiological, biochemical, and morphological changes in the organism (Balamatsia, 2006). This is directly related to the issues of «industrial extreme conditions», «magnetic deprivation», or «situational industrial chronic stress» (Wang, 2021).

Unlike ionizing radiation, non-ionizing radiation does not have the ability to break molecular bonds but can influence physiological and biochemical processes in animals (Gunes, 2020). Recent studies suggest a potential positive effect of such radiation on metabolism, immune response, and morphofunctional characteristics of tissues (Hashim, 2024). However, data on the effect of non-ionizing radiation on the meat productivity of chickens remain limited and contradictory, highlighting the need for further research in this area (Wang, 2021).

A key feature of the effect of electromagnetic radiation (EMR) on biological objects is its pronounced resonance (frequency-dependent) nature, which is considered one of the principal differences between informational and energetic influences, the latter being less sensitive to frequency (Chun, 2010; Lindberg, 2024).

Based on a review of the literature, it can be concluded that EMFs, especially with prolonged exposure, can cause physiological and morphological changes in animals (Indiarto, 2023). Changes in body weight and the condition of internal organs depend on the characteristics of the radiation (frequency, intensity, duration) and the sensitivity of the specific animal species. Given the inconsistency of the results, the topic requires further in-depth experimental research (Chun, 2010).

Electromagnetic radiation, as a non-specific stimulus that can trigger a range of adaptive responses and increase the overall adaptive resistance of the organism, allows the relevance of this research and the mechanisms of EMR action to extend from fundamental biological to applied agricultural aspects.

The aim of this study is to investigate the effect of extremely low-frequency pulsed electromagnetic fields (ELF-PEMF) on the quantitative (weight of the eviscerated carcass and internal organs) and qualitative (crude protein content in breast muscles) indicators of meat products in Dominant D959 crossbreed chickens. The results of this study may serve as a basis for the development of new biotechnological approaches in poultry farming aimed at improving product quality while maintaining safety and environmental sustainability.

Materials and methods. The study was conducted on Dominant crossbreed chickens, D959, raised under standard poultry farm conditions. One hundred clinically healthy 6-month-old chickens were selected for the experiment. They were divided into four experimental groups and one control group, each consisting of 20 chickens.

Irradiation and feeding of the chickens were carried out according to the experimental scheme (Table 1). In the magnetobiology laboratory, the room where the experiment was conducted was completely de-energized, and all external surfaces were covered with foil to isolate it from external magnetic radiation.

Variable pulse electromagnetic fields (VPEMFs) were generated using a signal generator capable of producing magnetic fields with individually set frequencies ranging from 0.01 to 20 kHz and oscillation amplitudes from 0 to 100 V, which is equivalent to a power of 150 W. Voltage and signal modulation control from the generator to the solenoid was performed using an S1-49 oscilloscope. The induction produced by the VPEMF was monitored with a G-49 microteslameter. Experimental studies involving low-frequency VPEMF were conducted at a frequency of 8 Hz, which is considered the fundamental frequency of the ionospheric waveguide and is close to the frequency of certain biorhythms.

After the experiment, 150 days later, the chickens were slaughtered and the carcasses eviscerated. The eviscerated carcass weight was determined by weighing after removing the head, legs up to the hock joint, digestive tract, and thoracic and abdominal organs. The mass of the internal organs was determined separately by weighing the liver, heart, lungs, kidneys, spleen, and muscular stomach. Before weighing, the organs were rinsed to remove feed residues and blood. All weighing procedures were carried out using electronic laboratory scales with an accuracy of 0.01 g.

Table 1

Experimental design

Group	Number of chickens	VLF-EMF irradiation regimen	Feeding regimen
Experimental I	20	Irradiation with VLF-EMF for 30 minutes daily for 150 days	Basic diet (BD) + 15% increased protein level
Experimental II	20	Irradiation with VLF-EMF for 30 minutes daily for 150 days	BD + 15% reduced protein level
Experimental III	20	Irradiation with VLF-EMF for 30 minutes daily every other week for 150 days	BD + 15% increased protein level
Experimental IV	20	Irradiation with VLF-EMF for 30 minutes daily every other week for 150 days	BD + 15% reduced protein level
Control	20	No irradiation	BD with protein content according to standard norms

Note: VLF-EMF – Very Low Frequency Electromagnetic Field; BD – Basic Diet

Crude protein in the breast muscles of chickens was determined in accordance with DSTU EN ISO 5983-2:2022 Animal feedstuffs — Determination of nitrogen content and calculation of crude protein content — Part 2: Block digestion and steam distillation method (EN ISO 5983-2:2009, IDT; ISO 5983-2:2009, IDT).

All obtained experimental data were processed using methods of descriptive and inferential statistics with the help of Microsoft Excel and Statistica 10.0 software. Results are presented as mean values (M) and standard deviations (SD). To determine the significance of differences between group means, the Student's t-test was used for normally distributed data, while the Mann–Whitney U test was applied when the data distribution deviated from normality. Differences between groups were considered statistically significant at $p < 0.05$. In cases where $p < 0.01$, the differences were considered highly significant.

Results. During slaughter, we performed a veterinary and sanitary assessment of the carcasses and internal organs of the experimental chickens, which revealed no deviations from the norm.

A comparative analysis of the eviscerated carcass weight of chickens from different experimental and control groups (Table 2) demonstrated significant changes under the influence of low-frequency variable pulse electromagnetic fields (VPEMF-LF). Specifically, the eviscerated carcass weight in birds from the first and second experimental groups exceeded that of the control group by 1.29 and 1.24 times, respectively ($p < 0.05$). This indicates a pronounced positive effect of VPEMF-LF on body weight gain in these groups.

At the same time, the carcass weight in chickens from the third and fourth experimental groups was lower than that of the control group, although these differences were not statistically significant.

As for the internal organ weights, although certain fluctuations were observed between the experimental groups and the control group, none of the differences were statistically significant. This indicates a stable physiological condition of the internal organs in all groups, which, on the one hand, confirms the safety of the applied VPEMF-LF exposure regimens, regardless of the protein content in the diet, and on the other hand, demonstrates the lack of substantial influence on the development of internal organs.

Table 2

Eviscerated carcass weight and internal organ weights in chickens (M ± m, n = 100)

Indicator		Control Group	Group I	Group II	Group III	Group IV
Eviscerated body weight, kg		1.55 ±0.09	2.00 ±0.11*	1.92 ±0.09*	1.50 ±0.12	1.51 ±0.08
Internal organ weights, g	Liver	38.72 ±0.93	38.23 ±1.16	37.91 ±1.02	38.47 ±0.81	37.98 ±1.40
	Heart	10.28 ±0.89	9.64 ±0.70	10.71 ±0.65	9.69 ±0.83	10.13 ±0.92
	Lungs	9.23 ±0.48	9.78 ±0.68	8.96 ±0.70	8.84 ±0.48	8.63 ±0.51
	Kidneys	8.03 ±0.39	8.25 ±0.35	7.99 ±0.30	7.88 ±0.29	7.80 ±0.44
	Spleen	2.99 ±0.19	3.25 ±0.31	2.73 ±0.22	3.04 ±0.43	3.10 ±0.20
	Muscular stomach	38.48 ±1.27	38.90 ±2.47	38.74 ±2.16	38.54 ±0.92	37.95 ±0.86

Note: *$p < 0.05$ – statistically significant difference compared to the control group.

