Приватна наукова установа
«Науково-дослідний інститут Єдиного Здоров'я»

Державний науково-дослідний інститут
з лабораторної діагностики та ветеринарно-санітарної експертизи

ГО «Інститут Єдиного Здоров'я»

Private Scientific Institution
«One Health Scientific and Research Institute»

State Scientific and Research Institute for Laboratory Diagnostics and Veterinary and Sanitary Expertise

«One Health Institute», NGO

ISSN (print): 2786-7420
ISSN (online): 2786-7439

One Health Journal

Healthy community, Healthy livestock,
Healthy wildlife, Health environment
One World – One Health

Том / Vol. 4
Номер / N 3
DOI: 10.31073/onehealthjournal2026-III

Київ / Kyiv – 2026

Contact information of One Health Journal Editorial Board:

State Research Institute for Laboratory Diagnostics and Veterinary and Sanitary Expertise
Mailing Address: Donetska, 30 (Entrance from Donetska str.), 03151, Kyiv, UA.
Phone: +38(067) 718-17-04; +38(067) 572-16-70; +38(044) 243-37-55; +38(044) 243-37-54
E-mail: prezyd.o.h.institute@gmail.com (*please indicate the e-mail topic ” for One Health Journal Editorial Board”*)

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 номера схвалені до друку Редакційною колегією 09.05.2026 р.

Dear colleagues,

As always, we are announcing:
Call for Papers – One Health Journal

The **One Health** approach fosters integrated collaboration across human, animal, plant, and environmental health sectors to effectively address complex global health challenges.

Founded in 2022 by the State Scientific and Research Institute for Laboratory Diagnostics and Veterinary and Sanitary Expertise (SSRILDVSE) in partnership with the Non-Governmental Organization *One Health Institute* (NGO OHI), the *One Health Journal* provides a platform for interdisciplinary scientific communication and knowledge exchange. The journal has been included in the State Register of Professional Scientific Journals since 2023 in the fields of veterinary and biological sciences, and since 2024 in medical sciences.

We invite researchers and practitioners to submit original research articles and review papers that contribute to the development and implementation of the One Health concept.

The forthcoming issue will focus on key challenges at the interface of human, veterinary, and environmental health, including:

- infectious diseases
- food safety and biosecurity
- environmental protection

We encourage you to join us in strengthening scientific collaboration and advancing the One Health paradigm.

We look forward to receiving your submissions and to your valuable contributions.

Sincerely yours,

Prof. Dr Anton Gerilovych,
Editor-in-Chief

Зміст /Table of contents

РОЗДІЛ 1. Емерджентні хвороби та Єдине здоров'я
SECTION 1. Emergent diseases and One Health

UDC 636.09:616-093/098
DOI: 10.31073/onehealthjournal2026-III-01

Multidrug-resistant *Enterobacteriaceae* in veterinary practice: current challenges

Murashko O.I.[1], Melnyk V.V.[2] (ORCID: 0000-0002-6958-2577)
1 – State Institution "L.V. Gromashevsky Institute of Epidemiology and Infectious Diseases of the National Academy of Medical Sciences of Ukraine", Kyiv, Ukraine, e-mail: sunsetmur@gmail.com
2 – National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine

Abstract. *Antimicrobial resistance in companion animals is of growing importance within the One Health approach due to close contact between humans and domestic animals and the risk of circulation of multidrug-resistant pathogens in households and veterinary clinics. The aim of the study was to evaluate the structure of clinically significant isolates of the Enterobacteriaceae family and the proportion of multidrug-resistant (MDR) strains in dogs and cats with clinical signs of bacterial infections in veterinary clinics in Kyiv in 2022–2025.*
Wound material was collected with sterile swabs from the depth/area of active inflammation and placed in Amies transport medium (AMIES). Samples (n=63: feces -17 (27.0%) and swabs from purulent-inflammatory wounds - 46 (73.0%)) were transported at +2...+8 °C. Cultivation was performed on MacConkey agar and Endo medium with incubation at 35–37 °C for 18–24 hours; identification was performed using standard biochemical tests. Sensitivity to antimicrobial drugs was determined using the Kirby–Bauer method on Mueller–Hinton agar with interpretation according to EUCAST; ESBL was detected using the DDST method. MDR was defined as insensitivity to at least one drug in three or more classes. The data were summarized descriptively (n, %).
The study was conducted as a single-moment observational study. Of the 63 clinical samples selected, the dominant isolate was Escherichia coli - 44/63 (69.8%), while Klebsiella spp. accounted for 19/63 (30.2%). MDR was detected in 15/63 isolates (23.8%), i.e., almost every fourth isolate was characterized by multidrug resistance. The detection of Klebsiella spp. as the second most frequent group is also clinically significant, since representatives of this genus are often associated with clinically associated infections and are capable of accumulating resistance factors, which complicates treatment.
In veterinary clinics in Kyiv in 2023–2025, E. coli predominated among clinical isolates of Enterobacteriaceae, and the proportion of MDR was 23.8%. The recorded proportion of MDR indicates an increased risk of ineffective empirical antibiotic therapy and justifies the need for wider use of bacteriological testing and susceptibility testing for clinically significant cases, particularly in purulent-inflammatory lesions of the skin and soft tissues. The results confirm the need for regular local microbiological monitoring, enhanced infection control, and rational use of antimicrobial drugs in the treatment of companion animals.
Keywords: antimicrobial resistance, multidrug resistance, *Enterobacteriaceae, Escherichia coli*, *Klebsiella* spp., companion animals, veterinary clinics, One Health

The issue of antimicrobial resistance (AMR) in domestic animals is becoming increasingly relevant for several reasons: the constant use of antibacterial drugs in veterinary practice, close contact between small pet owners and their animals, and intensive microbial exchange within homes and veterinary clinics. Under these conditions, animals can act not only as a factor reflecting the circulation of resistant strains in the shared environment, but also as a potential reservoir and "bridge" for the transmission of multidrug-resistant enterobacteria between humans, animals, and the environment, making the problem cross-sectoral from a One Health perspective (Walther, 2023; Menezes, 2024; Monteiro, 2025).

The transmission of multidrug-resistant bacteria (MDR/MDRO) among dogs and cats is considered an important component of the AMR problem, as carrier animals can maintain the circulation of resistant enterobacteria in households and veterinary facilities. Enterobacterales (in particular the Enterobacteriaceae family) are of particular importance in the structure of AMR in companion animals due to their prevalence in both normal microbiota and among pathogens of various etiologies (skin and soft tissue infections, postoperative complications). At the same time, they are characterized by a high capacity for horizontal transfer of resistance genes, which contributes to the formation of MDR/MDRO profiles. At the molecular level, the spread of plasmid-associated determinants plays a key role, primarily extended-spectrum β-lactamases (ESBL) and AmpC (Formenti, 2021; Menezes, 2024).

A systematic review of risk factors for antimicrobial-resistant Enterobacterales in dogs highlights the importance of previous antibiotic use, hospitalisation, and certain clinical conditions that increase the likelihood of detecting resistant isolates (Karalliu, 2024). The emergence of carbapenemase-producing *Enterobacteriaceae* in small companion animals is a particular concern, as such resistance mechanisms are associated with antimicrobial drugs that are critical for treatment and may reflect intense selective pressure in the clinical setting. It has been noted that the link between inadequate infection control in veterinary clinics correlates with environmental contamination with carbapenemase-producing *Enterobacterales* and other MDR bacteria (Schmidt, 2020), and recent reviews and reports from various European countries confirm the growing importance of this problem in the treatment of small animals (Silva, 2022; Silva, 2024; Perreten, 2025).

Additional attention is drawn to reports of decreased sensitivity to colistin among isolates obtained from companion animals, particularly within the Enterobacter cloacae complex, which emphasizes the need for rational use of antimicrobial drugs and laboratory monitoring of treatment (Sato, 2022). According to a population study in China, MDR *Escherichia coli* was detected in 35.77% of companion animals (dogs — 40.96%, cats — 27.28%), which indicates a significant prevalence of resistant strains even outside the veterinary clinic (Teng, 2023). At the same time, the risk of colonization increases in clinical settings: in Switzerland, the proportion of MDRO-carrying animals was 15.5% on admission and 32.1% on discharge, and MDRO acquisition during treatment was recorded in 28.3% of cases, emphasizing the role of veterinary clinics as an environment for intensive selection and transmission of resistant microorganisms (Dazio, 2021b). Data from a hospital in Spain show a similar trend: the carriage of ESBL-producing *Enterobacteriaceae* in dogs increased from 25.0% on admission to 45.5% on discharge (Ortiz-Díez, 2023). Available data also confirm the possibility of MDRO transmission between humans and animals under conditions of close contact, which increases the significance of the problem within households and clinics (Dazio, 2021a; Hackmann, 2024; Menezes, 2024).

On this basis, the aim of this study is to summarize current data on the prevalence of MDR/MDRO *Enterobacterales* in companion animal practice and to identify key risk factors and areas for prevention, in particular through increased microbiological monitoring and infection control measures in veterinary hospitals (Karalliu, 2024; Monteiro, 2025).

The aim of the study was to assess the structure of clinically significant isolates of the *Enterobacteriaceae* family and the proportion of multidrug-resistant (MDR) strains in dogs and cats with clinical signs of infections of bacterial etiology in veterinary clinics in Kyiv in 2022–2025.

Materials and methods. The study was conducted in 2022–2025 in veterinary clinics in Kyiv, included dogs and cats with clinical signs of bacterial infections. Biological material was collected during routine diagnostic procedures after obtaining informed consent from the animal owners. A total of 63 clinical samples (n=63) were collected, including 17 fecal samples (n=17) and 46 samples from purulent-inflammatory wounds (n=46). The laboratory stage was carried out at the Department of Veterinary Epidemiology and Animal Health of the National University of Life and Environmental Sciences of Ukraine, using the material and technical resources of the Laboratory of Molecular Virology and Medical Microbiology with the Museum of Microorganisms Pathogenic to Humans of the State Institution "L.V. Gromashevsky Institute of Epidemiology and Infectious Diseases of the National Academy of Medical Sciences of Ukraine."

Material from purulent-inflammatory wounds was collected with sterile swabs after preliminary cleaning of the wound surface with sterile saline solution (0.9% NaCl) to remove necrotic masses and surface exudate. The sampling was performed from the depth of the wound or from the area of active inflammation in order to obtain a clinically significant isolate. Fecal samples were collected in sterile containers in accordance with standard aseptic rules. The collected material was transported to the laboratory in thermal containers with cooling elements at a temperature of 2-8 °C, while maintaining the cold chain. Immediately after collection, swabs with wound material were placed in Amies transport medium (AMIES), which ensures the viability of Gram-negative bacteria during transport.

The initial bacteriological examination included cultivation of clinical material on selective and differential diagnostic medium recommended for the isolation of enterobacteria. For this purpose, MacConkey agar was used to assess lactose fermentation and Endo medium to differentiate between lactose-positive and lactose-negative colonies. If necessary, meat peptone agar (MPA) was used to obtain isolated colonies. The cultures were incubated under aerobic conditions at 35–37 °C for 18–24 hours. After incubation, the growth and morphological characteristics of the colonies (size, shape, edges, surface, pigmentation) were evaluated, as well as the nature of growth on MacConkey agar. Biochemical identification of the isolated cultures was performed using standard tests that allow differentiation between members of the Enterobacteriaceae family. The results of the reactions were recorded after incubation at 35–37 °C in accordance with the manufacturer's instructions and generally accepted microbiological reference books.

The sensitivity of isolates to antimicrobial drugs was determined by the disc diffusion method (Kirby–Bauer) with interpretation of the results in accordance with EUCAST recommendations. The panel of tested drugs included β-lactams (ampicillin, amoxicillin/clavulanic acid), third-generation cephalosporins (cefotaxime, ceftriaxone, or ceftazidime), fluoroquinolones (enrofloxacin, marbofloxacin), aminoglycosides (gentamicin, amikacin), tetracycline, and co-trimoxazole. Phenotypic detection of extended-spectrum β-lactamases (ESBL) was performed using the double disc synergy test (DDST). Multidrug resistance (MDR) was defined as the insensitivity of an isolate to at least one antimicrobial agent in three or more classes of antimicrobial agents. The results of the study were summarized descriptively in the form of absolute (n) and relative (%) indicators, followed by presentation in tables.

Results. The data obtained indicate that in the conditions of veterinary practice in Kyiv in 2022–2025, *Escherichia coli* (44/63; 69.8%) dominated among the isolated representatives of the *Enterobacteriaceae* family, while the proportion of *Klebsiella* spp. was 19/63 (30.2%). This structure of isolates is expected for clinical material from small domestic animals, since *E. coli* is widely represented in the intestinal microbiota and is opportunistic (Formenti, 2021; Monteiro, 2025).

The detection of *Klebsiella* spp. as the second most frequent group is also clinically significant, as representatives of this genus are often associated with clinically associated infections and are capable of accumulating resistance factors, which complicates treatment (Menezes, 2024; Monteiro, 2025).

The multidrug resistance (MDR) rate of 15/63 (23.8%) reflects the presence of a significant proportion of isolates for which the choice of antimicrobial drugs is potentially narrowed. For veterinary practice, this means an increased risk of ineffective therapy and the need to focus on laboratory diagnostic results, and also highlights the importance of rational prescribing of antimicrobial agents at the clinic level (Karalliu, 2024; Monteiro, 2025). Given that the study covered material from animals with clinical signs of bacterial infection, the recorded proportion of MDR may reflect the cumulative effect of previous antibiotic use, duration, or recurrence of infection, and possible factors related to the animal's admission to the clinic (Dazio, 2021b; Ortiz-Díez, 2023).

An important factor in interpretation is the structure of the biomaterial: swabs from purulent-inflammatory wounds predominated in the sample (46/63; 73.0%), while fecal samples accounted for 17/63 (27.0%). Skin and soft tissue infections, especially chronic or complicated ones, are often accompanied by mixed infection, surface contamination, and the formation of microbial communities, which can support the persistence of pathogens and increase the likelihood of detecting strains with acquired resistance (Monteiro, 2025).

These findings are of practical significance in the context of the One Health concept, since companion animals can act as reservoirs and epidemiological “bridges” facilitating the circulation of resistant Enterobacterales between households and veterinary clinics (Walther, 2023; Hackmann, 2024; Menezes, 2024). This reinforces the argument for systematic control in veterinary clinics: hand hygiene, routine disinfection of surfaces and instruments, separation of patient flows, proper handling pathogenic material (Schmidt, 2020; Dazio, 2021b; Monteiro, 2025).

The limitations of the study include the local nature of the sample , the moderate volume and uneven distribution of material types, which may affect the generalisation of results to other regions and other categories of patients. In addition, the lack of a detailed analysis of sensitivity profiles for each drug for all isolates limits the ability to detail practical recommendations for the choice of therapy; However, even under these conditions, the established proportion of MDR emphasizes the need for further studies with broader coverage and standardized data collection on antimicrobial susceptibility and resistance mechanisms (Karalliu, 2024; Menezes, 2024; Monteiro, 2025).

Conclusions. Escherichia coli dominated among the isolated enterobacteria — 44/63 (69.8%), while *Klebsiella* spp. accounted for 19/63 (30.2%), which characterizes the local microbiological profile of Enterobacteriaceae in clinical cases of bacterial infections in the city.

Multidrug resistance (MDR) was detected in 15/63 (23.8%) isolates, i.e., in almost every fourth case, which emphasizes the presence in practice of a significant proportion of strains with a potentially limited choice of antimicrobial agents. The recorded proportion of MDR indicates an increased risk of ineffective empirical antibiotic therapy and justifies the need for wider use of bacteriological testing and susceptibility testing for clinically significant cases, particularly in purulent-inflammatory lesions of the skin and soft tissues.

The results obtained indicate the importance of a systematic approach to combating AMR in veterinary institutions, which should include the rational use of antimicrobial drugs. Standardization of infection control measures (hand hygiene, disinfection, proper handling of wound material and excreta).

Further research should focus on expanding the sample (other cities/regions), balancing the structure of the material, and supplementing phenotypic screening (in particular ESBL) with molecular methods to clarify the mechanisms of resistance and assess possible routes of spread within the One Health approach.