Based on the conducted research, it was found that the crude protein content in the breast muscles of chickens from the fourth experimental group, where VPEMF-LF exposure alternated with weekly breaks and the protein level in the diet was 15% below the norm, reached its maximum level, exceeding that of the control group by 1.06 times, which is statistically significant at $p < 0.01$ (Fig. 1).

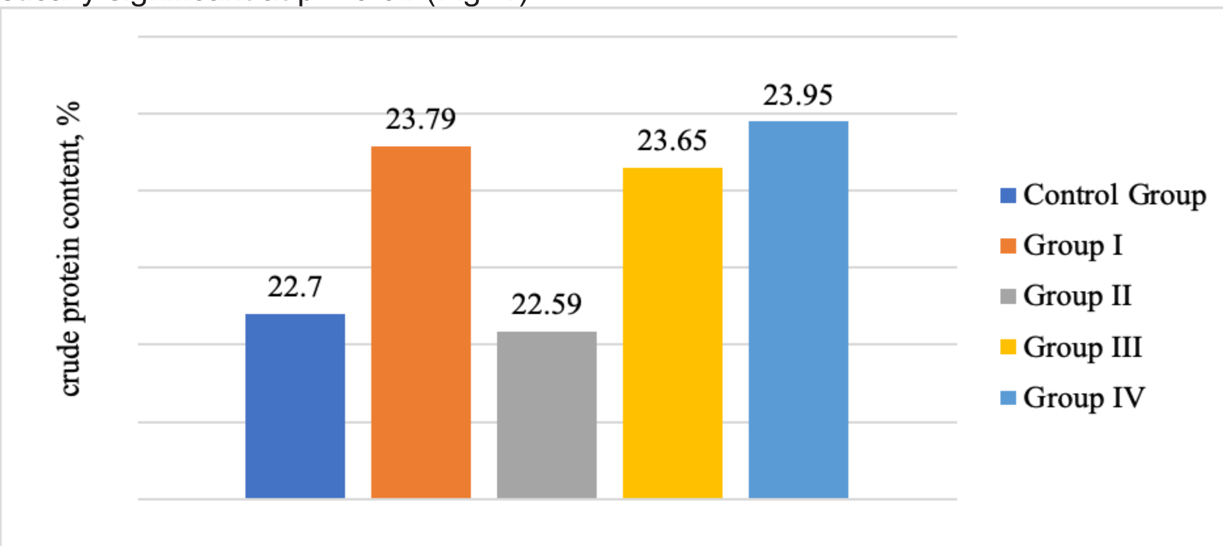

Fig. 1. Crude protein content in the hydrolysates of breast muscle proteins in experimental chickens

Under a similar VPEMF-LF exposure regimen but with a 15% increase in dietary protein, the crude protein content in the breast muscles of chickens from the third experimental group was slightly lower than in group IV, yet 1.04 times higher than the control group ($p < 0.05$).

Data analysis shows that in the first experimental group, where birds were exposed to VPEMF-LF continuously without interruption and received a protein-enriched diet, the crude protein content in breast muscles was 1.05 times higher ($p < 0.01$) compared to the control group.

Conversely, under the same exposure regimen but with a protein-deficient diet (second experimental group), the crude protein level in breast muscle hydrolysates was slightly lower than the control value, although the difference was not statistically significant.

Discussion. In today's context, with the active implementation of technologies that generate electromagnetic fields (EMF), it is important to study their effects on biological systems. Particular interest lies in changes in the morphofunctional state of the organism, including body weight and the condition of the internal organs of animals under the influence of EMF, specifically low-frequency alternating pulse electromagnetic radiation (Prosyanyi, 2021; Chun, 2010; Lindberg, 2024).

The results of studies on the effect of EMF on the body weight of animals are contradictory. It has been established that radiofrequency radiation causes a decrease in the mass of chicken embryos (Siddiqi, 2019). At the same time, research (Dyche, 2012) showed no significant changes in body weight under the influence of low-frequency EMF, suggesting that the effect depends on the intensity and duration of exposure.

Studies on laboratory animals indicate that chronic exposure to EMF can lead to structural and functional changes in internal organs. Specifically, researchers (Zymantienė, 2020) confirm structural and functional changes in the internal organs of laboratory animals after chronic exposure to electromagnetic fields.

Data on the effects of EMF on the cardiovascular system, kidney, and liver function show that prolonged exposure to low-intensity EMF can cause both adaptive and pathological changes in the body (Yakymenko, 2011). Studies also recorded structural changes in the heart muscle of chicken embryos after incubation under EMF conditions (Pawlak, 2018). Other studies point to the possible influence of EMF on the hormonal balance, which, in turn, may affect body weight growth. In animal experiments, slight fluctuations in weight were observed depending on the exposure parameters (Nisbet, 2016).

However, information on the specific effects of low-frequency extremely low-frequency EMF (ELF-EMF) on birds is rather limited.

Our comparative analysis of the slaughter weights of chickens from the experimental groups indicates significant changes under the influence of experimental ELF-EMF exposure regimes. Specifically, chickens from the first and second experimental groups showed a significant statistically reliable increase in this parameter compared to the control group, indicating a real biological effect of the conditions used in these groups.

The results suggest that continuous, uninterrupted, prolonged exposure to ELF-EMF for 30 minutes daily, as applied in the first and second experimental groups, positively affects muscle mass formation and overall growth intensity. Regardless of the protein content in the diet, ELF-EMF acts as a stimulating factor influencing the overall meat productivity of the poultry. Meanwhile, in the third and fourth experimental groups, the increase in slaughter weight was negligible, and statistical analysis revealed no significant difference compared to the control group. This may suggest that intermittent exposure, with weekly intervals of 30 minutes daily, did not significantly affect the growth intensity of the chickens in these groups.

These results allow us to hypothesize that continuous exposure to ELF-EMF without breaks contributed to more efficient nutrient absorption, intensified metabolic processes, and, as a result, more active muscle mass growth. On the other hand, the absence of significant changes in the third and fourth experimental groups compared to the control suggests that periodic but prolonged exposure to ELF-EMF had no substantial effect on the muscle mass increase in chickens.

Regarding the mass of internal organs, the analysis demonstrated certain fluctuations between the experimental and control groups. However, statistical processing of the data did not reveal significant differences, which may suggest no substantial impact of the experimental factors we used on the morphofunctional state of the internal organs. On the one hand, this indicates the biological safety of the applied approaches since no developmental disorders or hypertrophy of specific organs were detected, while on the other hand, it indicates a selective effect of ELF-EMF mainly on muscle tissue.

Thus, the results of the study point to the potential of the methods used in the first and second experimental groups for improving poultry productivity. Further research could focus on determining the mechanisms of these effects, optimizing their dosage or conditions of application.

The analysis of the conducted scientific studies allows us to conclude that the effect of EMF may have both positive and negative effects on protein metabolism in animal muscles, depending on the exposure parameters (frequency, intensity, duration) and the physiological state of the organism.

In particular, in the study (Morabito, 2024), the effect of extremely low-frequency electromagnetic fields on the skeletal muscles of adult mice was found to cause oxidative stress with short-term exposure, which is subsequently compensated by adaptive mechanisms in muscle tissue. Specifically, an increase in protein carbonyl content in muscles was observed after the first and fifth weeks of exposure, indicating oxidative protein damage.

Meanwhile, the effect of pulsed electromagnetic fields can stimulate muscle cell proliferation and accelerate muscle tissue regeneration processes without inducing apoptosis or significantly disrupting metabolic activity (Zhang, 2016).

Our research showed that the impact of ELF-EMF combined with different protein levels in the diet had a significant effect on the raw protein content in the breast muscles of chickens. The highest protein content was observed in the fourth experimental group, where exposure alternated with weekly breaks under conditions of protein deficiency (15% less than normal). In this case, protein content exceeded the control level by 1.06 times ($p<0.01$), which may indicate adaptive protein metabolism processes in response to intermittent stress caused by the electromagnetic field.

Similar results align with other researchers' data (Takegaki, 2017; Reed, 2023), which suggest that intermittent stress factor exposure can activate protein metabolism and stimulate protein synthesis in tissues. Likely, alternating periods of exposure with recovery phases contribute to the activation of compensatory metabolic rearrangement mechanisms, allowing the body to use available protein resources more efficiently, even with dietary protein deficiency. Thus, intermittent exposure creates a favorable environment for activating compensatory protein metabolism mechanisms, even in conditions of limited protein intake.

In the third experimental group, where the ELF-EMF exposure regime with breaks was also applied but the protein content in the diet was higher, a slight decrease in protein content was observed compared to the fourth group, yet the level still exceeded the control by 4% ($p<0.05$). This phenomenon can be explained by studies from other authors (Morabito, 2017; Morabito, 2024), who established that low-frequency electromagnetic fields can affect protein metabolism in muscle tissues. Specifically, the study (Gerardi, 2008) showed that low-frequency EMF exposure leads to an increase in protein content in rat muscles, likely due to the activation of protein synthesis processes through stress-induced adaptation. Similar data on other tissues is provided by Lin (2011), where osteoblasts (7F2) were exposed to low-frequency pulsed electromagnetic fields (75 Hz, 1.5 mT) for 9 hours under inflammatory conditions. The results showed a significant increase in cell proliferation (by 23%) and expression of type I collagen mRNA (by 3.4 times) compared to the control, indicating activation of protein synthesis processes in cells. This study demonstrates that low-frequency electromagnetic fields can affect protein metabolism in cells, not limited to muscle tissue.

Interestingly, in the first group (continuous exposure + increased protein content in the diet), protein levels were also high (1.05 times higher than the control, $p<0.01$). This aligns with data indicating that a high-protein diet promotes increased muscle protein synthesis (Fouad, 2014), and continuous exposure, although exerting some stress, when combined with optimal feeding, may stimulate anabolic processes.

On the other hand, in the second group (continuous uninterrupted exposure + protein deficiency), a decrease in raw protein content was observed to a level lower than the control, though the difference was not statistically significant. This indicates the destructive effect of continuous ELF-EMF exposure combined with protein deficiency, suppressing anabolism and promoting catabolic processes, as also indicated by some researchers (Gunes, 2020; Morabito, 2024).

Thus, the results suggest that alternating exposure periods with recovery phases (absence of ELF-EMF exposure) under even moderate protein deficiency may have a more pronounced positive effect on muscle protein synthesis than continuous exposure with adequate or excessive protein supply. This highlights the importance of the exposure regime as a factor modulating the metabolic response of the organism.

Conclusion. Continuous daily exposure of chickens to ELF-EMF for 30 minutes per day leads to a significant increase in the weight of the slaughtered carcass compared to the control, indicating a stimulating effect of such regimes on the meat productivity of the birds. In chickens subjected to intermittent exposure (30 minutes daily with weekly intervals), the increase in carcass weight was insignificant and did not reach statistical significance, indicating the insufficient effectiveness of this mode of ELF-EMF exposure. The absence of significant changes in the mass of internal organs in all groups suggests a selective action of ELF-EMF, with a predominant effect on muscle tissue without disrupting the morphological and functional state of internal organs.

The effect of ELF-EMF, combined with different protein levels in the diet, significantly influences the crude protein content in the breast muscles of chickens. The highest level of crude protein in muscle tissue (6% higher than the control, $p < 0.01$) was observed in the experimental chickens, where ELF-EMF exposure alternated with weekly breaks under conditions of protein deficiency (15% below the norm), indicating the activation of compensatory adaptive mechanisms in protein metabolism in response to intermittent stress.

Conditions of continuous ELF-EMF exposure combined with an increased protein level (+15%) also contributed to the increase in crude protein content in the muscles (5% higher than the control, $p < 0.01$). However, in the presence of protein deficiency, the protein level in such conditions decreased to values lower than the control, indicating the catabolic effect of prolonged ELF-EMF exposure in conditions of protein insufficiency. The obtained results suggest that intermittent exposure to ELF-EMF has a more pronounced positive effect on muscle protein synthesis, even under conditions of limited protein intake, compared to continuous exposure under optimal or excessive protein provision.

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ВПЛИВ НЕІОНІЗУЮЧОЇ РАДІАЦІЇ НА КІЛЬКІСНІ ТА ЯКІСНІ ПОКАЗНИКИ М'ЯСНОЇ ПРОДУКЦІЇ КУРЕЙ

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Резюме. *Зростаючий інтерес до неіонізуючої радіації та її можливого біологічного впливу сприяв активізації досліджень щодо її застосування у тваринництві. У статті розглянуто вплив неіонізуючої радіації (змінного імпульсного електромагнітного поля наднизької частоти (ЗІЕМП ННЧ) на продуктивні показники курей комбінованого напряму продуктивності кросу Домінант, Д$_{959}$. проаналізовано зміни в масі патраної тушки та окремих внутрішніх органів за умов впливу різних рівнів неіонізуючого випромінювання наднизької частоти. Окрему увагу приділено якісним характеристикам м'яса – вмісту сирого протеїну в гідролізатах білків грудних м'язів дослідних курей. Проведене дослідження засвідчило відсутність патологічних змін у внутрішніх органах курей після впливу ЗІЕМП ННЧ, що підтверджує біологічну безпечність застосованих режимів опромінення незалежно від вмісту протеїну в раціоні. Результати дослідження свідчать, що за певних використаних нами режимів опромінення, виникають статистично вірогідні зміни у досліджуваних показниках. Зокрема, виявлено, достовірне зростання маси патраної тушки курей під впливом неіонізуючої радіації за схемою опромінення по 30 хвилин щодоби впродовж 150 діб, годівля яких проводилась з підвищеним або пониженим на 15 % вмістом протеїну в раціоні. Проведені дослідження свідчать про потенційно позитивну роль періодичного впливу ЗІЕМП ННЧ як адаптогенного чинника, що сприяє збереженню або навіть підвищенню вмісту протеїну в м'язовій тканині, особливо за умов помірного стресу і адаптаційного білкового дефіциту. У той же час, постійна дія ЕМП при дефіциті поживних речовин має пригнічуваний ефект на білковий обмін. Отримані дані дають підстави вважати ЗІЕМП ННЧ перспективним фактором для стимуляції росту м'язової маси курей, а постійний режим опромінення – більш ефективним у порівнянні з періодичним. Режим впливу електромагнітного поля слід розглядати як важливий регулятор метаболічних процесів, здатний модулювати ефективність білкового обміну залежно від харчового статусу організму. Подальші дослідження доцільно спрямувати на вивчення механізмів впливу, а також оптимізацію параметрів застосування ЗІЕМП ННЧ у птахівництві для досягнення максимальної продуктивності.*