REFERENCES

1. Dazio V., Nigg A., Schmidt J. S., Brilhante M., Campos-Madueno E. I., Mauri N., Kuster S. P., Endimiani A. (2021a). Duration of carriage of multidrug-resistant bacteria in dogs and cats in veterinary care and co-carriage with their owners. *One Health; 13:*100322. https://doi.org/10.1016/j.onehlt.2021.100322
2. Dazio V., Nigg A., Schmidt J. S., Brilhante M., Mauri N., Kuster S. P., Endimiani A. (2021b). Acquisition and carriage of multidrug-resistant organisms in dogs and cats presented to small animal practices and clinics in Switzerland. *Journal of Veterinary Internal Medicine; 35*(2):970–979. https://doi.org/10.1111/jvim.16038
3. Formenti N., Grassi A., Parisio G., Romeo C., Guarneri F., Birbes L., Boniotti M. B. (2021). Extended-spectrum-β-lactamase- and AmpC-producing *Escherichia coli* in domestic dogs: Spread, characterisation and associated risk factors. *Antibiotics; 10*(10):1251. https://doi.org/10.3390/antibiotics10101251
4. Hackmann C., Genath A., Gruhl D., Weber A., Maechler F., Kola A., Bender J. K. (2024). The transmission risk of multidrug-resistant organisms between hospital patients and their pets—a case-control study, Germany, 2019 to 2022. *Eurosurveillance; 29*(39):2300714. https://doi.org/10.2807/1560-7917.ES.2024.29.39.2300714
5. Hong J. S., Song W., Jeong S. H. (2020). Molecular characteristics of NDM-5-producing *Escherichia coli* from a cat and a dog in South Korea. *Microbial Drug Resistance; 26*(8):1005–1008. https://doi.org/10.1089/mdr.2019.0382
6. Karalliu E., Chung K. Y., MacKinnon B., Haile B., Beczkowski P. M., Barrs V. R., Norris J. M. (2024). Risk factors for antimicrobial-resistant Enterobacterales in dogs: A systematic review. *Frontiers in Veterinary Science; 11:*1447707. https://doi.org/10.3389/fvets.2024.1447707
7. Menezes J., Frosini S.-M., Weese S., Perreten V., Schwarz S., Amaral A. J., Pomba C. (2024). Transmission dynamics of ESBL/AmpC and carbapenemase-producing Enterobacterales between companion animals and humans. *Frontiers in Microbiology; 15:*1432240. https://doi.org/10.3389/fmicb.2024.1432240
8. Monteiro H. I. G., Silva V., de Sousa T., Calouro R., Saraiva S., Igrejas G., Poeta P. (2025). Antimicrobial resistance in European companion animals practice: A One Health approach. *Animals; 15*(12):1708. https://doi.org/10.3390/ani15121708

9. Ortiz-Díez G., Rodríguez-Baño J., Mora A., López-Cerero L., Pascual Á., González-Zorn B. (2023). Prevalence, incidence and risk factors for acquisition and colonization of extended-spectrum beta-lactamase- and carbapenemase-producing Enterobacteriaceae from dogs attended at a veterinary hospital in Spain. *Comparative Immunology, Microbiology and Infectious Diseases; 92:*101922. https://doi.org/10.1016/j.cimid.2022.101922

10. Perreten V., Endimiani A. (2025). Carbapenemase-producing Enterobacterales in pets: A new threat from veterinary clinics. *Clinical Infectious Diseases*. Advance online publication. https://doi.org/10.1093/cid/ciaf544

11. Roscetto E., Varriale C., Galdiero U., Esposito C., Catania M. R. (2021). Extended-spectrum beta-lactamase-producing and carbapenem-resistant Enterobacterales in companion and animal-assisted interventions dogs. *International Journal of Environmental Research and Public Health; 18:*12952. https://doi.org/10.3390/ijerph182412952

12. Sato T., Harada K., Usui M., Yokota S.-I., Horiuchi M. (2022). Colistin susceptibility in companion animal-derived *Escherichia coli*, *Klebsiella* spp., and *Enterobacter* spp. in Japan: Frequent isolation of colistin-resistant *Enterobacter cloacae* complex. *Frontiers in Cellular and Infection Microbiology; 12:*946841. https://doi.org/10.3389/fcimb.2022.946841

13. Schmidt J. S., Kuster S. P., Nigg A., Brilhante M., Mauri N., Endimiani A. (2020). Poor infection prevention and control standards are associated with environmental contamination with carbapenemase-producing Enterobacterales and other multidrug-resistant bacteria in Swiss companion animal clinics. *Antimicrobial Resistance & Infection Control; 9*:93. https://doi.org/10.1186/s13756-020-00742-5

14. Silva J. M. D., Menezes J., Fernandes L., Costa S. S., Amaral A., Pomba C. (2024). Carbapenemase-producing Enterobacterales strains causing infections in companion animals—Portugal. *Microbiology Spectrum; 12*(4):e03416-23. https://doi.org/10.1128/spectrum.03416-23

15. Silva J. M. D., Menezes J., Marques C., Pomba C. F. (2022). Companion animals—an overlooked and misdiagnosed reservoir of carbapenem resistance. *Antibiotics; 11:*533. https://doi.org/10.3390/antibiotics11040533

16. Teng L., Feng M., Liao S., Zheng Z., Jia C., Zhou X., Wang Y. (2023). A cross-sectional study of companion animal-derived multidrug-resistant *Escherichia coli* in Hangzhou, China. *Microbiology Spectrum; 11:*e02113-22. https://doi.org/10.1128/spectrum.02113-22

17. Walther B., Schaufler K., Wieler L. H., Lübke-Becker A. (2023). Zoonotic and multidrug-resistant bacteria in companion animals challenge infection medicine and biosecurity. In A. Sing (Ed.), *Zoonoses: Infections affecting humans and animals*. Springer. https://doi.org/10.1007/978-3-031-27164-9_17

Мультирезистентні ентеробактерії у ветеринарній практиці: сучасні виклики

Мурашко О.І.[1], Мельник В.В.[2] (ORCID: 0000-0002-6958-2577)
1 – ДУ «Інститут епідеміології та інфекційних хвороб ім. Л.В. Громашевського НАМН України», м. Київ, Україна, e-mail: sunsetmur@gmail.com
2 – Національний університет біоресурсів і природокористування України, м. Київ, Україна

Резюме. *Антимікробна резистентність у тварин-компаньйонів має зростаюче значення в межах підходу One Health через тісний контакт людей і домашніх тварин та ризик циркуляції мультирезистентних збудників у домогосподарствах і ветеринарних клініках. Метою дослідження було оцінити структуру клінічно значущих ізолятів родини Enterobacteriaceae та частку мультирезистентних (MDR) штамів у собак і котів із клінічними ознаками інфекцій бактеріальної етіології у ветеринарних клініках м. Київ у 2022–2025 рр. Дослідження виконано як одномоментне обсерваційне. Відібрано 63 клінічні зразки (n=63): фекалії — 17 (27,0%) та мазки з гнійно-запальних ран — 46 (73,0%). Зразки транспортували при +2…+8 °C; рановий матеріал відбирали стерильними тампонами з глибини/ділянки активного запалення та поміщали в транспортне середовище Amies (AMIES). Культивування проводили на агарі МакКонкі та середовищі Ендо з інкубацією 35–37 °C протягом 18–24 год; ідентифікацію здійснювали стандартними біохімічними тестами. Чутливість до антимікробних препаратів визначали методом Kirby–Bauer на агарі Мюллера–Гінтона з інтерпретацією за EUCAST; ESBL виявляли методом DDST. MDR визначали як нечутливість щонайменше до одного препарату в трьох або більше* *класах. Дані узагальнювали описово (n, %). У результаті домінуючим ізолятом була Escherichia coli — 44/63 (69,8%), тоді як Klebsiella spp. становила 19/63 (30,2%). MDR виявлено у 15/63 ізолятів (23,8%), тобто майже кожен четвертий ізолят характеризувався мультирезистентністю.*

У ветеринарних клініках м. Київ у 2023–2025 рр. серед клінічних ізолятів Enterobacteriaceae переважала E. coli, а частка MDR становила 23,8%. Результати підтверджують потребу в регулярному локальному мікробіологічному моніторингу, посиленні інфекційного контролю та раціональному застосуванні антимікробних препаратів у практиці лікування тварин-компаньйонів.

Ключові слова: антимікробна резистентність, мультирезистентність, *Enterobacteriaceae*, *Escherichia coli*, *Klebsiella* spp., тварини-компаньйони, ветеринарні клініки, One Health.

DOI: 10.31073/onehealthjournal2026-III-01

DOI: 10.31073/onehealthjournal2026-III-02

PCR-based detection of *Erysipelothrix rhusiopathiae* using EvaGreen real-time PCR assay

Gerilovych A.[1, 2] (ORCID: 0000-0002-3280-4172), Korovin I.[1, 3], Vashchyk Ye. [3]
1 – PSI One Health Scientific and Research Institute, Kharkiv, Ukraine
2 – Institute for Problems of Cryobiology and Cryomedicine of NAS of Ukraine, Kharkiv, Ukraine
3 – National Scientific Center «Institute of Experimental and Clinical Veterinary Medicine», Kharkiv, Ukraine, email: korovin.vetbio@gmail.com

Abstract. *The present study aimed to develop and evaluate an EvaGreen real-time PCR assay for rapid, sensitive, and specific detection of Erysipelothrix rhusiopathiae, the causative agent of swine erysipelas and an important zoonotic pathogen.*
Species-specific primers targeting the spaA gene of E. rhusiopathiae were used for amplification on the iTower real-time PCR platform (Jena Analytik, Germany). Reaction conditions were optimized using EvaGreen fluorescent chemistry. Analytical sensitivity was determined using ten-fold serial dilutions of purified genomic DNA, while specificity was evaluated against non-target bacterial species commonly associated with swine infections. Clinical samples obtained from pigs with suspected erysipelas, including tonsils, spleen, lymph nodes, heart valves, blood, and synovial fluid, were tested to assess diagnostic applicability.
The developed assay successfully detected E. rhusiopathiae DNA in all positive controls and field-positive samples. The limit of detection was established at 10 fg/µL. Standard curve analysis demonstrated excellent linearity (R^2 = 0.998) and high amplification efficiency (98.4%). No amplification was observed with non-target bacterial DNA, confirming high analytical specificity. All positive samples produced a single characteristic melting peak at 81.7 ± 0.3°C, indicating specific amplification. Among 48 field specimens, 19 samples (39.6%) tested positive, with the highest detection rates observed in tonsillar and splenic tissues. Intra- and inter-assay variation remained below 3%, confirming strong repeatability and reproducibility.
The EvaGreen real-time PCR assay is a rapid, reliable, and cost-effective molecular method for detection of E. rhusiopathiae. Its high sensitivity, specificity, and robust performance make it suitable for routine veterinary diagnostics, outbreak investigations, and epidemiological surveillance of swine erysipelas. The assay may serve as an effective alternative to probe-based real-time PCR systems, particularly in laboratories with limited resources.
Keywords: *Erysipelothrix rhusiopathiae*; swine erysipelas; EvaGreen; real-time PCR; molecular diagnostics; *spaA* gene.

Erysipelothrix rhusiopathiae is a Gram-positive, non-spore-forming, facultatively intracellular bacterium with worldwide distribution and substantial veterinary as well as zoonotic importance. It is recognized as the principal causative agent of erysipelas in swine, a disease characterized by acute septicemia, urticarial cutaneous lesions (“diamond skin disease”), chronic arthritis, and endocarditis, resulting in considerable economic losses to the pig industry through mortality, reduced productivity, and carcass condemnation (Wood, 1999; Opriessnig, 2011). In addition to swine, the pathogen has been isolated from poultry, sheep, fish, reptiles, and numerous wildlife species, demonstrating a broad host spectrum and ecological adaptability (Brooke, 1999, Zhao, 2023).
Beyond its veterinary relevance, *E. rhusiopathiae* is an established zoonotic pathogen capable of causing localized erysipeloid, generalized cutaneous infection, and occasionally severe systemic disease such as septicemia or infective endocarditis in humans. Occupational exposure remains the principal risk factor, particularly among farmers, butchers, veterinarians, fishermen, and abattoir workers who frequently handle infected animals or contaminated animal products (Wang, 2010; Milton, 2025; Rajkhowa, 2023). The One Health significance of the organism is therefore increasingly recognized, linking animal health, food safety, occupational medicine, and environmental hygiene.
The epidemiology of *E. rhusiopathiae* is further complicated by its ability to persist in asymptomatic carrier animals and survive for prolonged periods in the environment. Clinically healthy pigs may harbor the bacterium in tonsillar tissues and shed the organism intermittently, serving as a continuous source of infection within herds (Wood, 1999). Environmental persistence

in soil, slurry, and organic matter enhances indirect transmission and contributes to recurrent outbreaks, especially under conditions of inadequate sanitation, stress, overcrowding, or concurrent infections (Opriessnig, 2011).

Traditional laboratory diagnosis is based on bacterial isolation, microscopic examination, biochemical characterization, and serological methods. However, culture-based identification may be hindered by slow growth, overgrowth by contaminating flora, prior antimicrobial treatment, or low bacterial concentration in samples. These limitations can delay diagnosis and compromise timely disease control measures (Makino, 1994).

Molecular diagnostic approaches, particularly polymerase chain reaction (PCR), have markedly improved the detection of *E. rhusiopathiae* by providing rapid, sensitive, and highly specific identification directly from clinical specimens. PCR assays targeting genes such as 16S rRNA**,** spaA**,** and other species-specific markers are widely used for confirmation of infection, differentiation from related bacteria, and epidemiological investigations (Makino, 1994; Pal, 2010; Nishikawa, 2022). Real-time PCR platforms further enhance diagnostic efficiency by enabling quantitative detection, reduced contamination risk, and high-throughput screening suitable for veterinary surveillance programs.

Considering the continuing economic burden of erysipelas, the zoonotic potential of the pathogen, and the necessity for rapid outbreak response, PCR-based methods have become indispensable tools in modern veterinary diagnostics. Therefore, the application and optimization of PCR for detection of *E. rhusiopathiae* remain highly relevant for disease monitoring, herd health management, and integrated One Health biosecurity strategies.

Therefore, the **aim of this study** was to develop and validate a real-time PCR assay based on EvaGreen chemistry using the iTOWER platform (Jena Analytik) for the detection of *E. rhusiopathiae* DNA targeting the *spaA* gene. The proposed method is intended to improve diagnostic efficiency and support integrated disease monitoring within the One Health framework.

Materials and Methods. The study was conducted to develop and evaluate a real-time PCR assay for the detection of *Erysipelothrix rhusiopathiae* using EvaGreen fluorescent chemistry. Reference strains of *E. rhusiopathiae* obtained from a certified microbial collection were used as positive controls. To assess analytical specificity, non-target bacterial species commonly associated with swine infections or capable of causing similar clinical manifestations were included, such as *Streptococcus suis*, *Staphylococcus aureus*, *Escherichia coli*, *Pasteurella multocida*, and *Trueperella pyogenes*.

Clinical samples were collected from pigs showing signs consistent with swine erysipelas, including fever, skin lesions, lameness, arthritis, and sudden death. Specimens included tonsils, spleen, liver, heart valves, lymph nodes, synovial fluid, and blood. Tissue samples were collected aseptically during necropsy and transported to the laboratory under refrigerated conditions (4°C) for further processing.

Sample preparation and DNA extraction. Approximately 25 mg of tissue was homogenized in sterile phosphate-buffered saline using disposable homogenizers. Blood and synovial fluid samples were vortexed thoroughly before extraction. Genomic DNA was extracted using a commercial silica membrane-based purification kit according to the manufacturer’s instructions. DNA was eluted in 100 µL of nuclease-free water and stored at −20°C until analysis.

DNA concentration and purity were measured spectrophotometrically at 260/280 nm. Only samples with acceptable purity ratios (1.7–2.0) were included for PCR testing.

Primer design. Species-specific primers were selected from conserved regions of the *spaA* gene encoding surface protective antigen A, commonly associated with virulent strains of *E. rhusiopathiae*. Primer sequences were analyzed for specificity using nucleotide database alignment tools and synthesized commercially.

Table 1

Primer set for *E. rhusiopathiae* target gene sequence amplifcation

Primer	Sequence (5′–3′)	Target Gene
ERH-F	AGT TTA CGC TGA TGA AGG TG	*spaA*
ERH-R	TCA ATC CTT GCA TTT CCA GC	*spaA*

Expected amplicon size by using of selected primers was 128 bp.

Real-time PCR assay. Amplification was performed using an iTower real-time PCR system (Jena Analytik, Germany) in a final reaction volume of 20 µL containing:

- 10 µL 2× EvaGreen qPCR Master Mix
- 0.4 µM forward primer
- 0.4 µM reverse primer
- 2 µL template DNA
- nuclease-free water to final volume

Thermal cycling conditions were as follows:

Initial denaturation: 95°C for 5 min, and 40 cycles of denaturation: 95°C for 15 s, annealing: 58°C for 20 s and extension: 72°C for 20 s

Fluorescence acquisition was performed at the end of each extension step.

Melting curve analysis. Following amplification, melt curve analysis was carried out from 65°C to 95°C with increments of 0.2°C every 5 s to verify product specificity. A single sharp melting peak was interpreted as specific amplification of the target fragment.

Analytical sensitivity. To determine the limit of detection (LOD), ten-fold serial dilutions of purified *E. rhusiopathiae* DNA were prepared from 10 ng/µL to 1 fg/µL. Each dilution was tested in triplicate. The lowest concentration consistently detected in all replicates was considered the analytical detection limit.

Analytical specificity. DNA extracted from non-target bacterial species was tested under identical reaction conditions to evaluate cross-reactivity. Absence of amplification or non-specific melt peaks was interpreted as assay specificity.

Standard curve and PCR efficiency. A standard curve was generated using serial DNA dilutions by plotting threshold cycle (Ct) values against the logarithm of DNA concentration. Amplification efficiency (E) was calculated using the formula:

$$E = (10^{-1/slope} - 1) \times 100\%$$

The correlation coefficient (R^2) was used to assess linearity of amplification.

Repeatability and reproducibility. Intra-assay repeatability was determined by testing three DNA concentrations in triplicate within a single run. Inter-assay reproducibility was evaluated by repeating experiments on three separate days. Mean Ct values, standard deviations, and coefficients of variation were calculated.

Data interpretation. Samples with Ct values ≤35 and a specific melting temperature corresponding to the positive control were considered positive. Samples with Ct values >35 were retested. No-template controls were included in each run to monitor contamination.

Statistical analysis. Descriptive statistics, regression analysis, and calculation of mean Ct values were performed using Microsoft Excel and statistical software. Differences were considered significant at $p < 0.05$ where applicable.

Results. *Performance of the EvaGreen real-Time PCR assay.* The developed EvaGreen real-time PCR assay successfully detected *Erysipelothrix rhusiopathiae* DNA in all positive control samples and in clinically suspected field specimens containing the target organism. Amplification curves demonstrated typical sigmoidal kinetics with clear fluorescence separation from the baseline and no signal in no-template controls.

The assay generated reproducible amplification over a wide dynamic range of DNA concentrations. Positive reactions were consistently observed from 10 ng/µL to the lowest detectable dilution, indicating high analytical sensitivity.

Analytical sensitivity and standard curve analysis. Ten-fold serial dilutions of purified *E. rhusiopathiae* DNA were used to determine the analytical sensitivity and amplification efficiency. The assay consistently detected target DNA down to 10 fg/µL, which was established as the limit of detection (LOD). At concentrations below this level, amplification became inconsistent and Ct values were highly variable.

A linear relationship was observed between Ct values and logarithmic DNA concentration across six dilution points. Standard curve analysis demonstrated excellent linearity with a correlation coefficient of $R^2 = 0.998$, indicating reliable quantitative performance. The slope of the standard curve was −3.36**,** corresponding to a PCR efficiency of 98,4 % (table 2).

$$E = (10^{-1/(-3.36)} - 1) \times 100\% = 98.4\%$$

These values indicate optimal amplification characteristics for diagnostic real-time PCR.

Table 2

Analytical sensitivity and standard curve parameters

Parameter	**Value**
Dynamic range	10 ng/μL – 10 fg/μL
Limit of detection (LOD)	10 fg/μL
Slope	−3.36
Efficiency (E%)	98.4%
Correlation coefficient (R²)	0.998

Specificity of the assay. No amplification signals were observed from DNA extracted from non-target bacterial species including *Streptococcus suis*, *Staphylococcus aureus*, *Escherichia coli*, *Pasteurella multocida*, and *Trueperella pyogenes*. These findings confirmed high analytical specificity of the primer pair and absence of cross-reactivity under optimized reaction conditions.

Melting curve analysis. All positive *E. rhusiopathiae* samples produced a single sharp melting peak at 81.7 ± 0.3°C, confirming specific amplification of the intended amplicon. No secondary peaks, primer-dimer signals, or abnormal melting profiles were detected.

*Detection in clinical samples.*A total of 48 field samples from pigs with suspected erysipelas were examined. Of these, 19 samples (39.6%) were positive by EvaGreen real-time PCR. Positive detections were most frequent in tonsils, spleen, lymph nodes, and heart valve tissues. Blood samples showed lower positivity rates, particularly in animals sampled after antimicrobial treatment (table 3).

Table 3.

Detection of *E. rhusiopathiae* in field specimens

Sample type	**Number tested**	**Positive**	**Positivity (%)**
Tonsils	12	7	58.3
Spleen	8	4	50.0
Lymph nodes	7	3	42.9
Heart valves	5	2	40.0
Blood	10	2	20.0
Synovial fluid	6	1	16.7
Total	**48**	**19**	**39.6**

Repeatability and reproducibility. The assay showed strong repeatability with intra-assay coefficient of variation (CV) values ranging from 0.8% to 1.9%. Inter-assay reproducibility across three independent runs demonstrated CV values between 1.4% and 2.7%, indicating robust assay stability.

Overall diagnostic utility. The EvaGreen real-time PCR assay provided rapid detection of E. rhusiopathiae within approximately 70 minutes, including amplification and melt curve analysis. The combination of high sensitivity, excellent specificity, and reproducible performance supports its suitability for routine veterinary diagnostics, outbreak investigations, and surveillance of swine erysipelas.

Conclusions. The developed EvaGreen real-time PCR assay proved to be a rapid, sensitive, and specific method for the detection of *Erysipelothrix rhusiopathiae*. The assay reliably identified target DNA over a broad dynamic range and demonstrated excellent analytical performance, including a low detection limit, high amplification efficiency, and strong linearity of the standard curve.

Melting curve analysis confirmed the specificity of amplification by producing a single characteristic melting peak for positive samples, while no cross-reactivity was observed with non-target bacterial species commonly associated with swine infections. These findings indicate that the selected primers and optimized reaction conditions are suitable for accurate laboratory diagnosis.

Application of the assay to clinical field samples enabled efficient identification of infected animals, with the highest detection rates observed in tonsils and internal organs, supporting the known role of carrier pigs and systemic dissemination during disease. The method also showed high repeatability and reproducibility, confirming its robustness for routine diagnostic use.

Overall, the EvaGreen real-time PCR assay represents a practical and cost-effective molecular tool for veterinary laboratories, particularly where probe-based systems are less accessible. Its implementation can significantly improve early diagnosis, outbreak response, epidemiological surveillance, and biosecurity management of swine erysipelas. Further validation using larger sample sets and comparison with bacteriological culture methods would strengthen its application in national monitoring programs and integrated One Health disease control strategies.

REFERENCES

1. Brooke C. J., Riley T. V. (1999). *Erysipelothrix rhusiopathiae*: Bacteriology, epidemiology and clinical manifestations of an occupational pathogen. *Journal of Medical Microbiology, 48*(9), 789–799. https://doi.org/10.1099/00222615-48-9-789
2. Gerilovych A. P. (2011). *Eksperymentalne i teoretychne obgruntuvannia ta rozrobka zasobiv epizootolohichnoho monitorynhu, diahnostyky virusnykh khvorob tvaryn ta molekuliarno-henetychnoho typuvannia yikh zbudnykiv (ortomikso-, paramikso-, herpes-, tsyrko- ta pestyvirusna infektsii)* [Experimental and theoretical substantiation and development of means of epizootological monitoring, diagnostics of viral diseases of animals and molecular genetic typing of their pathogens (orthomyxo-, paramyxo-, herpes-, circo- and pestivirus infections)] (Doctoral dissertation summary, National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine"). Kharkiv, Ukraine.
3. Makino S., Okada Y., Maruyama T., Ishikawa K., Takahashi T., Nakamura M., Ezaki T., Morita H. (1994). Direct and rapid detection of *Erysipelothrix rhusiopathiae* DNA in animals by PCR. *Journal of Clinical Microbiology, 32*(6), 1526–1531.
4. Milton A. A. P., Khan S., Srinivas K., Basar T., Hussain Z., Priya G. B., Saminathan M., Puii L. H., Ghatak S., Das S. (2025). Novel multiplex PCR approach for the detection of *Erysipelothrix rhusiopathiae*, *Streptococcus suis*, and *Staphylococcus hyicus* in swine. *Frontiers in veterinary science*, *12*, 1605316. https://doi.org/10.3389/fvets.2025.1605316
5. Nishikawa S., Shiraiwa K., Shimoji Y. (2022). A PCR assay to specifically detect serovar 1a strains of Erysipelothrix rhusiopathiae and differentiate them from serovar 2 strains possessing an intact ERH_1440 gene. *The Journal of veterinary medical science*, *84*(1), 90–93. https://doi.org/10.1292/jvms.21-0528
6. Opriessnig T., Wood R. L., Eamens G. J. (2011). *Erysipelas*. In B. E. Straw J. J. Zimmerman, S. D'Allaire D. J. Taylor (Eds.), *Diseases of Swine* (10th ed., pp. 750–759). Wiley-Blackwell.
7. Pal N., Bender J. S., Opriessnig T. (2010). Rapid detection and differentiation of *Erysipelothrix* species by multiplex real-time PCR. *Journal of Applied Microbiology, 108*(1), 285–294. https://doi.org/10.1111/j.1365-2672.2009.04400.x
8. Rajkhowa S., Sonowal J., Borthakur U., Pegu S. R., Deb R., Das P. J., Sengar G. S., Gupta V. K. (2023). Meta-Analysis of the Prevalence of Porcine Zoonotic Bacterial Pathogens in India: A 13-Year (2010-2023) Study. *Pathogens (Basel, Switzerland)*, *12*(10), 1266. https://doi.org/10.3390/pathogens12101266
9. Wang Q., Chang B. J., Riley T. V. (2010). *Erysipelothrix rhusiopathiae*. *Veterinary Microbiology, 140*(3–4), 405–417. https://doi.org/10.1016/j.vetmic.2009.08.012
10. World Organisation for Animal Health. (2021). *Manual of diagnostic tests and vaccines for terrestrial animals* (8th ed.). https://www.woah.org/en/what-we-do/standards/codes-and-manuals/#chapter/?rid=298&volume_no=3&ismanual=true&language=102&standard_type=6&animal_type=7
11. Zhao L., Wen X. H., Jia C. L., Zhou X. R., Luo S. J., Lv D. H., Zhai Q. (2023). Development of a multiplex qRT-PCR assay for detection of classical swine fever virus, African swine fever virus, and *Erysipelothrix rhusiopathiae*. *Frontiers in veterinary science*, *10*, 1183360. https://doi.org/10.3389/fvets.2023.1183360.

Виявлення *Erysipelothrix rhusiopathiae* методом ПЛР у реальному часі із використанням EvaGreen

Герілович А.[1, 2] (ORCID: 0000-0002-3280-4172), Коровін І.[1, 3], Ващик Є.[3]
1 – ПНУ «Науково-дослідний інститут єдиного здоров'я», м. Харків, Україна
2 – Інститут проблем кріобіології та кріомедицини НАН України, м. Харків, Україна
3 – Національний науковий центр «Інститут експериментальної і клінічної ветеринарної медицини», м. Харків, Україна, електронна пошта: korovin.vetbio@gmail.com

***Резюме**. Метою даного дослідження було розроблення та оцінка методу ПЛР у реальному часі з використанням EvaGreen для швидкого, чутливого та специфічного виявлення Erysipelothrix rhusiopathiae, збудника бешихи свиней та важливого зоонозного патогену.*
Для ампліфікації на платформі ПЛР у реальному часі iTower (Jena Analytik, Німеччина) використовували видоспецифічні праймери, спрямовані на ген spaA E. rhusiopathiae. Умови реакції були оптимізовані з використанням флуоресцентного барвника EvaGreen. Аналітичну чутливість визначали за допомогою десятикратних послідовних розведень очищеної геномної ДНК, тоді як специфічність оцінювали щодо нецільових видів бактерій, які зазвичай зумовлюють інфекції у свиней. Клінічні зразки, отримані від свиней з підозрою на бешиху, включаючи мигдалики, селезінку, лімфатичні вузли, серцеві клапани, кров та синовіальну рідину, були протестовані для оцінки діагностичної застосовності.
Розроблений тест успішно виявив ДНК E. rhusiopathiae у всіх позитивних контролях та польових позитивних зразках. Межа виявлення була встановлена на рівні 10 фг/мкл. Аналіз стандартної кривої продемонстрував чудову лінійність (R^2 = 0,998) та високу ефективність ампліфікації (98,4%). Ампліфікації не спостерігалося з ДНК нецільових бактерій, що підтвердило високу аналітичну специфічність. Усі позитивні зразки дали один характерний пік плавлення при 81,7 ± 0,3 °C, що вказує на специфічну ампліфікацію. Серед 48 польових зразків 19 (39,6 %) дали позитивний результат, причому найвищі показники виявлення спостерігалися у тканинах мигдаликів та селезінки. Внутрішньо- та міжтестова варіація залишалася нижче 3 %, що підтверджує високу повторюваність та відтворюваність.
Аналіз EvaGreen у режимі реального часу є швидким, надійним та економічно ефективним молекулярним методом виявлення E. rhusiopathiae. Його висока чутливість, специфічність та надійність роблять його придатним для рутинної ветеринарної діагностики, розслідування спалахів та епідеміологічного нагляду за еризепелою свиней. Цей аналіз може слугувати ефективною альтернативою системам ПЛР у режимі реального часу на основі зондів, особливо в лабораторіях з обмеженими ресурсами.
Ключові слова: *Erysipelothrix rhusiopathiae*; бешиха свиней; EvaGreen; ПЛР у реальному часі; молекулярна діагностика; ген spaA.

DOI: 10.31073/onehealthjournal2026-III-02

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

DOI: 10.31073/onehealthjournal2026-III-03

Monitoring of radiation contamination of wild mushrooms in the Polissia region

Kochetova H. (ORCID: 0000-0003-3234-1355), Prokopenko T. (ORCID: 0000-0003-3234-1355), Gusak L. (ORCID: 0000-0001-7570-2574), Molodyk A. (ORCID: 0009-0003-9719-5560)
State Scientific and Research Institute for Laboratory Diagnostics and Veterinary and Sanitary Expertise, Kyiv, Ukraine, email: kochetovag@ukr.net

Abstract. *Radiological control plays a key role in ensuring the safety of food products, especially freshly picked wild mushrooms, which actively absorb radioactive elements, in particular Cesium-137 and Strontium-90. Because of this ability, mushrooms can pose a significant threat if radiation contamination levels are not properly controlled. Scientific studies show that the ecological features of Polissia, in particular the sandy soils of this region, contribute to the accumulation of radionuclides in the upper layers of the earth. The natural migration of such pollutants leads to their concentration in wild mushrooms. Mechanisms of migration and accumulation of radionuclides are complex processes that depend on many factors. Among them, the most important are the type of soil, climatic conditions of the territory, reclamation measures, methods of land use, as well as the biological specificity of vegetation. At the same time, the situation is aggravated by modern socio-ecological challenges, such as military actions and mass fires. These phenomena disturb the upper layers of the soil, causing the release of previously bound radionuclides from contaminated areas, in particular those affected by the accident at the Chernobyl nuclear power plant. Radioactive dust and ash rise into the air and are carried over considerable distances, settling even in regions that were previously considered ecologically clean. This creates an additional threat to the safety of food products and forest resources in "clean" zones. In response to these challenges, Ukraine introduced a food safety control system designed to reduce the impact of radiation on the population. Its key component has become regular radiological monitoring of food products, which allows for prompt assessment of the level of radiation contamination and timely response to potential risks. In addition, radiological control performs an important function of raising public awareness and ensuring environmental and industrial safety.*
Keywords: radiological control, fresh wild mushrooms, Cesium-137, Strontium-90, radiation load, environment, accumulation.

Even decades after the accident at the Chernobyl nuclear power plant, the issue of radiological control of food products of plant and animal origin remains extremely relevant and important both for science and for practical activities. Since radioactive pollution of the environment remains one of the biggest environmental hazards, because its consequences can affect not only the environment but also human health for a long time.

According to statistical data, two artificial radionuclides — Cesium-137 and Strontium-90 — stand out among the most dangerous sources of biosphere pollution, including food products. The harmful effect of which is primarily caused by a long half-life: for Cesium-137 it is 30 years, and for Strontium-90 it is 29 years. Therefore, the research conducted in this field will continue to be relevant because it has practical and scientific value and is aimed at protecting the population from the consequences of radioactive pollution. In particular, systematic monitoring of food products, especially products of forest origin, is a key measure to reduce the level of radiation exposure to humans, as it prevents food products from entering the market that do not meet safety standards (Gudkov, 2012; Zdrojewicz, 2016; Kashparov, 2019; Malimon, 2023).

Considering all of the above, the goal of our research was to establish the current state of safety of fresh wild mushrooms, regarding the content of Cesium-137 and Strontium-90 radionuclides collected on the territory of the Polissia region in the context of food safety. Because it is scientifically stated that the unique ecosystem of Polissia contributes to the accumulation of radionuclides in plants, primarily Cesium-137, by their migration from the soil. For example, soils are able to retain up to 87% of the radionuclide Cesium-137, and shrubs, herbaceous plants, and mushrooms are able to accumulate almost 90% of radionuclides (Dutov, 2015; Jeskovsky, 2019; Kotelevych, 2019).

Materials and methods. Radiological control of forest food products was carried out by the method of spectrometric analysis. To determine the specific activity of radionuclides, universal spectrometric complexes "Gamma-Plus" with "Progress" software were used.

The obtained results were analyzed and systematized using generally accepted statistical and analytical methods. All measurements and data are presented according to International System of Units (SI) standards.

Results and their discussion. On the basis of the of the Research Radiology Department of the State Scientific and Research Institute for Laboratory Diagnostics and Veterinary and Sanitary Expertise, the results of research obtained with the participation of regional state laboratories of the State Production and Consumer Service of Volyn, Kyiv, Zhytomyr and Rivne regions were studied, systematized and analyzed.

It was established that despite the decades that have passed since the accident at the Chernobyl NPP and the natural decay of radionuclides in the environment, wild mushrooms continue to intensively absorb radionuclides from the soil as in previous years, while remaining the main source of internal exposure of the population.

It is shown the regions in a section of regions in the territories of which exceeding the permissible levels of the radionuclide Cesium-137 in samples of fresh wild mushrooms was most often detected (Fig.1).