Ключові слова: неіонізуюча радіація, ЗІЕМП ННЧ, кури кросу Домінант Д959, продуктивність, маса тушки, внутрішні органи, м'язова тканина, сирий протеїн
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STUDY OF INTEGRAL INDICES FOR DIAGNOSING INSULIN RESISTANCE IN HORSES

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Abstract. *Since insulin resistance is a component of the pathogenesis of many diseases, including the development of metabolic syndrome, its detection and elimination should be considered as an important and necessary means of preventing diseases and their complications. Unlike many complex methods, the data of integral indices are the main ones for detecting insulin resistance disorders. Thus, the aim of the work was to diagnose insulin resistance in horses using homeostatic indices and to establish their practical significance. For the study, 18 horses were used, which were divided into 2 groups according to the scoring of physical condition, which was carried out by two independent veterinary specialists. The calculation of integral indices: HOMA-IR index, HOMA-β index, Caro index, metabolic index, atherogenicity index was performed according to the literature. It was found that the HOMA-β index and the HOMA-IR index were significantly increased by 18.1% ($p<0.01$) and 3.7 times ($p<0.01$) compared to the control; Caro index in horses with insulin resistance was reduced by 39.4% ($p<0.05$); metabolic index increased by 82.5% ($p<0.01$); atherogenicity index in horses with insulin resistance was 54.9% ($p<0.05$) higher than in control. However, when studying the ALT/AST ratio and the Matsuda index, no correlation was found. Thus, in order to assess the development of insulin resistance, appropriate indices have been proposed, the calculation of which is based on data on carbohydrate and lipid metabolism and which complement the data on the pathogenesis of this condition. However, in horses, the choice of a method for studying insulin resistance requires a thorough study.*

Keywords: insulin resistance, integral indices, horses, metabolic syndrome, obesity.

Introduction. In modern medicine and veterinary medicine, the metabolic syndrome is one of the most widely discussed problems. This syndrome is a major problem in cardiology, endocrinology, and therapy (Girvalaki, 2014). Comparative analysis of the pathophysiological mechanisms of metabolic syndrome in humans and animals indicates the presence of common pathogenesis, in particular, disorders of insulin sensitivity, glucose homeostasis and lipid metabolism. In animals, as in humans, lifestyle factors play a key role in the development of insulin resistance: physical inactivity, excessive energy intake, and chronic stress. Establishing these parallels allows the use of veterinary models to study human metabolic disorders and substantiates the prospects of translational approaches to assessing risk factors and pathogenic targets (Alemu, 2021; Bamford, 2019).

Animal models, in particular horses, dogs and rodents, have significant potential in the study of insulin resistance, given the similarity of clinical manifestations and metabolic reactions to similar processes in humans. For example, the use of the oral glucose tolerance test to assess insulin sensitivity in horses allows non-invasive monitoring of glucose metabolism, which can be adapted for screening programs in high-risk human populations. Such an interdisciplinary approach enhances the scientific understanding of the mechanisms of metabolic disorders and contributes to the improvement of preventive and therapeutic strategies in human medicine (Durham, 2021; Hukkanen, 2022).

Metabolic syndrome is a pathogenetically interrelated metabolic disorder in the body. A large complex of factors involved in the onset of this syndrome hinders accurate scientific understanding of its pathophysiological mechanisms. It was first described as an independent symptom complex of clinical manifestations in 1980 (da Silva, 2020). In 1985, S. Eaton and M. Konnor hypothesized that the increase in the number of diseases of civilization in the second half of the twentieth century was due to the fact that the genes of modern humans, adapted by a long evolutionary process to the nutritional behavior of ancestors, are not able to ensure health in the face of a dramatic change in lifestyle over the past 100 years. The intensity of stress against the background of an unbalanced diet has significantly reduced the ability to adequately adapt, not only in humans but also in animals, and formed the basis for many diseases (Daradics, 2021). Insulin resistance (IR) is closely related to the metabolic syndrome, which includes dyslipidemia, hypertension, and obesity, leading to an increased risk of cardiovascular disease. Originally proposed to describe patients with diabetes who require high doses of insulin (Er, 2016), is now defined as reduced sensitivity (maximum insulin effect) or sensitivity (insulin concentration required for a half-maximal response) to the metabolic actions of insulin, such as insulin-mediated glucose utilization by muscle and adipose tissue and inhibition of gluconeogenesis in the liver (Muniyappa, 2008). Insulin, a peptide hormone consisting of 51 amino acids, is the most important hormone for energy metabolism, such as glucose, fat, and protein, as well as for maintaining homeostasis. The most difficult to perform is the clamp test, also called the gold standard (DeFronzo, 2004). Insulin resistance is determined in different ways (Borai, 2011). The simplest ones include simultaneous determination of fasting insulin and glucose levels, which allows to assess the effectiveness of the body's tissue response to insulin, which is a key component in assessing insulin dysregulation, which is characteristic of the pathological state of insulin resistance (Park, 2021). Unlike many complex methods, the main ones for detecting insulin resistance disorders are the data of the HOMA-IR, HOMA-β index, glucose-to-insulin and glucose-to-C-peptide ratios (Goswami, 2010), as well as elevated levels of alanine aminotransferase and aspartate aminotransferase, which indicate the presence of IR (Mather, 2001). Since IR is a component of the pathogenesis of many diseases (type 2 diabetes mellitus, atherosclerosis, congestive heart failure, laminitis, etc.), its detection and elimination should be considered an important and necessary means of preventing diseases and the development of their complications (Gomez-Serrano, 2016). In our previous studies, an oral glucose tolerance test was chosen to assess insulin and non-insulin glucose dynamics because of its practicality and physiological relevance. The test assesses hyperinsulinemia and insulin dysregulation after glucose ingestion, and it has been actively used to determine insulin sensitivity in horses and ponies (Borovkov, 2024). However, modern diagnostics includes the evaluation of HOMA-IR, HOMA-β, de Ritis, metabolic index, and atherogenicity data due to their practicality and physiological significance. Thus, the metabolic syndrome as a consequence of insulin resistance is a complex problem, the solution of which should be aimed at reducing the prevalence of obesity, early detection to modify risk factors.

The aim of study. To diagnose insulin resistance in horses using homeostatic indices and to establish their practical significance.

Materials and methods. Horses of different breeds, sexes and physical condition were used for the study. Feeding and housing conditions met the physiological needs of the animals. The animals' diet was balanced in terms of essential nutrients, and all animals had free access to water and enjoyed walking. A total of 18 horses were used in the study and were divided into 2 groups based on a scoring assessment of physical condition, which was conducted by two independent veterinary specialists. All horses were kept on pasture with free access to grass hay without concentrate feeding. The horses were placed in a stall the evening before testing and given free choice of hay, grass, and water overnight; the next morning, 2 hours before testing, access to feed was restricted and a halter was placed on the horse. The calculation of integral indices, which are relevant in the study of insulin resistance, was performed according to the literature (Melnyk, 2024; Gutch, 2015).

All animal studies were conducted in accordance with the basic principles of bioethics, in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986) and the General Ethical Principles for Animal Experiments adopted by the First National Congress on Bioethics (2012).

The obtained results were processed by methods of variation statistics using the analysis of variance (ANOVA) software package StatPlus 5.9.8.5 (AnalystSoft Inc., USA). The reliability of the results was assessed by the Tukey's test (HSD difference of means) at the level of probability $p<0.05$, $p<0.01$ and $p<0.001$.

Results. During the studies described in detail in the previous work (Borovkov et al., 2024), all horses were divided into 2 groups according to their physical condition using a scoring system. The first group included 9 horses with an average BCS score of 4.75 (4.5-6.5), while the experimental group was formed of animals with a BCS score of 6.5 (5.5-8.0). There was no significant difference between the groups. The average age in the first group was 10.3±2.3 (8-15) years, and in the experimental group 13.5±2.6 (10-18) years. The assessment is carried out using points from 1 to 9, where 1 is a very thin horse and 9 is morphologically overweight. During the evaluation, researchers pay attention to several key areas: in healthy horses, the outline of the spine is clearly visible, while in thin horses it is more noticeable, and in obese horses, fat is felt. In good physical condition, the ribs should not be visible, but you can feel them with your fingers. In thin horses, the ribs protrude, and in overweight horses, they can be completely invisible. Hair coat and coat quality are also an indicator of physical condition: a healthy horse has a shiny, supple coat, while a thin horse has dull and tousled hair. It is also important to assess the presence and intensity of fat deposits on the sides, neck and behind the ears (Borovkov, 2024).

In the present study, the HOMA model and a set of interrelated risk factors for cardiovascular disease and insulin resistance were used to calculate the indices, which are presented in Table 1. The animal serum parameters necessary for the calculation of the relevant indices were obtained in advance and described in our previous work (Borovkov, 2024)

Table 1

Integral blood serum indices in horses with insulin resistance (n=10)

Animal groups	HOMA-IR index	HOMA-β index	Caro index	Metabolic index	Atherogenicity index
horses with IR	11,2±0,34**	75,5±0,52**	0,20±0,01*	2,30±0,03**	0,79±0,02*
control	3,0±0,15	89,2±0,48	0,33±0,02	1,26±0,01	0,51±0,03

Notes: * - $p<0.05$, ** - $p<0.01$, compared to the control

To calculate the HOMA index, the ratio of basal insulin and blood glucose concentrations is used, which reflects the balance between endogenous glucose production in the liver and insulin secretion by β-cells, which is maintained by the principle of negative feedback. This homeostatic model has two pillars: the first is the degree to which basal glucose concentration increases in response to insulin deficiency, which reflects the shape of the normal insulin secretory response to glucose; the second is that basal insulin levels are directly proportional to insulin resistance (Lim, 2019; Park, 2021). When assessing β-cell function and peripheral tissue IR using the HOMA model, significant differences between the groups were obtained. The HOMA-β and HOMA-IR indices were significantly increased by 18.1% ($p<0.01$) and 3.7 times ($p<0.01$) compared to the control. It is likely that high insulin requirements in insulin-resistant animals lead them to the manifestation of the disease and a decrease in the functional activity of β-cells (Thunander, 2011). According to the literature, in case of insulin resistance, the endocrine part of the pancreas reacts with compensatory changes in the function and mass of β-cells, which leads to hyperinsulinemia and excessive insulin production to maintain normal glucose levels (Movahedi, 2016).

Other calculated indices have been proposed to determine insulin resistance. The most famous is the F index. Caro is the ratio of fasting blood glucose to fasting insulin concentration (Caro, 1991). Thus, as a result of our study, it was found that the Caro index in horses with insulin resistance was reduced by 39.4% ($p<0.05$) compared to healthy animals. It should also be noted the well-known Bennett's index, which is calculated as 1 divided by fasting insulin and multiplied by fasting glucose (Anderson, 1995), but in horses this index is less informative. The Matsuda index should also be taken into account, which allows you to calculate insulin sensitivity. The index is calculated on the basis of fasting serum glucose (mg/dl) and insulin (mIU/l) concentrations. However, the results of the study indicate that in female animals with a history of metabolic disorders, this index is less sensitive to detect early changes associated with insulin resistance (Melnyk, 2024). This may indicate a complex mechanism that regulates glucose homeostasis and correlates with hormonal variations, which requires further investigation (Lechner, 2021).

The state of insulin resistance, in turn, causes dysregulation of lipid metabolism, which is manifested not only in changes in the lipid spectrum of the blood (which is usually determined in patients), but also in changes in the fatty acid spectrum of circulating lipid fractions (Lonardo, 2005). According to the current literature, it is known that the development of cardiovascular disease is a consequence of insulin resistance, which affects the depth of the processes. Since IR is of high clinical importance, the ability to assess the depth of IR in animals before the development of cardiometabolic disorders is of great importance. Based on this, the determination of the index of metabolic disorders in insulin resistance reflects a functional disorder of the cardiovascular system. Its advantages are increased sensitivity to the initial changes in lipid composition, as well as to disorders of glucose metabolism. Analyzing the results of the table, it was found that in horses with insulin resistance, the metabolic index was increased by 82.5% ($p<0.01$) compared to healthy animals (Tahapary, 2022)

Information about the balance between atherogenic and antiatherogenic lipoproteins is provided by an index that reflects the ratio between low-density lipoprotein, LDL, and high-density lipoprotein, HDL, and blood cholesterol (Lonardo, 2005). The atherogenicity index complements the data of the metabolic index and indicates the risk of atherosclerotic plaque formation, which in turn reflects the functional state of the cardiovascular system (Lee, 2021). According to our data, the atherogenicity index in horses with insulin resistance is 54.9% ($p<0.05$) higher than in controls, indicating a high level of atherosclerotic changes.

There is evidence in the literature that the assessment of the ALT/AST ratio reflects the prediction of insulin resistance regardless of comorbidities, and their activity consistently correlated with the above-described insulin resistance indices. The authors found that the ALT/AST ratio is a more useful prognostic marker than HOMA-IR (Han, 2024). As a result of our research on insulin resistance in horses, no such correlation was found, which requires more detailed study.

Conclusion. Thus, in order to assess the development of insulin resistance, appropriate indices have been proposed, the calculation of which is based on data on carbohydrate and lipid metabolism and which complement the data on the pathogenesis of this condition. However, in horses, the choice of a method for studying insulin resistance requires a thorough study.