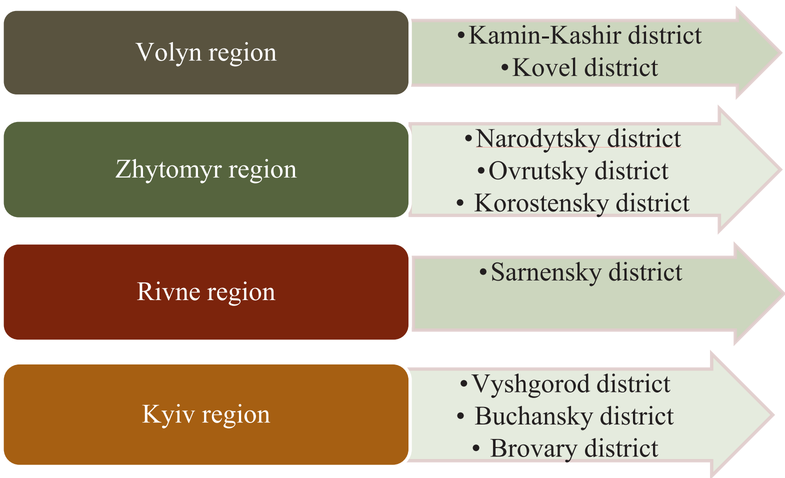


Fig.1. Territories where fresh wild mushrooms contaminated with radioactive Cesium were most often found

It should be noted that the level of content of radionuclides in forest mushrooms can vary significantly even in the territory of one district, depending on the specific place of collection. Such differences are explained by the uneven fallout of radioactive fallout after the Chernobyl disaster. As a result, mushrooms can be completely safe in one area, and the level of pollution can significantly exceed permissible levels in just half a kilometer, such a phenomenon is called "spotted pollution" (Jeskovsky, 2019; Konoplev, 2020; Grodzynska, 2023). In addition, the highest concentration of radionuclides is usually observed in lowlands, streams and places of water stagnation, where Cesium is washed away with precipitation (Grodzynska, 2017; Kashparov, 2019; Holiaka, 2020).

It is shown the highest levels of contamination with the radionuclide Cesium-137 found in samples of fresh wild mushrooms collected on the territory of the Polissky region in the period from 2020 to 2025 (Fig.2).

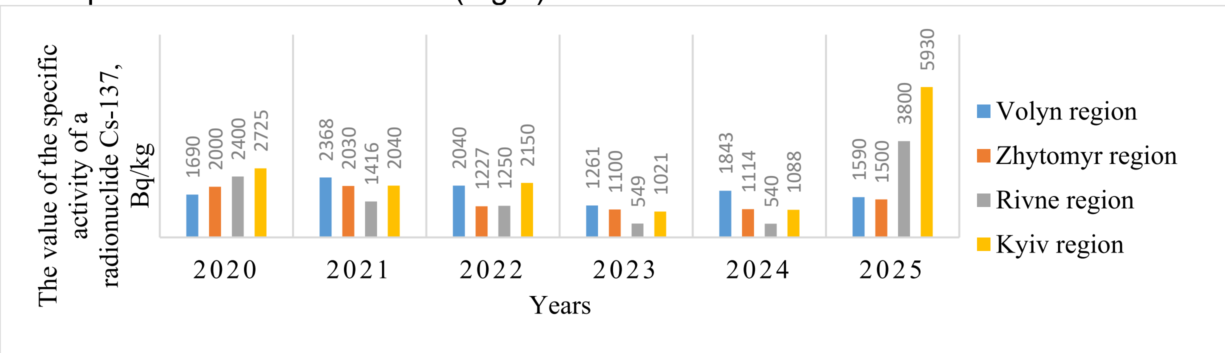


Fig. 2. The highest levels of Cesium-137 contamination were found in samples of fresh wild mushrooms

During a detailed analysis of the obtained data, it was established that forest mushrooms will continue to steadily accumulate significant concentrations of radiocesium and remain "critical" food products, and the main dose-forming radionuclide that significantly affects the formation of the dose of internal exposure of the population remains Cesium-137.

As of 2025, the maximum recorded specific activity of radiocesium in fresh wild mushrooms was 5930 Bq/kg. These mushrooms were collected on the territory of the Kyiv region. Other regions also show notable concentrations of radiocesium. Thus, in the Volyn region, the radionuclide activity reached the level of 1,590 Bq/kg, in Zhytomyr region — 1,500 Bq/kg, and in the Rivne region — 3,800 Bq/kg.

The high content of radioactive cesium-137 in wild mushrooms has a scientific explanation and is caused by a combination of various factors. First of all, cesium is able to accumulate for a long time in the upper layer of soil humus, which creates favorable conditions for its active assimilation by mycelium. This is especially characteristic of fungi with a surface mycelium system, such as Polish mushroom, buttercups or bryophytes, which effectively absorb the radionuclide (Russell, 2015; Ernst, 2022; Gabriel, 2023).

The biological mechanism of assimilation of Cesium isotopes by fungi is also important. Its penetration occurs due to chemical similarity with potassium ions, which is vital for their development. This similarity leads to the fact that radioactive Cesium is absorbed by fungi as a substitute for a useful element. It should be noted that the distribution of radioactive Cesium in the structure of the mushroom has its own characteristics: in the cap, its concentration is usually 1.5–2 times higher than the content in the stem. An additional danger is old, overripe mushrooms that accumulate much more harmful substances, which makes them especially dangerous for consumption (Orita, 2018; Gupta, 2018; Romanchuk, 2019).

Exceeding the permissible levels of Strontium-90 in forest mushroom samples was not detected. This is primarily due to the fact that strontium is a chemical analogue of calcium, and most forest mushrooms have a low physiological need for calcium, so they practically do not absorb this isotope from the soil. (Gupta, 2018; Kashparov, 2019; Grodzinskaya, 2022).

Also, the state of food safety, primarily of forest origin, is significantly affected by modern environmental challenges, in particular, large-scale forest fires and damage to the surface layers of the soil as a result of military operations. Such phenomena contribute to the repeated release of radionuclides from the contaminated soil. During the burning of wood and grass, radioactive particles, together with smoke and ash, can be carried by the wind for tens of kilometres, settling on agricultural and forest vegetation in "clean" territories and making them dangerous for consumption even in those regions that were previously considered radiation-safe.

So, summarizing the obtained data, it should be noted that the specific activity of the radionuclide Cesium-137 in fresh wild mushrooms collected on the territory of the Polissia region in 2025 exceeds the permissible levels of Cesium-137 content (≤ 500 Bq/kg) established by the current Hygienic Standards in the Volyn Region in 3.2 times, 11.9 times in Kyiv region, 3.0 times in Zhytomyr region, and 7.6 times in Rivne region, respectively.

Conclusions. It was established that fresh wild mushrooms collected in the Polish region continue to be "critical" products from the point of view of radioactive contamination. The level of cesium-137 contamination in such mushrooms exceeds the permissible norms from 3 to almost 12 times. Therefore, it is recommended to carry out mandatory, permanent radiological control of food products, in particular wild mushrooms, in radiological laboratories in local markets or in the institutions of the State Production and Consumer Service of Ukraine.

REFERENCES

1. Dutov O. I., Landin V. P., Melnychuk A. O., Grynyk O. I. (2015). Radiation-ecological aspects of the use of contaminated lands in the remote period after the Chernobyl disaster. *Agroecological Journal*; 1, 115–121. http://nbuv.gov.ua/UJRN/agrog_2015_1_16
2. Ernst A.-L., Reiter G., Piepenbring M., Bässler C. (2022). Spatial risk assessment of radiocesium contamination of edible mushrooms: Lessons from a highly frequented recreational area. *Science of the Total Environment*; 807(2), 150861. https://doi.org/10.1016/j.scitotenv.2021.150861
3. Gabriel J., Grodzynska G. A., Nebesnyi V. B., Landin V. P. (2023). Radioactive contamination of mushrooms from Polis’ke Forestry (Kyiv Region, Ukraine) long after the Chornobyl accident. *Czech Mycology*; 75(2), 117–137. https://doi.org/10.33585/cmy.75202
4. Grodzynska G. A. (2017). Radionuclide contamination of macromycetes. *Visnyk of the National Academy of Sciences of Ukraine*; 6, 61–76. https://doi.org/10.15407/visn2017.06.061
5. Grodzinskaya A. A., Nebesnyi V. B., Landin V. P., Gabriel J. (2022). Radioactive contamination of wild mushrooms from Ukraine under conditions of contrasting radiation loads: 36 years after the Chernobyl nuclear power plant catastrophe. *International Journal of Medicinal Mushrooms*; 24(9), 25–40. https://doi.org/10.1615/IntJMedMushrooms.2022044725
6. Grodzynska G., Nebesnyi V., Teslenko I. (2023). Radioactive contamination of wild mushrooms in Chernihiv Polesie. *Biota. Human. Technology*; 2, 55–72. https://doi.org/10.58407/bht.2.23.5
7. Gudkov I. M., Kashparov V. O. (2012). Actual problems of radioecology a quarter century after the Chernobyl disaster. *Bulletin of Zhytomyr National Agroecological University*; 1(1), 27–36
8. Gupta D. K., Schulz W., Steinhauser G., Walther C. (2018). Radiostrontium transport in plants and phytoremediation. *Environmental Science and Pollution Research*; https://doi.org/10.1007/s11356-018-3088-6

9. Holiaka D. M., Levchuk S. E., Yoschenko V. I., Kashparov V. A., Yoschenko L. V., Holiaka M. A., Pavliuchenko V. V., Diachuk P. P., Zadorozhniuk R. M., Morozova V. S. (2020). 90Sr and 137Cs inventories in forest stands in the Chernobyl exclusion zone. *Nuclear Physics and Atomic Energy*; 21(3), 256–264. https://doi.org/10.15407/jnpae2020.03.256
10. Jeskovsky M., Kaizer J., Kontul I., Lujaniene G., Mullerova M., Povinec P. (2019). Analysis of environmental radionuclides. In *Handbook of Radioactivity Analysis* (Vol. 2, pp. 137–261). Academic Press. https://doi.org/10.1016/b978-0-12-814395-7.00003-9
11. Kotelevych V. A. (2019). Actual problems of quality and safety of food products in the context of food security in the Zhytomyr region. *Scientific Messenger of Lviv National University of Veterinary Medicine and Biotechnologies. Series: Veterinary Sciences*; 21(93), 155–159. https://doi.org/10.32718/nvlvet9327
12. Konoplev A. (2020). Mobility and bioavailability of Chernobyl-derived radionuclides in soil water environment: A review. In *Behavior of Radionuclides in the Environment II: Chernobyl* (pp. 157–193). Springer. https://doi.org/10.1007/978-981-15-3568-03
13. Kashparov V., Salbu B., Levchuk S., Protsak V., Maloshtan I. (2019). Environmental behaviour of radioactive particles from Chernobyl. *Journal of Environmental Radioactivity*; 208–209, 106025. https://doi.org/10.1016/j.jenvrad.2019.106025
14. Malimon Z., Kochetova H., Gusak L., Shuliak S. (2023). Radiation situation in contaminated territories of Ukraine (2013–2022). *One Health Journal*; 1(IV), 70–76. https://doi.org/10.31073/onehealthjournal2023-IV-07
15. Orita M., Kimura Y., Taira Y., Fukuda T., Takahashi J., Gutevych O., Chornyi S., Kudo T., Yamashita S., Takamura N. (2018). Activity concentration of radiocesium in wild mushrooms collected in Ukraine 30 years after the Chernobyl accident. *PeerJ*; 6, e4222. https://doi.org/10.7717/peerj.4222
16. Russell B. C., Croudace I. W., Warwick P. E. (2015). Determination of 135Cs and 137Cs in environmental samples: A review. *Analytica Chimica Acta*; 890, 7–20
17. Romanchuk O., Lopatiuk Y., Kovalchuk S. (2019). Evaluation of 137Cs content in forest food products. *Scientific Horizons*; 11(84), 108–112. https://doi.org/10.33249/2663-2144-2019-84-11-108-112
18. Ministry of Health of Ukraine. (2006). *Permissible levels of 137Cs and 90Sr radionuclides in food and drinking water (DR-97:2006)*. https://zakon.rada.gov.ua/rada/show/v0255282-97
19. Zdrojewicz Z., Szlagor A., Wielogorska M., Nowakowska D., Nowakowski J. (2016). Influence of ionizing radiation on the human body. *Family Medicine & Primary Care Review*; 18, 174–179. https://doi.org/10.5114/fmpcr/43945

Моніторинг радіаційного забруднення дикорослих грибів у Поліському регіоні

Кочетова Г.С. (ORCID: 0000-0003-3234-1355), Прокопенко Т.О. (ORCID: 0000-0003-3234-1355), Гусак Л.М. (ORCID: 0000-0001-7570-2574), Молодик А.Г. (ORCID: 0009-0003-9719-5560)
Державний науково–дослідний інститут з лабораторної діагностики та ветеринарно–санітарної експертизи, м. Київ, Україна, email: kochetovag@ukr.net

Резюме. *Радіологічний контроль відіграє ключову роль у забезпеченні безпеки харчових продуктів, особливо свіжозібраних дикорослих грибів, які активно поглинають радіоактивні елементи, зокрема цезій-137 і стронцій-90. Через цю здатність гриби можуть становити значну загрозу, якщо рівень радіаційного забруднення не контролювати належним чином.*
Наукові дослідження свідчать, що екологічні особливості Полісся, зокрема піщані ґрунти цього регіону, сприяють накопиченню радіонуклідів у верхніх шарах землі. Природна міграція таких забруднювачів призводить до їх концентрації у

дикорослих грибах. Механізми міграції та накопичення радіонуклідів є складними процесами, що залежать від багатьох чинників. Серед них найважливішими є тип ґрунту, кліматичні умови території, меліоративні заходи, методи землекористування, а також біологічна специфіка рослинності.

Водночас ситуація загострюється через сучасні соціально-екологічні виклики, такі як військові дії та масові пожежі. Ці явища порушують верхні шари ґрунту, спричиняючи вивільнення раніше зв'язаних радіонуклідів із забруднених зон, зокрема тих, що постраждали внаслідок аварії на Чорнобильській АЕС. Радіоактивний пил і попіл підіймаються в повітря та переносяться на значні відстані, осідаючи навіть у регіонах, які до цього вважалися екологічно чистими. Це створює додаткову загрозу для безпеки харчових продуктів і лісових ресурсів у "чистих" зонах.

У відповідь на ці виклики в Україні запроваджено систему контролю безпеки харчових продуктів, покликану зменшити вплив радіації на населення. Її ключовим компонентом став регулярний радіологічний моніторинг харчових продуктів, що дозволяє оперативно оцінювати рівень радіаційного забруднення та своєчасно реагувати на потенційні ризики. Крім цього, радіологічний контроль виконує важливу функцію підвищення обізнаності громадян та забезпечення екологічної та промислової безпеки.

Ключові слова: радіологічний контроль, дикорослі гриби свіжі, цезій-137, стронцій- 90, радіаційне навантаження, навколишнє середовище, акумуляція

DOI: 10.31073/onehealthjournal2026-III-03

UDC 636.2:579.84:637.11
DOI: 10.31073/onehealthjournal2026-III-04

A comprehensive assessment of the quality of raw cow's milk based on somatic cell count and the presence of mesophilic aerobic and facultative anaerobic microorganisms as indicators of the safety and processing properties of raw milk

Togachynska L. (ORCID: 0009-0005-5032-5940), Kuriata N. (ORCID: 0000-0002-6958-1064), Musiiets I. (ORCID: 0000-0002-2456-560X), Pishchansky O. (ORCID: 0009-0002-0111-4977), Halka I. (ORCID: 0000-0001-8701-3506), Balanchuk L. (ORCID: 0000-0003-0989-5886), Kulykova V. (ORCID:0009-0008-8827-030X)
State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise, Kyiv, Ukraine, e-mail: tog.liya888@gmail.com

Abstract. *This study conducted a comparative evaluation of methods for determining the somatic cell count and the number of mesophilic aerobic and facultative anaerobic microorganisms MAFAnM) in raw cow's milk. The aim of the study was to establish the analytical agreement, accuracy, and reproducibility of results obtained by different methods, as well as to justify the feasibility of using the TEMPO® automated system as an alternative method for microbiological control.*

The study material consisted of 39 samples of raw cow's milk collected in 2025 from clinically healthy Black-and-White and Red dairy cows aged 4–8 years on private farms in various regions of Ukraine. Somatic cell counts were determined using two microscopic methods in accordance with the requirements of ISO 13366-1:2008: by counting cells in smears across fifty fields of view and in ten parallel strips. The number of total aerobic bacteria was determined using two independent methods: an automated fluorometric method using the TEMPO® system and a reference method of deep plating on Plate Count Agar medium in accordance with ISO 4833-1:2013.

Statistical analysis of the results was performed using Student's paired t-test and Pearson's correlation analysis. It was found that the mean values of somatic cell counts obtained by different methods were 301,795 and 294,641 cells/cm³, respectively, and for MAFAnM—43,041 and 43,513 CFU/cm³. The calculated t-test values (t = 1.80 for somatic cells and t = –1.32 for MAFAnM) did not exceed critical values ($p > 0.05$), indicating the absence of statistically significant differences between the methods. The Pearson correlation coefficient was r = 1.0 for somatic cells and r = 0.9996 for MAFAnM ($p < 0.05$), indicating a strong direct linear relationship and high consistency of results.

The research demonstrated that TEMPO® automated system provides results for the detection of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM) in raw milk that are statistically equivalent to the reference method, with high accuracy and reproducibility and minimal human influence. The use of this system is recommended for implementation in production laboratories as a rapid and reliable tool for monitoring the microbiological parameters of raw milk.

Keywords: somatic cells, mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM), cow's milk, species, automated TEMPO® method, correlation analysis, Student's t-test.

Cow's milk is one of the most valuable and nutritionally balanced foods in the human diet, as it contains a wide range of nutrients essential for the body's normal functioning. It is characterized by high nutritional and biological value due to its optimal combination of proteins, fats, carbohydrates, minerals, and vitamins. Milk proteins consist of casein and whey proteins, which are complete proteins containing all the essential amino acids necessary for the synthesis of body tissues, with a digestibility of up to 95–98%. Milk fat is a source of energy and a carrier of fat-soluble vitamins, while lactose meets the body's energy needs and promotes better calcium absorption (Vranješ, 2015; Mykhailiutenko, 2025).

Milk is particularly important as a source of minerals, specifically calcium and phosphorus, which are present in an optimal ratio that supports the effective formation and maintenance of bone tissue and teeth. The presence of vitamin D further enhances calcium absorption, which is important for children during periods of rapid growth and for adults to help prevent osteoporosis. In addition, milk contains B vitamins, which have a positive effect on nervous system function, as well as potassium, which helps regulate water-salt balance and blood pressure (Li, 2025).

The food industry makes extensive use of cow's and goat's milk, as well as dairy products (hard and processed cheeses, butter, fermented milk products, etc.). Milk and dairy products are an important part of the human diet (Sadvari, 2024).

The nutritional value of cow's milk is also due to the presence of biologically active components, such as enzymes, immunoglobulins, lysozyme, and lactoferrin, which play an important role in building the body's immune defenses and maintaining normal intestinal microflora. As a result, milk helps increase the body's resistance to infectious diseases (Miftari, 2026).

Cow's milk is an accessible source of energy and nutrients, making it particularly important in the diets of children, adolescents, individuals with high physical activity levels, and the elderly. At the same time, individual physiological characteristics should be taken into account, as some people may have lactose intolerance or allergic reactions to milk proteins. Thus, when consumed in moderation, cow's milk is an important component of a balanced diet and contributes to maintaining good health (Zhao, 2025).

The dairy industry in Ukraine is of great economic importance, as it provides the population with essential food products of high nutritional value. The growing demand for dairy products in Ukraine is confirmed by statistical data, which indicates stable consumption of milk and dairy products. This demonstrates the continued importance of dairy products in the diet of citizens, which in turn stimulates the development of the dairy industry, as well as milk production and processing. The high quality of milk and dairy products directly impacts the industry's profitability and its competitiveness in domestic and international markets (Yang, 2025; Curci, 2025).

The primary goal of dairy farms is to provide environmental conditions that meet the needs of the breed being raised in order to support profitable and sustainable production. Given that cattle productivity is the result of a complex interaction between genotype and environmental conditions, it is crucial to provide appropriate environmental conditions to enhance productivity. Sustainable and profitable production depends on the number of calves born per year and optimal milk yield from each cow. Around the world, breeding research on dairy cows has focused primarily on increasing milk yield for many years. However, most researchers note that increased milk yield has a negative impact on the fertility of dairy cows. Additionally, the elevated metabolic rate in high-producing cows tends to negatively affect their metabolic status. A fast metabolism can lead to an imbalance in energy and nutrients (Ermetin, 2025; Nuzzi, 2026).

Because milk production is the primary source of income for dairy farms, it is important to consider factors that can directly affect milk production, such as mastitis. Mastitis is the most common disease of the mammary gland in cattle, goats, sheep, and other animals (Nagy, 2025).

Mastitis is an inflammatory disease of the mammary gland in animals, usually caused by a bacterial infection, although other causes (trauma, stress, poor hygiene) may also be involved. It results in significant economic losses in the dairy industry due to its direct impact on milk production. Mastitis is a major problem in terms of reduced productivity and the quality and safety of milk. Somatic cells serve as a criterion for determining the quality and safety of raw milk and the health status of the mammary gland in animals (Kotelevich, 2023; Kukeyeva, 2023).

The somatic cell count is widely used as an indicator of udder inflammation, since both subclinical and clinical mastitis result in high levels of these cells.

Somatic cells in raw cow's milk are an important biological and diagnostic indicator that characterizes the physiological condition of the mammary gland, the sanitary quality of milk, and its technological properties. The term "somatic cells" refers to the totality of cells present in milk, which include, on the one hand, mammary gland epithelial cells that are shed during milk secretion, and on the other hand, cells of the immune system (leukocytes) (Fonseca, 2025; Fonseca, 2025).

Leukocytes are vital to the immune system of animals, playing a key role in protecting the mammary gland from environmental pathogens, including bacteria, viruses, and fungi. The composition and relative proportions of these leukocytes vary among different animal species, reflecting the unique immune response mechanisms of different breeds. Although all animals have the same basic types of white blood cells (neutrophils, monocytes, eosinophils, and basophils). In cattle, lymphocytes predominate—they account for approximately 50–70%, while neutrophils are less common (20–40%). This type of blood is called lymphocytic (Desidera, 2025).

In physiologically healthy cows, the somatic cell count in milk is relatively low and falls within the limits established by regulatory documents, indicating a normal udder condition and the absence of inflammatory processes. The majority of these cells are macrophages and epithelial cells, which perform protective and regulatory functions. Macrophages participate in the phagocytosis of microorganisms and cellular debris, providing local immunity, while epithelial cells reflect the physiological renewal of mammary gland tissues (Besteiro, 2025).

When inflammatory processes develop, particularly mastitis, there is a sharp increase in the number of somatic cells in milk, which is caused by the migration of leukocytes to the site of infection. In such cases, neutrophils become the dominant cell type, playing a key role in neutralizing pathogenic microorganisms. The increase in somatic cell count is a protective response of the body; however, it is accompanied by changes in the physical, chemical, and technological properties of milk (Pan, 2025).

Elevated somatic cell counts negatively affect milk quality; specifically, they lead to a decrease in casein content, an imbalance in protein fractions, and increased activity of proteolytic and lipolytic enzymes, which causes the hydrolysis of proteins and fats. This, in turn, impairs the taste properties of milk, reduces its heat stability and processing suitability, particularly in the production of cheese and fermented milk products. Furthermore, a high somatic cell count may indicate the presence of pathogenic microorganisms and a decline in the sanitary quality of the raw material (Mikulec, 2024; Smistad, 2025).

From a diagnostic standpoint, determining somatic cell count is one of the key criteria for monitoring udder health and detecting subclinical mastitis at an early stage. This parameter is widely used in veterinary practice and the dairy industry as an indicator of raw milk quality and compliance with regulatory requirements.

Thus, somatic cells in raw cow's milk play a dual role: on the one hand, they are a key component of the mammary gland's local immune defense, and on the other, they serve as a sensitive indicator of the gland's physiological condition and milk quality. Controlling their numbers is crucial for ensuring the safety, nutritional value, and technological suitability of raw milk (Mikulec, 2024).

The analysis of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM) in raw cow's milk plays a different role. It is one of the main microbiological indicators characterizing its sanitary quality, safety, and technological suitability. It characterizes the level of milk contamination and provides an assessment of milking hygiene and equipment cleanliness. Unlike somatic cells, the MAFAnM count is not a direct indicator of udder health. A high MAFAnM count may result from external contamination, even in animals with healthy udders. At the same time, the number of microorganisms may increase in the presence of mastitis, but this is not always the case and does not always correlate directly (Ryzhkova, 2023).

MAFAnM consists of various groups of microorganisms capable of growing under aerobic or facultatively anaerobic conditions at a temperature of approximately 30 °C. It comprises mainly saprophytic bacteria, as well as opportunistic pathogens that may enter milk from the environment.

The main sources of milk contamination, as measured by the MAFAnM index, include the animal's udder surface, milking equipment, tools, water, air, and the hands of the staff. In addition, microorganisms can enter the milk if sanitary and hygiene requirements are not met during milking, transportation, and storage. An important factor affecting the MAFAnM level is storage temperature: if milk is not cooled promptly, conditions favorable for the rapid multiplication of microorganisms are created (Ryzhkova, 2023).

This indicator provides a comprehensive assessment of the overall microbial contamination of milk and is widely used in laboratory practice for quality control. A low level of total bacterial count indicates proper sanitary conditions in production, compliance with hygiene requirements during milking, and effective milk cooling. Such milk is characterized by high storage stability, preservation of organoleptic properties, and suitability for further processing. Conversely, a high MAFANM content is a sign of microbial contamination and a disruption in the production process, which can lead to the rapid growth of microflora (Yuan, 2022).

An increase in the number of MAFAnM is accompanied by an intensification of enzymatic processes, particularly proteolysis and lipolysis, which cause the breakdown of milk proteins and fats. This leads to the formation of decomposition products that impair the taste, odor, and consistency of milk, reduce its nutritional value, and shorten its shelf life. In addition, high microbial contamination of milk can complicate technological processes, particularly pasteurization and the production of dairy products, since some microorganisms or their enzymes may remain active even after heat treatment (Ryzhkova, 2023).

From a safety perspective, the MAFAnM value is important as an indicator of the overall sanitary condition of milk, although it does not provide direct information about the presence of specific pathogenic microorganisms. However, high MAFAnM values increase the likelihood of the presence of undesirable or potentially hazardous microflora, which may pose a risk to consumer health. Regular monitoring of this indicator allows for the timely detection of deviations in the production process, the prediction of milk shelf life, and the assurance of its compliance with established regulatory requirements (Micules, 2025).

Thus, the analysis of somatic cells allows for an assessment of the udder's health, while the count of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM) reflects the sanitary and hygienic standards of milk production. Both indicators are important and are used in conjunction to provide a comprehensive assessment of raw milk quality.

Materials and methods. The experimental studies were conducted at the Laboratory for Microbiological Analysis of Food and Feed within the Research Bacteriology Department of the State Research Institute for Laboratory Diagnostics and Veterinary and Sanitary Expertise (DNDILDVSE), which is accredited by the National Accreditation Agency of Ukraine for competence in accordance with the requirements of DSTU ISO/IEC 17025-21, Kyiv.

The study utilized samples of raw cow's milk collected throughout 2025 from private farms located in various regions of Ukraine. The milk was obtained from clinically healthy animals. At the time of sampling, the cows were not pregnant and were not in the lactation period, which helped minimize the influence of physiological factors on milk composition. Cow's milk was obtained from Black-and-White and Red dairy breeds aged 4 to 8 years.The animals were kept on private farms and were in a physiologically normal condition. The fat content in cow's milk averaged 3.2 g per 100 g of product (3.2%). The collected raw milk samples were transported to the laboratory in special insulated containers with cooling packs, which ensured that the temperature was maintained + 4 °C ± 2 °C.

During the research, 39 samples of raw milk were analyzed. The study was conducted based on two main parameters:

- determination of somatic cell count;
- determination of the number of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM).

The somatic cell count was determined using two microscopic methods (to compare results) by counting cells in stained smears in accordance with the requirements of the international standard ISO 13366-1:2008 (ISO 13366, 2008).

The number of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM) in raw milk samples was determined using two independent methods for the purpose of comparing the results. The first method involved the use of the TEMPO® automated system. The second method for determining MAFAnM was performed in accordance with the requirements of ISO 4833:2013 (ISO 4833, 2013).

Prior to microscopic examination, the milk was first incubated in a water bath at +40 °C for 20 minutes to determine somatic cell count; it was then cooled to room temperature and thoroughly mixed to ensure the homogeneity of the sample. For each test sample, at least two microscopic slides were prepared, from which the slide of the highest quality was selected for counting.

To prepare a smear, 0.01 cm³ of pre-mixed milk was applied to a clean microscope slide using a microsyringe. The drop was placed within a pre-marked area measuring 1 cm³ ± 5% (from 95 mm² to 105 mm²) (Fig. 1).

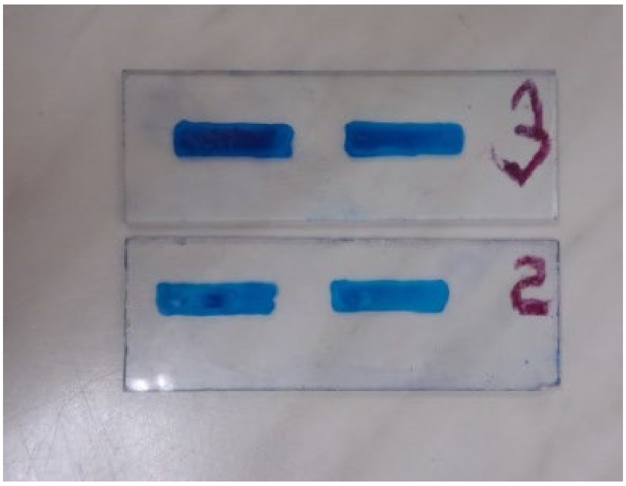


Fig.1 A smear of raw milk stained with a Newman-Lampert dye solution

The material was evenly spread over the surface of the area in a thin layer using a dissecting needle. Afterward, the specimen was left to dry at room temperature until completely dry.

After drying, the slides were immersed in a staining bath containing a modified Newman–Lampert stain and left for 15 minutes. After staining, the slides were removed, dried at room temperature, and then rinsed with distilled water to remove any residual stain.

Somatic cell counts (leukocytes, lymphocytes, monocytes, and epithelial cells) were performed using a microscope at the most appropriate magnification (ranging from 500× to 1000×), (Fig. 2).

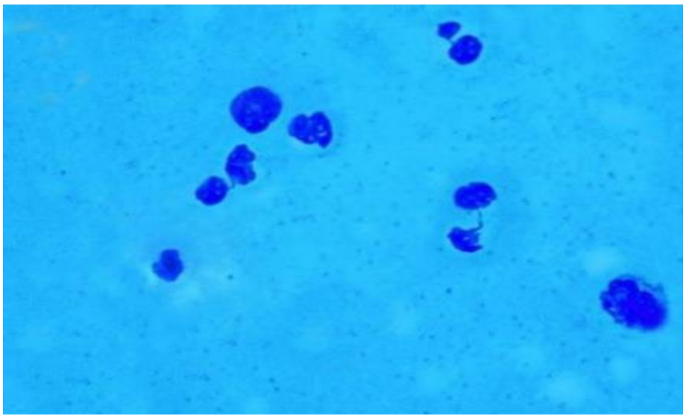


Fig. 2. Somatic cells in the smear (magnification ×1000)

The obtained values were then used to calculate their concentration in the tested milk sample.

To determine the number of mesophilic aerobic and facultative anaerobic microorganisms in raw milk using the TEMPO® system, the test samples were prepared in stages. From each batch of test material, 10 cm^3 of raw milk was sampled and aseptically transferred into a sterile filter bag (Bag Filter). We added 90 cm^3 of sterile primary solvent—peptone water—to the bag, resulting in an initial dilution of 1:10. The resulting suspension was thoroughly homogenized using a homogenizer (Bag Mixer) until a homogeneous mixture suitable for further processing was formed.

Using the dispenser on the TEMPO® Aerobic Count unit, 9 ml of secondary solvent (saline solution) and 1 ml of the sample from the primary dilution were added to the vial containing the dry culture medium to fully dissolve the dry culture medium and prepare the mixture for further analysis (Fig. 3).

Fig. 3. Dry growth medium TEMPO® AC

The mixture was homogenized for 3 seconds.

Further preparation for analysis was performed using the TEMPO® FILLER sample preparation station (Fig. 4).

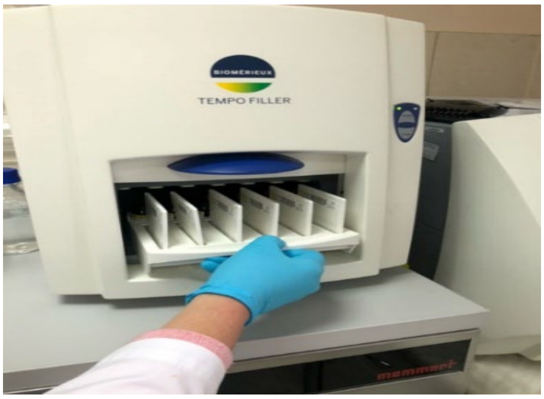


Fig. 4. TEMPO® Filler Sample Preparation Station (card filling and sealing)

After logging into the instrument's software, the corresponding working module was opened to enter information about the test samples.

The identification data for each sample were read using a barcode scanner. Information such as the sample number, test date, type of matrix being tested, and the selected analytical method was automatically entered into the system.

Special TEMPO® AC cards, designed for determining MAFAnM, were attached to each vial containing the prepared inoculated culture medium. Before starting the procedure, the barcodes on the vial and the card were verified by scanning them. After that, the vials and cards were placed in a rack. One rack is designed to hold up to six vials and six cards at a time.

The prepared rack was placed into the TEMPO® FILLER device, where the card wells were automatically filled with the inoculated culture medium. During this process, the contents of the vial were completely drawn into the card's micro-well system. After the card filling process was complete, the TEMPO® FILLER automatically cut off the transport tubes and hermetically sealed the cards (Fig. 5).

After the approximately three-minute cycle was complete, the rack with the filled cards was removed from the sample preparation station. An empty vial indicated that the inoculated medium had been completely and correctly transferred to the card.

The prepared plates were placed in a special incubation rack, oriented so that the labels faced the rack handle. The plates were then transferred to a thermostat, where the microorganisms were cultured at a temperature of 30 °C ± 1 °C for 40–48 hours.

Microbiological examination of the test samples, processing of the obtained data, and interpretation of the results for determining the number of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM) were performed using the second method in accordance with the requirements of the international standard ISO 4833-1:2013.

Buffered peptone water was used to prepare the initial suspension in a 1:10 ratio. When using the standard method, a series of consecutive decimal dilutions of the test material (10^2, 10^3, etc.)

was prepared. From the corresponding dilutions, 1 cm³ of suspension was collected using a sterile pipette and transferred to sterile Petri dishes.