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ВИВЧЕННЯ ІНТЕГРАЛЬНИХ ІНДЕКСІВ ДІАГНОСТИКИ ІНСУЛІНОРЕЗИСТЕНТНОСТІ У КОНЕЙ

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Резюме. *Оскільки інсулінорезистентність є складовою патогенезу багатьох хвороб у тому числі і розвитку метаболічного синдрому, її виявлення та усунення слід розглядати як важливий і необхідний засіб профілактики захворювань та розвитку їх ускладнень. На відміну від багатьох складних методик основними при виявленні порушення інсулінорезистентності є дані інтегральних індексів. Таким чином метою роботи стало провести діагностику інсулінорезистентності коней з використанням гомеостатичних індексів та встановити їх практичну значимість. Для дослідження було використано 18 коней які були розділені на 2 групи за бальною оцінкою фізичного стану, котра була проведена двома незалежними фахівцями ветеринарної медицини. Розрахунок інтегральних індексів: індекс HOMA-IR, індекс HOMA-β, індекс Caro,* *метаболічний індекс, індекс атерогенності вели згідно літературним даним. Встановлено, що індекс HOMA-β та індекс HOMA-IR були вірогідно підвищені на 18,1% (р<0,01) та у 3,7 рази (р<0,01) відносно контролю; індекс Caro у коней з інсулінорезистентністю знижено на 39,4% (р<0,05); метаболічний індекс підвищено на 82,5% (р<0,01); індекс атерогенності у коней з інсулінорезистентністю на 54,9% (р<0,05) вищий ніж у контролі. Проте, при вивченні співвідношення АЛТ/АСТ та індекса Matsuda кореляції не встановлено. Таким чином, з метою оцінки розвитку інсулінорезистентності запропоновано відповідні індекси, розрахунок яких оснований на даних вуглеводного та ліпідного обмінів та які доповнюють дані патогенезу цього стану. Проте, у коней вибір методу дослідження інсулінорезистентності потребує досконалого вивчення.*

Ключові слова: інсулінорезистентність, інтегральні індекси, коні, метаболічний синдром, ожиріння.

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NOSEMATOSIS IN BEEKEEPING IN UKRAINE (2022–2024): THE ONLY POLLINATOR HEALTH PROBLEM IN THE FOCUS OF RESEARCH

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Abstract. *This article presents the results of a monitoring study on the spread of nosematosis among honey bees in Ukraine from 2022 to 2024. This parasitic disease, caused by Nosema apis and Nosema ceranae, significantly disrupts bee physiology, reduces immunity and lifespan, weakens colonies, and leads to substantial losses in beekeeping. The article analyzes veterinary reports and laboratory data, allowing researchers to trace the dynamics of infection and identify regional patterns. The highest infection rates were observed in Kherson, Khmelnytskyi, and Volyn regions. It was found that Nosema ceranae is gradually displacing Nosema apis in bee populations, complicating disease control strategies. The study emphasizes the need to improve diagnostic systems, ensure regular laboratory monitoring, develop effective preventive programs, and conduct educational outreach for beekeepers. The authors stress the importance of the «One Health» approach in protecting pollinators as a vital component of agroecosystems. Directions for future research are proposed, including adapting control measures to climate change, local environmental conditions, and the biological characteristics of the pathogens*.

Keywords: nosematosis, *Nosema ceranae*, *Nosema apis*, honey bees, disease, monitoring, infestation, biosecurity, pollinators, diagnostics, prevention

Introduction. Ukraine is one of the countries with developed beekeeping, ranking third in the world in honey production (Agro Perspectiva, 2025). However, the effective development of beekeeping is hampered by infectious and invasive bee diseases.

Nosematosis is a slow invasive disease of bee colonies caused by the microsporidia *Nosema apis* and *Nosema ceranae*, which infects the epithelial cells of the midgut, causing intestinal disorders, weakening of bee colonies and their death.

Nosematosis is an urgent disease of adult European honey bees, including queen bees. According to scientific data, the disease reduces honey collection by 36-50% and negatively affects the reproductive capacity of bee colonies, which emphasizes its economic and environmental significance. For example, Neuman P. (2010) emphasizes the global nature of bee mortality, linking it to pathogens such as *Nosema* and other factors that exacerbate the problem. Thus, the study of nosematosis, its diagnosis and prevention, as emphasized by modern authors, is critical for the preservation of beekeeping and food security.

The disease is caused by the spore-forming microsporidia *Nosema apis* and *Nosema ceranae*. Morphologically, the spores are difficult to distinguish between these two species, and this synergy ensures the pathogen's persistence and the form of disease spread (Palazzetti, 2007). The causative agent of *neomatosis N. apis* and *N. ceranae* have a stable spore, with stressors acting in association and all of them having an important synergistic or complementary effect on the occurrence of parasitic infection (Galajda, 2021).

Numerous studies by Ukrainian scientists show that «Asian» nosematosis is predominantly registered in Ukraine, with *N. ceranae* being the causative agent, which was detected in 74.5% of the analyzed samples (Odnosum, 2023).

Nosema ceranae spores are very resistant to the environment: they can withstand very low temperatures (down to minus 20 °C) and high temperatures (up to 60 °C), which contributes to the re-infection of bee colonies and the recurrence of the disease after a long time (Efimenko, 2014). Spores can remain infectious for several days to five years at temperatures below 0 °C (in particular, at minus 12 °C for up to 5 years). *N. apis* spores are less resistant to the environment.

In contrast to the Ukrainian researchers, colleagues from Germany showed that in their territory there is no dominance of *N. ceranae* over *N. apis* and a general replacement of *N. apis* with *N. ceranae* in the studied honey bee population (Gisder, 2017).

Transmission occurs mainly by fecal-oral route (Martin-Hernandez, 2007). Within the family, the spores are spread mainly by worker bees, which clean the combs, feed the queen, drones, and exchange food with each other. Spores of nosema contaminate hives and all equipment.

Mostly, nosematosis manifests itself in late winter, spring and autumn. In summer, the disease is asymptomatic. Infection of bees occurs when they consume honey, perga, water, which are infected with nosema spores, as well as kept on fallen honey. The development of the disease is facilitated by sharp temperature fluctuations, high humidity inside the hives, lack of perga, and late bee flight. Important factors are pesticide exposure and nutritional stress, which impair the immunity of bees (Galajda, 2021).

The disease can occur in overt and latent forms.

Among adult bees infected with nosema, an increase in the incidence of dysentery is recorded (Agriculture Victoria Connect, 2025).

Neomatosis does not have any distinct symptoms, but is accompanied by slow depopulation and a decrease in bee productivity. Bees that cannot fly appear in the hives, and they have a wing defect with a «K» shape. Paralyzed individuals can also be found, while other bees gather in small groups (Chemurot, 2017).

The latent form is unnoticeable, with no obvious signs of the disease.

In the overt form, the disease is observed:

- Nurse bees lose the ability of their hypopharyngeal glands to produce royal jelly, which is used to feed the honey bee brood.
- A significant portion of the eggs laid by the diseased queen may not produce mature larvae.
- Young infected queen bees stop raising brood and switch to guarding and foraging, usually done by older bees.
- Reduced life expectancy of infected bees. In spring and summer, infected bees live half as long as uninfected bees.
- Infected queens stop laying eggs and die within a few weeks.

The study on the epizootic situation with nosematosis is relevant and helps to control the spread of the disease, the importance of its diagnosis, treatment and prevention, as well as the development of bee species that will be resistant to pathogen spores. The loss of bee colonies due to nosematosis will lead not only to economic losses but also to an environmental disaster, as the agricultural sector, which is directly dependent on bee pollination of crops, is a priority for our country.

The aim of the study. Monitoring of bee infestation with nosematosis spores in the regions of Ukraine by analyzing veterinary reports for the period 2022 – 2024.

Materials and methods. Statistical analysis was performed on the basis of annual reporting forms No. 2-Vet. Report on the work of the laboratories of the State Service of Ukraine for Food Safety and Consumer Protection, which was analyzed using standard epizootological and biostatistical approaches. The laboratories of the State Service of Ukraine for Food Safety and Consumer Protection conducted microscopy tests in compliance with DSTU ISO/IEC 17025:2019 General requirements for competence of testing and calibration laboratories.