Fig. 5. Cards with cut-off straws

To each Petri dish containing the material, approximately 15 cm³ of Plate Count Agar medium was added, which had been pre-cooled in a water bath to a temperature of (47 ± 2) °C. After that, the contents of the dishes were gently mixed with light circular motions to ensure uniform distribution of the sample in the medium.

After the agar medium had completely solidified, the plates were incubated in a thermostat at a temperature of (30 ± 1) °C for (72 ± 3) hours. Upon completion of incubation, all microbial colonies on the plates suitable for counting were counted, i.e., those containing between 15 and 300 colonies on at least one of the plates of the corresponding dilution.

Results and Discussion. A study was conducted on 39 samples of raw milk to determine somatic cell count and the presence of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM).

Somatic cell counts were determined using two calculation methods:

1) The number of rectangular somatic cells in the smear was counted and calculated in fifty consecutive, evenly spaced fields of view using the following formula:

$$\boldsymbol{C} = \frac{\boldsymbol{W_S} \times \boldsymbol{L_S} \times \boldsymbol{N_t}}{\boldsymbol{\pi} \times (\frac{\boldsymbol{D_f}}{2})^2 \times \boldsymbol{N_f} \times \boldsymbol{V_m}} \times \frac{\boldsymbol{1}}{\boldsymbol{d}},$$

where:

C **-** total cell concentration per cm³;

W_S **-** horizontal diameter of the smear, mm;

L_S **-** vertical diameter of the smear, mm;

N_t **-** total number of cells counted;

D_f **-** diameter of the microscope’s field of view, mm;

N_f **-** number of all fields of view in which cells were counted;

V_m **-** volume of the test sample in the smear;

V_m = 0,01 (if the Newman-Lampart colorant was used for coloring);

d **-** sample dilution factor;

d = 1 (if the sample was not diluted).

2) The number of rectangular somatic cells in the smear was counted and calculated in consecutive fields across ten parallel vertical strips using the following formula:

$$\boldsymbol{C} = \frac{\boldsymbol{L_S} \times \boldsymbol{N_t}}{\boldsymbol{D_f} \times \boldsymbol{N_b} \times \boldsymbol{V_m}} \times \frac{\boldsymbol{1}}{\boldsymbol{d}},$$

with:

N_b **-** the total number of counted lanes.

Calculations of the somatic cell count in the test samples showed that both calculation methods are reliable and practical for use (Fig. 6), (Table 1).

The study and counting of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM) were conducted using the automated TEMPO® method and the reference method of deep plating on Petri dishes containing Plate Count Agar medium.

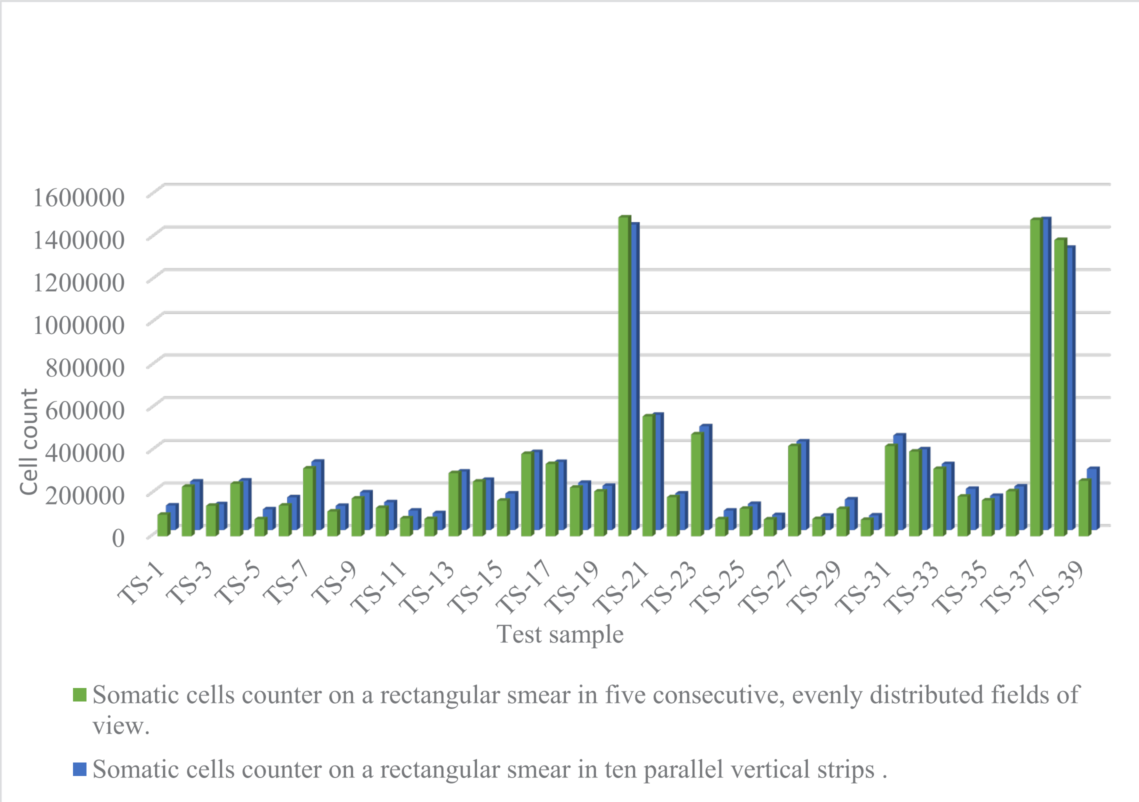


Fig. 6. Results of somatic cell count in raw milk using two calculation approaches

After incubation, the TEMPO® AC cards used in the study at the MAFAnM via the automated TEMPO® method were scanned to interpret the results. A rack containing the cards was placed in the TEMPO® READER (card reading station). The device automatically read the barcode on each card. Next, the fluorescence intensity in individual wells of the card was measured. Based on the obtained fluorescence signals, the software processed the data, calculated the quantitative content of mesophilic aerobic and facultative anaerobic microorganisms in the samples, and displayed the results electronically for further interpretation.

In individual wells of the card, the system automatically identified positive and negative wells and performed mathematical calculations to determine the bacterial concentration in the samples.The results were then recorded.

The calculated test results were displayed on the computer screen, converted to the original product in CFU/cm³, taking into account the dilutions applied, which ensured an accurate representation of the quantitative content of microorganisms in the sample (Fig. 2), (Table 2).

Following the inoculation of samples onto Petri dishes containing Plate Count Agar, characteristic colonies were observed to grow. The colonies were white to cream in color; yellowish-gray colonies were also noted. The surface of the colonies was smooth, and their consistency was soft and slimy (Fig. 7).

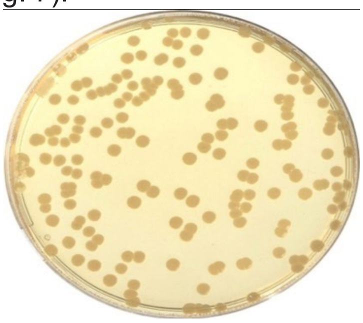


Fig. 7. Growth of MAFAnM colonies on the medium Plate Count Agar

Table 1

Analysis of the results of a study on the somatic cell count in raw milk (M ± m, n = 5)

Test samples (TS)	Results of the count of rectangular somatic cells in a smear across fifty consecutive, evenly spaced fields of view, cells/cm^3	Results of the count of rectangular somatic cells in a smear across consecutive fields on ten parallel vertical strips, cells/cm^3
TS № 1	101 000 ± 31	115 000 ± 35
TS № 2	232000 ± 69,0	227 000 ± 68
TS № 3	143 000 ± 43	121 000 ± 36
TS № 4	246 000 ± 74	232 000 ± 70
TS № 5	80 000 ± 24,0	97 000 ± 29
TS № 6	144 000 ± 43	153 000 ± 46
TS № 7	318 000 ± 95	320 000 ± 96
TS № 8	116 000 ± 35	113 000 ± 34
TS № 9	177 000 ± 53	176 000 ± 53
TS № 10	133 000 ± 40	130 000 ± 39
TS № 11	84 000 ± 42	91 000 ± 27
TS № 12	81 000 ± 24	79 000 ± 24
TS № 13	296 000 ± 89	274 000 ± 82
TS № 14	256 000 ± 77	235 000 ± 71
TS № 15	167 000 ± 50	171 000 ± 51
TS № 16	386 000 ± 116	365 000 ± 110
TS № 17	339 000 ± 102	319 000 ± 96
TS № 18	228 000 ± 68	221 000 ± 66
TS № 19	211 000 ± 63	207 000 ± 62
TS № 20	1495 000 ± 299	1432 000 ± 430
TS № 21	562 000 ± 169	540 000 ± 162
TS № 22	183 000 ± 55	171 000 ± 51
TS № 23	478 000 ± 143	486 000 ± 146
TS № 24	80 000 ± 24	91 000 ± 27
TS № 25	129 000 ± 39	122 000 ± 37
TS № 26	79 000 ± 24	70 000 ± 21
TS № 27	423 000 ± 127	415 000 ± 125
TS № 28	81 000 ± 24	67 000 ± 20
TS № 29	128 000 ± 39	143 000 ± 43
TS №30	77 000 ± 23	68 000 ± 20
TS №31	423 000 ± 127	443 000 ± 133
TS №32	397 000 ± 119	378 000 ± 113
TS № 33	316 000 ± 95	309 000 ± 93
TS № 34	186 000 ± 56	193 000 ± 58
TS № 35	168 000 ± 50	160 000 ± 48
TS № 36	211 000 ± 63	204 000 ± 61
TS № 37	1483 000 ± 445	1457 000 ± 437
TS № 38	1389 000 ± 417	1323 000 ± 397
TS № 39	260 000 ± 78	286 000 ± 86

$p < 0,05$

The number of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM) that grew on Petri dishes in the test samples was calculated using the appropriate formula.

$$\boldsymbol{N} = \frac{\sum c}{(\boldsymbol{n_1}+\mathbf{0{,}1}\cdot\boldsymbol{n_2})\cdot\boldsymbol{d}},$$

with:
N **-** number of microorganisms (CFU/cm³);
∑c **-** the total number of colonies counted in all Petri dishes;
n_1 **-** the number of cups in which colonies grew in the first culture;
n_2 **-** the number of cups in which colonies grew in the second culture;
d **-** dilution ratio.

The results obtained regarding the number of MAFAnM were expressed in colony-forming units (CFU/cm³) (Fig. 8), (Table 2).

A comparison was conducted of selected values from the study of somatic cell count and the number of MAFAnM present in raw milk.

The somatic cell count results were compared between the two counting methods in rectangular smears, specifically across fifty consecutive, evenly spaced fields of view and across ten parallel vertical strips.

For this purpose, Student's t-test for paired samples was used, which allows comparing the means of two dependent samples and determining whether they differ statistically significantly.

The study included 39 paired samples, for each of which the analyses were performed using both methods simultaneously.

Calculations of Student's t-test (t) to assess differences between the results of determining somatic cell count and the number of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM) in raw milk were performed using a paired t-test, since the results obtained for the same samples using different methods were being compared. The calculation was performed using the formula:

$\overline{\boldsymbol{d}} = \frac{1}{n}\sum_{i=1}^{n} (\mathrm{x_i} - y_\mathrm{i}),$

$\overline{\boldsymbol{d}} \approx$ 7230,77 somatic cells/cm^3

$\overline{\boldsymbol{d}} \approx$ -217,95 CFU/cm^3 (MAFAnM),

where x_i **-** is the number of somatic cells counted in rectangular smears across fifty consecutive, evenly spaced fields of view (cells/cm³);
y_i is the number of somatic cells counted in rectangular smears across ten parallel vertical strips (cells/cm³);
d- the standard deviation of the values between the two methods;
n - the number of test samples

Standard deviation:

$\boldsymbol{S} = \sqrt{\frac{\sum(\mathrm{x}_i-\overline{\mathrm{x}})^2}{n-1}},$

$\boldsymbol{S_1} \approx$ 44725 somatic cells /cm^3**;**
$\boldsymbol{S_2} \approx$ 42462 somatic cells /cm^3**;**
$\boldsymbol{S_1} \approx$ 36360 CFU/cm^3 (MAFAnM)
$\boldsymbol{S_2} \approx$ 36630 CFU/cm^3(MAFAnM)

where,
S **-** standard deviation, which characterizes the degree of variability (dispersion) of the results of somatic cell count and MAFAnM measurements relative to their arithmetic mean;
x **-** the arithmetic mean of the indicator under study, calculated based on the aggregate of the results obtained:

- for somatic cells—based on the results of two counts;
- for MAFAnM - based on the results obtained using different research methods (the automated TEMPO® method and the reference method of inoculation on PCA agar);

$\mathrm{x_i}$ **-** a separate (individual) value of the indicator under study:
for somatic cells—the value obtained from two different counts;

Table 2

Analysis of the results of the study on the concentration of MAFAnM in raw milk (M ± m, n = 5)

Test samples	Results of the determination of the concentration of MAFAnM in raw milk using the TEMPO® system, CFU/cm³	Results of the determination of the number of total aerobic bacteria in raw milk using the standard method on Plate Count Agar, CFU/cm³
TS № 1	(1,2 ± 0,006) × 10^4 CFU/cm³	(1,30 ± 0,017) ×10^4 CFU/cm³
TS № 2	(3,2 ± 0,016) × 10^4 CFU/cm³	(3,3 ± 0,013) × 10^4 CFU/cm³
TS № 3	(1,4 ± 0,007) × 10^4 CFU/cm³	(1,5 ± 0,006) × 10^4 CFU/cm³
TS № 4	(1,0 ± 0,005) × 10^3 CFU/cm³	(1,1 ± 0,003) × 10^3 CFU/cm³
TS № 5	(1,2 ± 0,006) × 10^4 CFU/cm³	(1,1 ± 0,09) × 10^4 CFU/cm³
TS № 6	(3,3 ± 0,016) × 10^4 CFU/cm³	(3,2 ± 0,012) × 10^4 CFU/cm³
TS № 7	(1,9 ± 0,009) × 10^4 CFU/cm³	(2,0 ± 0,008) × 10^4 CFU/cm³
TS № 8	(3,2 ± 0,016) × 10^4 CFU/cm³	(3,3 ± 0,013) × 10^4 CFU/cm³
TS № 9	(2,9 ± 0,014) × 10^4 CFU/cm³	(3,1 ± 0,012) × 10^4 CFU/cm³
TS № 10	(3,7 ± 0,018) × 10^4 CFU/cm³	(3,6 ± 0,0144) × 10^4 CFU/cm³
TS № 11	(1,1 ± 0,055) × 10^5 CFU/cm³	(1,2 ± 0,044) × 10^5 CFU/cm³
TS № 12	(2,6 ± 0,013) × 10^4 CFU/cm³	(2,7 ± 0,011) × 10^4 CFU/cm³
TS № 13	(5,4 ± 0,027) × 10^4 CFU/cm³	(5,5 ± 0,022) × 10^4 CFU/cm³
TS № 14	(1,5 ± 0,007) × 10^4 CFU/cm³	(1,4 ± 0,005) × 10^4 CFU/cm³
TS № 15	(3,6 ± 0,018) × 10^4 CFU/cm³	(3,7 ± 0,014) × 10^4 CFU/cm³
TS № 16	(5,5 ± 0,027) × 10^4 CFU/cm³	(5,6 ± 0,022) × 10^4 CFU/cm³
TS № 17	(2,0 ± 0,010) × 10^4 CFU/cm³	(1,9 ± 0,007) × 10^4 CFU/cm³
TS № 18	(1,7 ± 0,008) × 10^5 CFU/cm³	(1,8 ± 0,068) × 10^5 CFU/cm³
TS № 19	(6,0 ± 0,030) × 10^4 CFU/cm³	(5,9 ± 0,023) × 10^4 CFU/cm³
TS № 20	(5,4 ± 0,027) × 10^4 CFU/cm³	(5,5 ± 0,022) × 10^4 CFU/cm³
TS № 21	(1,4 ± 0,012) × 10^3 CFU/cm³	(1,5 ± 0,003) × 10^3 CFU/cm³
TS № 22	(1,5 ± 0,007) × 10^4 CFU/cm³	(1,4 ± 0,014) × 10^4 CFU/cm³
TS № 23	(3,7 ± 0,018) × 10^4 CFU/cm³	(3,8 ± 0,012) × 10^4 CFU/cm³
TS № 24	(9,0 ± 0,045) × 10^4 CFU/cm³	(9,1 ± 0,036) × 10^4 CFU/cm³
TS № 25	(3,5 ± 0,017) × 10^4 CFU/cm³	(3,6 ± 0,014) × 10^4 CFU/cm³
TS № 26	(2,1 ± 0,010) × 10^4 CFU/cm³	(2,0 ± 0,008) × 10^4 CFU/cm³
TS № 27	(1,2 ± 0,006) × 10^4 CFU/cm³	(1,1 ± 0,004) × 10^4 CFU/cm³
TS № 28	(9,0 ± 0,045) × 10^4 CFU/cm³	(9,1 ± 0,036) × 10^4 CFU/cm³
TS № 29	(3,7 ± 0,018) × 10^4 CFU/cm³	(3,6 ± 0,014) × 10^4 CFU/cm³
TS № 30	(2,1 ± 0,010) × 10^4 CFU/cm³	(2,0 ± 0,008) × 10^4 CFU/cm³
TS № 31	(1,0 ± 0,005) × 10^4 CFU/cm³	(1,1 ± 0,013) × 10^4 CFU/cm³
TS № 32	(1,1 ± 0,051) × 10^5 CFU/cm³	(1,3 ± 0,041) × 10^5 CFU/cm³
TS № 33	(1,5 ± 0,076) × 10^5 CFU/cm³	(1,6 ± 0,061) × 10^5 CFU/cm³
TS № 34	(3,9 ± 0,019) × 10^4 CFU/cm³	(4,0 ± 0,016) × 10^4 CFU/cm³
TS № 35	(8,9 ± 0,044) × 10^4 CFU/cm³	(9,0 ± 0,036) × 10^4 CFU/cm³
TS № 36	(3,7 ± 0,018) × 10^4 CFU/cm³	(3,6 ± 0,014) × 10^4 CFU/cm³
TS № 37	(8,6 ± 0,043) × 10^4 CFU/cm³	(8,5 ± 0,034) × 10^4 CFU/cm³
TS № 38	(1,3 ± 0,006) × 10^4 CFU/cm³	(1,4 ± 0,011) × 10^4 CFU/cm³
TS № 39	(9,1 ± 0,045) × 10^4 CFU/cm³	(9,0 ± 0,036) × 10^4 CFU/cm³

$p < 0,05$

- for MAFAnM: the value obtained for each sample using the TEMPO® automated system and the reference method of plating on PCA agar.
- n – number of observations (sample size)

t - Statistics for a paired t-test:

$$t = \frac{\bar{d}}{s_d/\sqrt{n}},$$

$t \approx$ 1,80 somatic cells/cm^3
$t \approx$ -1,32 CFU/cm^3 (MAFAnM)
where,
s_d - standard deviation of the differences
Critical value at α = 0,05, df = 38:

$t_{cr} \approx$ 2,024 somatic cells/cm^3
$t_{cr} \approx$ 2,02 CFU/cm^3 (MAFAnM)
Comparison:|*tcount*| = 1,80 < *tcr* = 2,024 somatic cells/cm^3
Comparison:|*tcount*| = 1,32 < *tcr* = 2,02 CFU/cm^{33} (MAFAnM)

Thus, the mean values from the two somatic cell counts (301,795 and 294,641 cells/cm³) and the standard deviations (44,725 cells/cm³ and 42,462 cells/cm³) are similar, indicating comparable variability in the results. The calculated t-value (t = 1.80) at df = 38 is less than the critical value (t*cr* = 2.024, α = 0.05, two-tailed test). Thus, no statistically significant differences were found between the methods for determining somatic cell counts (p > 0.05). The methods are consistent and equivalent in terms of measurement results.