Results. Control and supervision of the spread of nosematosis in Ukraine is carried out by the State Service of Ukraine on Food Safety and Consumer Protection in accordance with the Plan of Anti-Epizootic Measures for the Prevention of Major Infectious and Parasitic Animal

Diseases in Ukraine. Planning is carried out in the amount of 10% of the total number of bee colonies of registered market operators.

Diagnostic tests for bee nosema are carried out microscopically and only by authorized laboratories of the State Service of Ukraine for Food Safety and Consumer Protection that are accredited in the ISO 17025 system. The results of the research are submitted to 2-VET Report on the work of state laboratories of veterinary medicine, quarterly, and recorded in the passport for the apiary.

According to this report, the staff analyzed the dynamics of the epizootic process of nosematosis in bees. During 2022 – 2024, 315141 bee samples were tested. Of these, a positive result was obtained in 4664 cases, which is 2.05% of the invasiveness (Fig. 1). during the study period, an increase in the number of positive samples for the pathogen of nosema was observed. In 2022, the laboratories of the State Service of Ukraine for Food Safety and Consumer Protection examined 140362 samples, which is 1.3% of the invasiveness rate. In 2023, 170988 samples were examined, the percentage of infection was 1.9%, in 2024 52034 studies were conducted, 2.9% (Fig. 1).

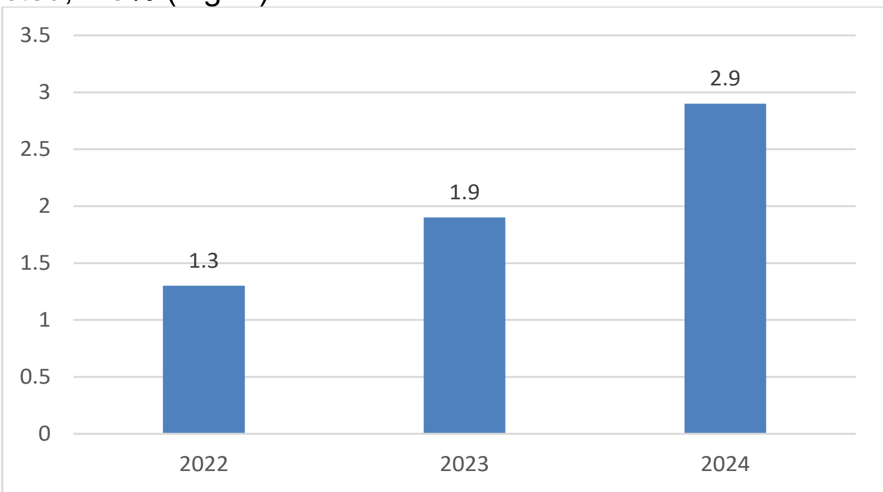

Fig. 1. Percentage of bees infected with nosematosis for the period 2022 –2024

These statistics are not complete due to the occupation of a large part of our country. The obtained indicators indicate an increase in nosematosis infection in the regions of Ukraine.

The distribution of nosematosis infection by region is shown in Fig. 2.

Discussion of the results. Based on the above map of the spread of bee nosematosis in Ukraine, it is possible to analyze the situation, taking into account regional characteristics and the level of bee colonies' susceptibility. The map shows the percentage of bees affected by nosema in different regions of Ukraine, where the values vary from 0.1% to 28% (Fig. 3).

The highest rate of damage is recorded in Kherson region – 28%. This may be due to the climatic conditions of the region, in particular high humidity and warm winters, which favor the development of *Nosema ceranae*, which is better adapted to such conditions than *Nosema apis*. It may also be due to insufficient use of preventive measures at local apiaries.

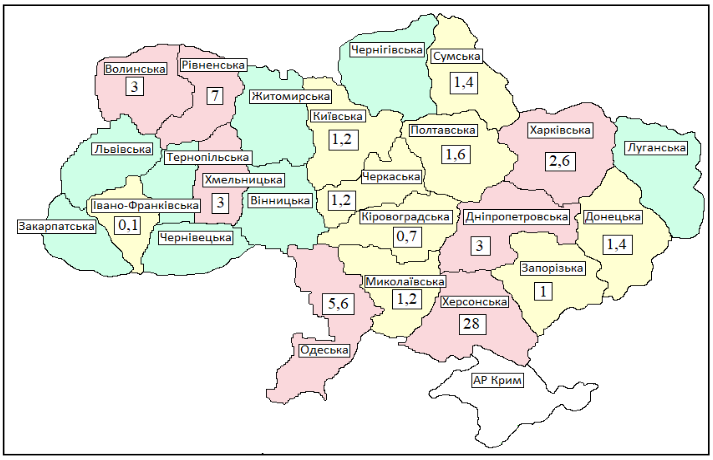

Fig. 2. Registration of bee nosematosis by regions of Ukraine for the period 2022 – 2024 (%)

Khmelnytskyi region (5.6%) and Volyn region (3%) also show high levels of infestation. These regions have developed beekeeping, but are likely to face problems in managing bee health, possibly due to insufficient timely treatment of bee colonies.

Regions with low levels of incidence include: Ivano-Frankivsk region, where only 0.1% is recorded (Fig. 3). This may indicate effective preventive measures, such as timely treatment of bees with drugs (e.g., fumagilin), or favorable environmental conditions that reduce the risk of disease spread.

Kirovohrad (0.7%), Cherkasy (1.2%), and Poltava (1.6%) regions also have relatively low rates. This may be the result of a lower density of bee colonies in these regions or better control over the condition of apiaries.

Average level of damage. Most regions, such as Vinnytsia (1.2%), Dnipropetrovs'k (3%), and Zhytomyr (1.2%), have an average level of damage (1-3%). This indicates a typical situation for Ukraine, where nosema is widespread but not critical. In such regions, basic preventive measures are likely to be applied, but they may not be sufficient to fully control the disease.

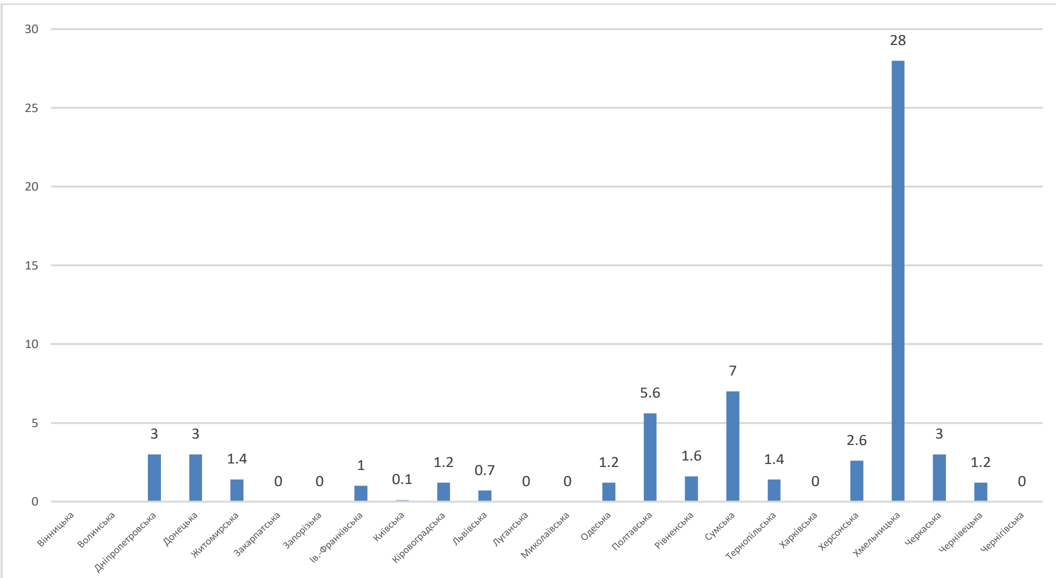

Fig. 3. Percentage of bees infected with nosema in the regions of Ukraine for the period 2022 – 2024

Higher rates of infestation (Kherson, Khmelnytsky, Volyn) are observed in different geographical zones – south, west and north, which indicates that there is no clear dependence on climatic zones. High infestation rates may be associated with *Nosema ceranae* infestation, which, unlike *Nosema apis*, is more active throughout the year and less dependent on seasonal fluctuations. This may indicate that the main factor in the spread is not climate, but the level of beekeeping practices and awareness of beekeepers.

Eastern regions, such as Kharkiv and Donetsk oblasts, have an average level of infestation, which may be due to the smaller number of apiaries due to the difficult socio-economic situation in the region. Thus, nosematosis is a serious problem for the Ukrainian beekeeping industry, as the disease is present even in regions with low levels of infection. This confirms its endemic nature.

Nosematosis is mainly transmitted by the fecal-oral route. The causative agent of nosematosis is easily spread through the droppings of diseased bees, especially in the hive. Spread from hive to hive and from apiary to apiary can occur through: movement of infected worker bees; high humidity inside the hive; moving infected combs from one hive to another; feeding infected honey to bees; use of infected materials or beekeeping equipment; frequent inspections of hives in unfavorable weather conditions (e.g., winter season, windy or rainy weather) can trigger the onset of the disease and its spread due to stress; the presence of other diseases (e.g., varroa, amoebiasis, or viruses) exacerbates the symptoms of nosema (Martin-Hernandez, et al., 2007; Galajda, et al., 2021; Galatyk, 2022). Lack of awareness among beekeepers, limited access to modern diagnostic and prevention methods, and the possible use of contaminated honeycombs or feed may contribute to the spread of the disease.

It is not easy to diagnose the disease in the early stages; the only suspicious sign is the presence of liquid excrement on the hive floor. A field test is to examine the color of the end part of the digestive system of some bees: in healthy bees it is reddish, while in diseased bees it is milky white. This sign, however, is observed only when the disease has already reached a certain severity. The main method of diagnosis is microscopy of spores at the intestinal level or directly in the feces (Galatyk, 2022, Forsgren & Fries, 2010).

Conclusions. The results of the study indicate a gradual increase in the level of nosematosis in bees in Ukraine during 2022 – 2024, which is confirmed by official veterinary statistics. The most affected regions are Kherson, Khmelnytsky and Volyn regions, which indicates an uneven spread of the disease and the need for regionally focused preventive measures.

The peculiarities of the nosematosis pathogen, its ability to survive in adverse conditions and the complexity of early diagnosis pose serious challenges for beekeeping. Risk factors, such as climatic conditions, non-compliance with sanitary and preventive standards, nutritional stress and comorbidities, contribute to the spread of the infestation.

Effective control of nosematosis requires an integrated approach: strengthening laboratory control, informing beekeepers, improving diagnostic methods and developing safe treatment alternatives. The preservation of beekeeping in Ukraine directly depends on a timely response to the challenges posed by this invasive disease.

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НОЗЕМАТОЗ У БДЖІЛЬНИЦТВІ УКРАЇНИ (2022–2024): ЄДИНА ПРОБЛЕМА ЗДОРОВ'Я ЗАПИЛЮВАЧІВ У ФОКУСІ ДОСЛІДЖЕНЬ

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Резюме. *У статті подано результати моніторингового дослідження поширення нозематозу серед медоносних бджіл в Україні у 2022–2024 рр. Це паразитарне захворювання, зумовлене збудниками Nosema apis та Nosema ceranae, що викликає серйозні порушення фізіологічних процесів у бджіл, призводить до зниження імунітету, скорочення тривалості життя, ослаблення бджолиних сімей, а також до економічних втрат у бджільництві. У статті проаналізовано ветеринарну звітність та результати лабораторних досліджень, що дозволило простежити динаміку захворюваності та виділити регіональні особливості розповсюдження інфекції.*

Найвищі показники виявлено у Херсонській, Хмельницькій і Волинській областях. У роботі акцентовано увагу на потребі вдосконалення системи діагностики, регулярного лабораторного контролю, розробки профілактичних програм і просвітницьких заходів для бджолярів. Автори підкреслюють важливість підходу «Єдине здоров'я» у контексті захисту запилювачів як невід'ємного компоненту агроекосистем. Запропоновано напрями подальших досліджень щодо адаптації протиепізоотичних заходів до змін клімату, регіональних умов та біологічних особливостей збудників.

Ключові слова: нозематоз, *Nosema ceranae*, *Nosema apis*, бджоли, захворювання, моніторинг, інвазійність, біобезпека, запилювачі, діагностика, профілактика.

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The private scientific institution «One Health Scientific and Research Institute» and «One Health Institute», NGO present an original Ukrainian translation of the CDC manual «Biosafety in Microbiological and Biomedical Laboratories», 6th edition.

Against the backdrop of the consequences of the full-scale military aggression of the Russian Federation against the Ukrainian people, Ukrainian independence, and statehood, and in light of the growing threats of military and terrorist actions aimed at destroying critical infrastructure, food, and energy security, the aspects of biological safety and biological protection have become extremely important.

A critical component of developing modern systems of state regulation of biosafety and bioprotection is the implementation of proper practices to prevent the spread of pathogens and ensure compliance with safety requirements in the laboratories of research institutes, healthcare institutions, the public health system, and bioproduction facilities.

It is significant that a draft Law of Ukraine «On Biological Safety and Biological Protection» is under consideration, with experts from the One Health Institute contributing to its development. There is much work ahead in creating subordinate legislation for the future law and developing institutional policies.

In response to the demands of the present day, there is an urgent need to strengthen the system of state regulation of biological safety and biological protection in Ukraine, including aspects of controlling the circulation of pathogens, creating conditions for safe diagnostic work, and producing specific protective measures for humans, animals, and plants.

Previously, a team of specialists from the PSI «One Health Scientific Research Institute» and the «One Health Institute», NGO, with the kind permission of the World Health Organization, completed an adapted Ukrainian translation of the WHO Biosafety Manual, which is based on a risk-oriented approach to ensuring biological safety and providing biological protection.

Now, with permission from the U.S. Centers for Disease Control and Prevention and the National Institutes of Health, and in collaboration with our scientific consultants, we have prepared an adapted Ukrainian translation of the manual **«Biosafety in Microbiological and Biomedical Laboratories», 6th edition**, which is based on a directive approach to creating laboratory and production conditions for biomedical research while adhering to biosafety and bioprotection standards.

In presenting this document, we sincerely hope that this initiative will benefit the medico-biological scientific and practical community in building and strengthening national standards for biological safety and biological protection in Ukraine.

Sincerely yours
Anton Gerilovych,
Director General of the One Health
Scientific and Research Institute, PSI

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