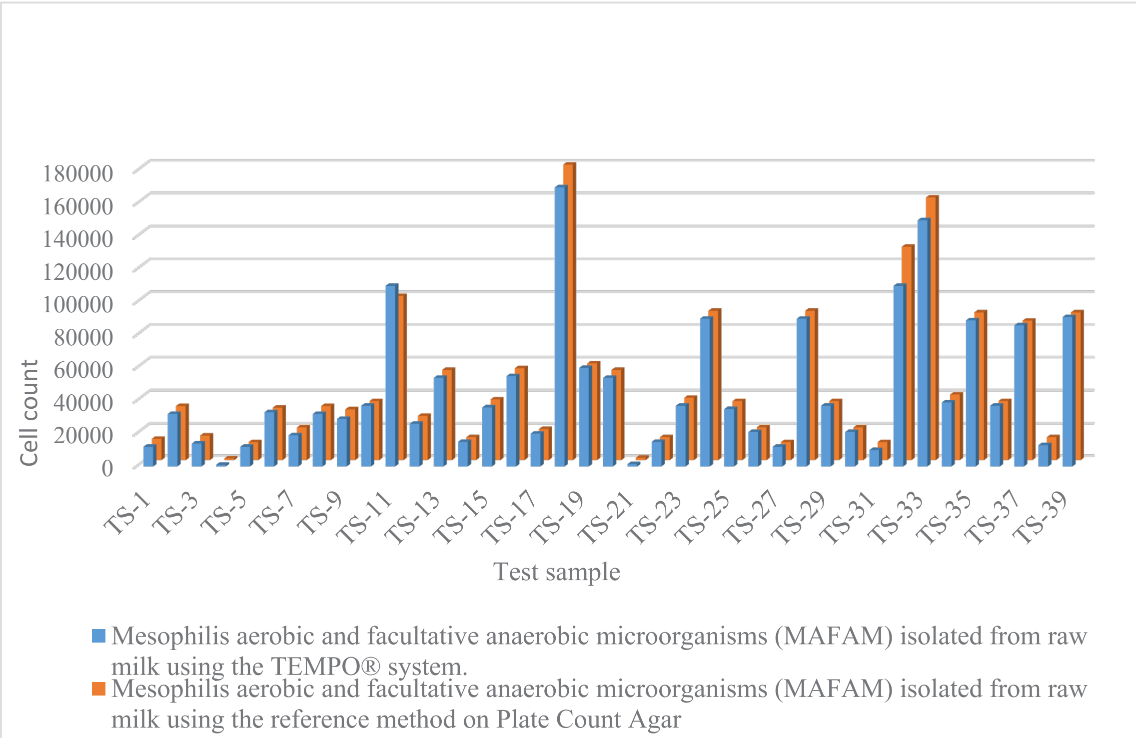


Fig. 8. Results of isolation and enumeration of MAFAM in raw milk using two research method

The mean values obtained from milk testing using the MAFAnM system via two different methods (43,041 CFU/cm³ and 43,513 CFU/cm³) and standard deviations (36,360 CFU/cm³³ and 36,630 CFU/cm³) are practically identical, indicating a high degree of consistency in the results. The calculated t-value (*t* = –1.32) at *df* = 38 is less than the critical value *tcr* = 2.020, α = 0.05, two-tailed test). Thus, no statistically significant differences were found between the automated TEMPO® method and the reference method of inoculation on PCA agar (p > 0.05). The methods are statistically equivalent for the determination of MAFAnM.
The analysis was based on the results of a study of 39 paired samples.
The formula for Pearson's correlation coefficient:

$$\boldsymbol{r} = \sqrt{\frac{\sum_{i=1}^{n}(x_i-\bar{x})\times(y_i-\bar{y})}{\sum_{i=1}^{n}(x_i-\bar{x})^2\times\sum_{i=1}^{n}(y_i-\bar{y})^2}},$$

n = 39

For 39 paired samples, the following results were obtained: $r = 1{,}0$ somatic cells/cm^3

r = 0.9996 CFU/cm^3 (MAFAnM)

Thus, the results of the correlation analysis show that there is a very strong direct linear relationship between the results of somatic cell counts obtained using different counting methods and the results of MAFAnM determination using the automated TEMPO® method and the reference method of plating on PCA agar. For somatic cells, the correlation coefficient is r = 1.0—indicating perfect agreement of the results—while for MAFAnM, r = 0.9996, indicating virtually complete agreement between the methods. In both cases, the correlation is statistically significant ($p < 0.05$), which confirms the reliability of the results obtained and indicates the equivalence of the research methods used.

Following experimental analyses of 39 raw milk samples, they were classified by grade in accordance with the requirements of DSTU 3662:2018. The samples were classified based on quality indicators, specifically somatic cell count and the number of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM). The results obtained made it possible to assess the sanitary and hygienic condition of the milk and classify each sample into the appropriate grade in accordance with established regulatory criteria. The proportion of each grade (extra, premium, first, and ungraded) was determined as a percentage of the total number of samples tested (Figs. 9-10).

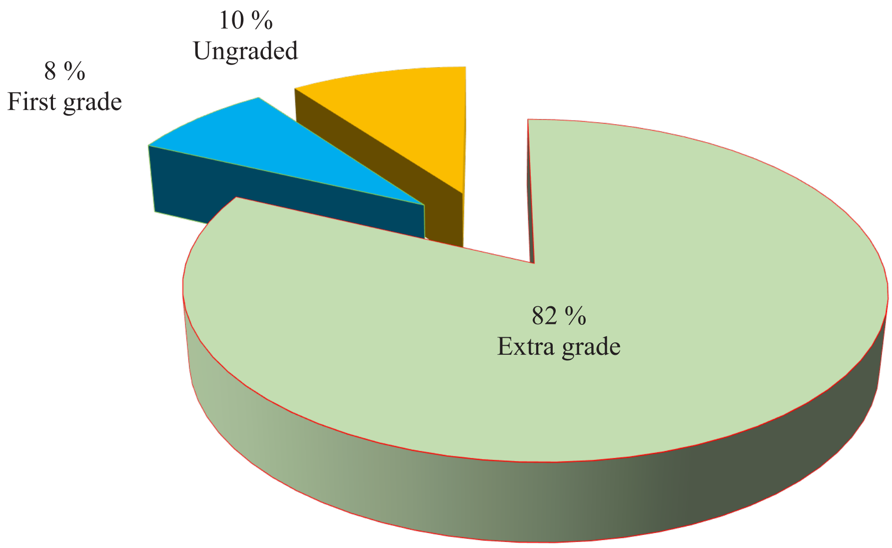


Fig. 9. Distribution of raw milk samples by grade based on somatic cell count, %

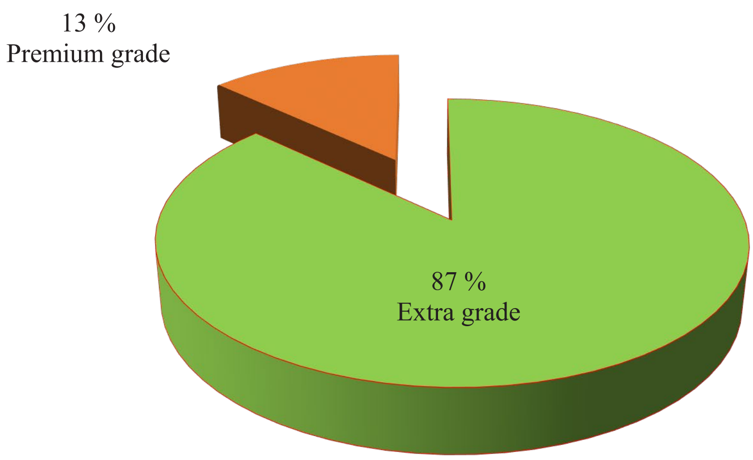


Fig. 10. Distribution of raw milk samples by grade based on the percentage of MAFAnM, %

Conclusions. The study analyzed 39 samples of raw cow's milk from clinically healthy Black-and-White and Red dairy cows aged 4–8 years, collected from private farms in various regions of Ukraine.

The determination of somatic cells in raw milk was performed using two microscopic methods with Newman–Lampert staining in accordance with ISO 13366-1:2008. Both methods allow for the quantitative assessment of epithelial cells and leukocytes, reflecting the physiological condition of the udder and milk quality. The results demonstrated the high accuracy and practical applicability of both approaches. Pearson's correlation analysis revealed a strong positive linear relationship between the results of the two methods, indicating their analytical equivalence. Student's paired t-test did not reveal any statistically significant differences between the mean values of the samples ($p > 0.05$), confirming the reliability, reproducibility, and stability of the results obtained by both methods.

An analysis of 39 raw milk samples showed that most of the milk met high-quality standards in terms of somatic cell count. Specifically, 82% of the samples met the requirements for extra-grade milk, 8% for first-grade milk, and 10% were classified as ungraded milk. The results indicate an overall adequate level of milk production hygiene; however, the presence of ungraded raw milk points to the need to improve animal housing conditions and strengthen health monitoring.

The testing of raw milk for the presence of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM) was conducted using the TEMPO® automated system and conventional deep plating on Plate Count Agar in accordance with ISO 4833-1:2013. Statistical analysis revealed no significant differences between the results of the automated TEMPO® method and the reference plating method ($p > 0.05$). Correlation analysis showed a high positive correlation between the results of the two methods, with the MAFAnM correlation coefficient indicating virtually complete agreement ($p < 0.05$), confirming the reliability of the data obtained. Regression analysis demonstrated a proportional relationship between the quantitative indicators obtained by different methods, without any systematic bias in the results, indicating the absence of significant methodological error when using the automated TEMPO® system.

The results obtained using the TEMPO® system confirmed the feasibility of using the system as an alternative method for determining MAFAnM in raw milk in production laboratory settings. The TEMPO® system ensured the reliability, accuracy, and reproducibility of results comparable to those of the classical culture method. The TEMPO® system demonstrated significant advantages, including reduced analysis time, ease of use, and minimized human factor.

The results of the assessment of raw milk for MAFAnM content showed that 87% of the samples met the "Extra" grade standards, while 13% met the "Superior" grade standards, indicating a high overall quality of the raw material tested.

The low somatic cell counts in the samples analyzed fell within the normal range for physiologically healthy milk, confirming the absence of inflammatory processes in the udder and the animals' good health. The MAFAnM levels varied depending on housing conditions, animal care, and milking techniques. Low MAFAnM values indicated high milk hygiene, while elevated values may indicate hygiene violations or issues with the processing technology. Regular monitoring of somatic cells and MAFAnM is an effective tool for assessing the physiological condition of the udder, the sanitary quality of milk, and its technological suitability, which is of direct importance for ensuring the safety and nutritional value of dairy products.

REFERENCES

1. Ryzhkova T. (2023). Comparative evaluation of methods for determining the number of mesophilic aerobic and facultative anaerobic microorganisms and coliforms in cow and goat milk and brined cheese. *Agrarian Bulletin of the Black Sea Region*; 107, 96–104. doi.: 10.37000/abbsl.2023.107.17
2. Fonseca M., Kurban D., Roy J. P., Santschi D. E., Molgat E., Dufour S. (2025). Usefulness of differential somatic cell count for udder health monitoring: Association of differential somatic cell count and somatic cell score with quarter-level milk yield and milk components. *Journal of Dairy Science*; 108(4), 3003–3016. doi.: 10.3168/jds.2024-25402
3. Desidera F., Skeie S. B., Devold T. G., Inglingstad R. A., Porcellato D. (2025). Fluctuations in somatic cell count and their impact on individual goat milk quality throughout lactation. *Journal of Dairy Science*; 108, 2291–2304. doi.: 10.3168/jds.2024-25310
4. Nagy S. Á., Csabai I., Varga T., Póth-Szebenyi B., Gábor G., Solymosi N. (2025). Neural network-aided milk somatic cell count increase prediction. *Veterinary Sciences*; 12(5), 420. doi.: 10.3390/vetsci12050420
5. Besteiro R., Fouz R., Diéguez F. J. (2025). Influence of heat stress on milk production, milk quality, and somatic cell count in Galicia (NW Spain). *Animals*; 15(7), 945. doi.: 10.3390/ani15070945
6. Smistad M., Inglingstad R. A., Vatne M. K., Franklin F. V., Hansen B. G., Skeie S., Porcellato D. (2025). Somatic cell count in dairy goats II: Udder health monitoring at goat and herd level. *BMC Veterinary Research*; 21(1), 157. doi.: 10.1186/s12917-025-04556-8
7. Ermetin O., Okuyucu İ. C., Kul E. (2025). Effect of somatic cell count on fertility and milk yield traits during different lactation periods in Holstein cows. *Turkish Journal of Agriculture: Food Science and Technology*; 13(5), 1285–1291. doi.: 10.24925/turjaf.v13i5.1285-1291.7633
8. Fonseca M., Kurban D., Roy J. P., Santschi D. E., Molgat E., Yang D. A., Dufour S. (2025). Usefulness of differential somatic cell count for udder health monitoring: Identifying referential values for differential somatic cell count in healthy quarters and quarters with subclinical mastitis. *Journal of Dairy Science*; 108(4), 3917–3928. doi.: 10.3168/jds.2024-25403
9. Pan L., Chen X., Han D., Li N., Chen D., Wang J., Huo X. (2025). Machine learning-based clinical mastitis detection in dairy cows using milk electrical conductivity and somatic cell count. *Frontiers in Veterinary Science*; 12, 1671186. doi.: 10.3389/fvets.2025.1671186
10. Mikulec N., Špoljarić J., Plavljanić D., Lovrić N., Oštarić F., Gajdoš J., Kljusurić K., Sarim M., Zdolec N., Kazazić S. (2024). MALDI-TOF mass spectrometry-based identification of aerobic mesophilic bacteria in raw unpreserved and preserved milk. *Processes*; 12(4), 731. doi.: 10.3390/pr12040731
11. Miftari H., Nikolovska Nedelkoska D., Rampanti G., Harasym J., Ferrocino I., Ferati I., Cardinali F., Orkusz A., Milanović V., Franciosa I., Garofalo C., Aquilanti L., Osimani A. (2026). A taste of North Macedonia: Seasonal variation in the microbiota, physico-chemical traits, and morpho-textural profile of a traditional brined raw goat's milk cheese. *Food Research International*; 231, 118806. doi.: 10.1016/j.foodres.2026.118806
12. Sadvari V. Y., Shevchenko L. V., Slobodyanyuk N. M., Tupitska O. M., Gruntkovskyi M. S., Furman S. V. (2024). Microbiome of craft hard cheeses from raw goat milk during ripening. *Regulatory Mechanisms in Biosystems*; 15(3), 483–489. doi.: 10.15421/022468
13. Zhao X., Zheng N., Zhang Y., Wang J. (2025). The role of milk urea nitrogen in nutritional assessment and its relationship with dairy cow phenotypes: A review. *Animal Nutrition*; 20, 33–41. doi.: 10.1016/j.aninu.2024.08.007

14. Vranješ P., Popović M., Jevtić M. (2015). Raw milk consumption and health. *Srpski Arhiv za Celokupno Lekarstvo*; 143(1–2), 87–92. doi.: 10.2298/SARH1502087P
15. Li Y., Jia H., Zheng Y., Sun X., Zhang Y., Liu W., Zhang W., Jiang Y., Zhao Q., Guo L. (2025). The nutritional composition and multi-omics analysis of raw milk from Normande and Holstein cows. *Food Frontiers*; 6(6), 3042–3058. doi.: 10.1002/fft2.70115
16. Yang Y., Wu X., Xu Y., Shuang Q., Xia Y. N. (2025). Influence of feeding systems on the microbial community and flavor characteristics of raw milk: A comparative analysis. *Journal of Dairy Science*; 108(5), 4693–4708. doi.: 10.3168/jds.2024-25959
17. Mykhailiutenko S. M., Kuzmenko L. M., Gutyj B. V., Mykhailiutenko Y. E. (2025). Seasonal fluctuations in individual milk parameters in free-range cows. *Ukrainian Journal of Veterinary and Agricultural Sciences*; 8(1), 85–89. doi.: 10.32718/ujvas8-1.12
18. Kotelevich V., Guralska S., Honcharenko V. (2023). Actual problems of the quality and safety of milk and dairy products. *Naukovyi Visnyk Veterynarnoi Medytsyny*; 1(180), 24–39. doi.: 10.33245/2310-4902-2023-180-1-24-39
19. Nuzzi C., Pasinetti S., Bassi I., Bello V. (2026). On the applicability of speckle pattern imaging combined with AI for raw milk classification. *Measurement*; 258, 119246. doi.: 10.1016/j.measurement.2025.119246
20. Curci D., Sundaram T. S., Ghidini S., Arioli F. (2025). What we know about per- and polyfluoroalkyl contamination levels in milk: A review from the last decade. *Foods*; 14(13), 2274. doi.: 10.3390/foods14132274
21. Kukeyeva A., Abdrakhmanov T., Yeszhanova G., Bakisheva Z., Kemeshov Z. (2023). The use of a homeopathic preparation in the treatment of subclinical mastitis in cows. *Open Veterinary Journal*; 13(8), 991–1002. doi.: 10.5455/OVJ.2023.v13.i8.5
22. Micules N., Spoljaric J., Plavljanic D., Darrer M., Ostaric F., Kljusuris J., Sarin K., Zdolec N., Kazazic S. (2025). Microbiota composition in raw drinking milk from vending machines: A case study in Croatia. *Fermentation*; 11(2), 55. doi.: 10.3390/fermentation11020055
23. Yuan H., Han S., Zhang S., Xue Y., Zhang Y., Lu H., Wang S. (2022). Microbial properties of raw milk throughout the year and their relationships to quality parameters. *Foods*; 11(19), 3077. doi.: 10.3390/foods11193077
24. International Organization for Standardization. (2008). *Milk—Enumeration of somatic cells—Part 1: Microscopic method (Reference method) (ISO 13366-1:2008 | IDF 148-1:2008)*. https://www.iso.org/standard/40259.html.
25. International Organization for Standardization. (2013). *Microbiology of the food chain—Horizontal method for the enumeration of microorganisms—Part 1: Colony count at 30 °C by the pour plate technique (ISO 4833-1:2013)*. https://www.iso.org/standard/53728.html

Комплексна оцінка стану сирого коров'ячого молока за вмістом соматичних клітин і мезофільних аеробних та факультативно-анаеробних мікроорганізмів як індикаторів безпечності та технологічних властивостей молочної сировини

Тогачинська Л. (ORCID: 0009-0005-5032-5940), Курята Н. (ORCID: 0000-0002-6958-1064), Мусієць І. (ORCID: 0000-0002-2456-560X), Піщанський О. (ORCID: 0009-0002-0111-4977), Галка І. (ORCID: 0000-0001-8701-3506), Баланчук Л. (ORCID: 0000-0003-0989-5886), Куликова В. (ORCID:0009-0008-8827-030X)
Державний науково-дослідний інститут з лабораторної діагностики та ветеринарно-санітарної експертизи, м. Київ, Україна, e-mail: tog.liya888@gmail.com

Резюме. *У роботі проведено порівняльну оцінку методів визначення вмісту соматичних клітин та кількості мезофільних аеробних і факультативно-анаеробних мікроорганізмів (МАФАнМ) у сирому коров'ячому молоці. Метою дослідження було встановлення аналітичної узгодженості, точності та відтворюваності результатів, отриманих різними методами, а також обґрунтування можливості застосування автоматизованої системи TEMPO® як альтернативного методу мікробіологічного контролю.*
Матеріалом дослідження слугували 39 зразків нативного коров'ячого молока, відібраних у 2025 році від клінічно здорових корів чорно-рябої та червоної молочних порід віком 4–8 років у приватних господарствах різних регіонів України. Визначення кількості соматичних клітин здійснювали двома мікроскопічними методами відповідно до вимог ISO 13366-1:2008: шляхом підрахунку клітин у мазках у п'ятдесяти полях зору та у

десяти паралельних смугах. Кількість МАФАнМ визначали двома незалежними методами: автоматизованим флуориметричним методом із використанням системи TEMPO® та еталонним методом глибинного посіву на поживне середовище Plate Count Agar згідно з ISO 4833-1:2013.

Статистичну обробку результатів здійснювали із застосуванням парного t-критерію Стьюдента та кореляційного аналізу Пірсона. Встановлено, що середні значення показників соматичних клітин, отримані різними методами, становили 301795 та 294641 клітин/см³ відповідно, а для МАФАнМ — 43041 та 43513 КУО/см³. Розраховані значення t-критерію (t = 1,80 для соматичних клітин та t = –1,32 для МАФАнМ) не перевищували критичних значень (p > 0,05), що свідчить про відсутність статистично значущих відмінностей між методами. Коефіцієнт кореляції Пірсона становив r = 1,0 для соматичних клітин та r = 0,9996 для МАФАнМ (p < 0,05), що вказує на наявність тісного прямого лінійного зв'язку та високу узгодженість результатів.

Дослідження показали, що автоматизована система TEMPO® забезпечує результати визначення мезофільних аеробних та факультативно-анаеробних мікроорганізмів (МАФАнМ) у сирому молоці, статистично еквівалентні еталонному методу, з високою точністю та відтворюваністю і мінімальним впливом людського фактору. Використання цієї системи доцільне для впровадження у виробничих лабораторіях як оперативного та надійного інструменту контролю мікробіологічних показників молочної сировини.

Ключові слова: соматичні клітини, мезофільних аеробних і факультативно-анаеробних мікроорганізмів (МАФАнМ), молоко коров'яче, ґатунок, автоматизований метод TEMPO®, кореляційний аналіз, t-критерій Стьюдента

DOI: 10.31073/onehealthjournal2026-III-04

UDC: 619.22.28:614.48:615.9:636.065
DOI: 10.31073/onehealthjournal2026-III-05

Experimental evaluation of the bactericidal activity and stability of the biocidal agent "Krezonid"

Kovalenko V.[1,2] (ORCID: 0000-0002-2416-5219), Romanko M.[1] (ORCID: 0000-0003-0285-5603), Ihnatieva T.[2,5] (ORCID: 0000-0001-9905-4807), Liniichuk N.[1] (ORCID: 0000-0001-6745-307X), Popov D.[4], Miahka K.[1] (ORCID: 0000-0002-3089-4012), Stupak O.[1] (ORCID: 0000-0001-5391-3530), Ponomaryova S.[3]
1 – State Research Institute for Laboratory Diagnostics and Veterinary and Sanitary Expertise, Kyiv, Ukraine, e-mail: kovalenkodoktor@gmail.com
2 – Institute of Veterinary Medicine, Kyiv, Ukraine
3 – State Scientific Research Control Institute of Veterinary Medicinal Products and Feed Additives, Lviv, Ukraine
4 – SANFORT company, Kyiv, Ukraine
5 – State Biotechnological University, Kharkiv, Ukraine

Abstract. *The article presents the results of an experimental assessment of the bactericidal activity and stability of the biocidal agent "Krezonid," whose main active ingredient is meta-cresol (3-methylphenol). The study was conducted to substantiate the effectiveness of the biocidal agent and confirm the preservation of its antimicrobial properties during long-term storage in accordance with the requirements. Bactericidal activity was evaluated using a quantitative suspension method with determination of the number of viable microorganisms (CFU) after exposure to the preparation. Streptococcus faecalis and Salmonella enteritidis strains were used as test cultures. The preparation was studied at concentrations of 0.1, 0.5, and 1.0% with exposure times of 10–30 minutes. The stability of the biocidal agent was studied under real storage conditions for 30 months by monitoring organoleptic indicators, pH values, and the preservation of bactericidal activity. It was found that the biocidal agent "Krezonid" exhibits pronounced bactericidal activity against both test microorganism cultures.*

Complete inactivation of Streptococcus faecalis and Salmonella enteritidis was achieved at a concentration of 0.5% and exposure of 20 minutes. During storage, the drug "Krezonid" retained its physicochemical homogeneity, stable pH value, and complete bactericidal activity throughout the observation period. The results obtained indicate the high efficacy and stability of the biocidal agent Krezonid, which justifies its use in veterinary practice and the possibility of establishing a shelf life of at least 24 months under regulated storage conditions.

Keywords: biocidal agents, meta-cresol, bactericidal activity, stability, veterinary disinfection

Prevention and control of infectious diseases in animal husbandry are fundamental components of the epizootic welfare system and ensuring the safety of food products of animal origin. The effectiveness of sanitary and hygienic measures largely depends on the use of biocidal agents capable of ensuring a stable reduction in the number of pathogenic and conditionally pathogenic microorganisms in the external environment (Maillard, 2024).

Among chemical compounds with proven antimicrobial activity, phenolic derivatives occupy a special place, characterized by their ability to disrupt the structural and functional integrity of microbial cell membranes. The mechanism of antimicrobial action of phenols consists in destabilizing the lipid layer of the cytoplasmic membrane, denaturing protein structures, and disrupting energy metabolism, which leads to the death of the bacterial cell (Russell, 2002; Rutala, 2019).

Modern experimental studies confirm the high effectiveness of phenolic compounds against Gram-positive and Gram-negative bacteria. In particular, phenolic disinfectants provide a pronounced bactericidal effect at relatively low working concentrations, maintaining their activity even in difficult conditions of exposure to organic impurities. Similar conclusions regarding the promise of phenolic compounds as biocides are cited by Oulahal (2022), emphasizing their stability and broad spectrum of antimicrobial activity.

One of the important representatives of phenolic compounds used in disinfectants and biocidal agents is meta-cresol (3-methylphenol). According to Maillard, J.-Y., Pascoe, M., and Russell, meta-cresol exhibits bactericidal activity by disrupting the permeability of the cytoplasmic membrane and inhibiting key enzymatic processes in microbial cells. An important advantage of phenolic biocides, in particular meta-cresol, is the preservation of antimicrobial activity in conditions of organic contamination, which is of fundamental importance for veterinary practice (Maillard, 2024; Russell, 2002).

In the biocidal product "Krezonid," meta-cresol is the main active ingredient, while auxiliary ingredients ensure the stability of the composition, maintain optimal pH, and reproduce bactericidal activity throughout the entire storage period. This approach to the formulation of biocidal products is in line with current recommendations for the creation of effective veterinary disinfectants, as outlined in the works of Tyski (2019) and Wales (2020).

According to current requirements for state registration of veterinary biocidal products, experimental evaluation should include not only confirmation of bactericidal activity, but also monitoring of the stability of the active substance during storage using quantitative microbiological indicators. The need for such an approach is indicated by (Tarka, 2021), as well as (Wales, 2020), who emphasize the importance of determining the logarithmic reduction of viable microorganisms to justify the effectiveness of biocides (Ponomarenko, 2020).

Aim of the study was to experimentally investigate the bactericidal activity and stability of the biocidal agent "Krezonid," whose main active ingredient is meta-cresol, using quantitative microbiological methods in accordance with current requirements.

Materials and methods. The object of the study was the biocidal agent "Krezonid" – a composite preparation based on meta-cresol with the addition of auxiliary components that ensure the stability and technological properties of the preparation. The studies were conducted at the laboratory of the Institute of Veterinary Medicine of the National Academy of Agrarian Sciences of Ukraine. Standard test strains of microorganisms were used in the studies: *Streptococcus faecalis*; *Salmonella enteritidis*.

The cultures were grown on meat-peptone agar at a temperature of 37±1°C for 18–24 hours.

The bactericidal activity of the preparation was determined by a quantitative suspension test in accordance with the generally accepted approaches used in EN 1040 and EN 1656 standards for evaluating the action of chemical disinfectants (Harkavenko et al., 2020).

Working suspensions of microorganisms were prepared at a concentration of 107–108 CFU/cm^3. The drug "Krezonid" was studied at working concentrations of 0.1%, 0.5%, and 1.0% for exposure times of 10, 20, and 30 minutes at a temperature of 37±1°C.

After exposure, the effect of the preparation was neutralized, serial dilutions were performed, and the preparation was seeded onto a nutrient medium, followed by counting the number of colonies (CFU). Samples without the addition of a biocidal agent served as controls.

The stability of the biocidal agent was evaluated under real storage conditions for 24 months. The preparation was stored in a hermetically sealed factory container at a temperature of 5 to 25°C in a place protected from light.

Stability control was carried out after 0, 6, 12, 24, and 30 months of storage by: evaluating organoleptic indicators (appearance, color, smell, presence of sediment or stratification); determining pH values; re-determining bactericidal activity using generally accepted methods (Kovalenko, 2011).

Results. Experimental studies have shown that the drug "Krezonid" exhibits pronounced bactericidal activity even at low concentrations. At a concentration of 0.1% and exposure of 10 minutes, the number of viable Streptococcus faecalis cells decreased from 1.2×10^8 CFU/cm^3 to 4.6×10^5 CFU/cm^3, indicating a pronounced but incomplete reduction in the microbial population. Increasing the exposure time to 20 minutes at the same concentration led to a further decrease in the number of CFU/cm^3 to 1.3×10^4, which can be characterized as a significant suppression of bacterial viability (Table 1).

When the concentration is increased to 0.5% and the exposure time is 20 minutes, Streptococcus faecalis is completely inactivated, as no colonies were found in the test samples. This indicates that a working concentration of 0.5% is optimal for effective destruction of bacteria in a short period of time. Complete inactivation is also observed at a concentration of 1.0% and an exposure time of 30 minutes, confirming a direct relationship between concentration, exposure time, and product efficacy.

Table 1

Bactericidal activity of the biocidal agent "Krezonid" against *Streptococcus faecalis*

Concentration of the drug, %	Exposition, min.	Number of CFU/cm³ (control)	CFU/cm³ (test)	Decrease CFU/cm³
0,1	10	$1,2 \times 10^8$	$4,6 \times 10^5$	pronounced
0,1	20	$1,2 \times 10^8$	$1,3 \times 10^4$	significant
0,5	20	$1,2 \times 10^8$	Not detected	complete
1,0	30	$1,2 \times 10^8$	Not detected	complete

The results obtained are consistent with the literature data (Russell, 2002) on the effect of phenolic compounds on Gram-positive bacteria: the mechanism of action consists in disrupting the integrity of the cell membrane and denaturing proteins, which leads to the rapid death of microorganisms.

Salmonella enteritidis showed slightly higher resistance to the drug than *Streptococcus faecalis*, which corresponds to the known properties of Gram-negative bacteria with an outer membrane that provides an additional barrier against chemical agents (Table 2). At a concentration of 0.1% and an exposure time of 10 minutes, the number of viable cells decreased from 9.8×10^7 CFU/cm³ to 6.1×10^5 CFU/cm³, i.e., there was a marked but incomplete inhibition. Increasing the exposure to 20 minutes reduced the CFU/cm³ to 2.4×10^4, which can be assessed as a significant but not complete inhibition of bacterial viability.

Table 2

Bactericidal activity of the biocidal agent "Krezonid" against *Salmonella enteritidis*

Concentration of the drug, %	Exposition, min.	Number of CFU/cm³	Concentration of the drug, %	Exposition, min.
0,1	10	$9,8 \times 10^7$	$6,1 \times 10^5$	pronounced
0,1	20	$9,8 \times 10^7$	$2,4 \times 10^4$	significant
0,5	20	$9,8 \times 10^7$	Not detected	complete
1,0	30	$9,8 \times 10^7$	Not detected	complete

The use of a concentration of 0.5% and an exposure time of 20 minutes ensured complete inactivation of Salmonella enteritidis, similar to Streptococcus faecalis. A concentration of 1.0% and an exposure time of 30 minutes also ensured complete destruction of the bacteria.

These results confirm that Krezonid is effective against both test strains, but for Gram-negative bacteria such as Salmonella enteritidis, a minimum working concentration of 0.5% with an exposure time of at least 20 minutes is recommended to achieve complete bactericidal action.

To assess the stability of "Krezonid", monitoring was carried out for 30 months under real storage conditions (at a temperature of 5 to 25°C, protected from light, in sealed containers) (Table 3). The results showed that the product retains: A uniform appearance without sediment or separation at all stages of observation.

Table 3

Stability indicators of the biocidal agent "Krezonid" during storage

Term of storage, months	External appearance	Sediment / stratification	pH 5,8–6,2	Meta-cresol content	Density 1.10–1.20 g/cm³	Bactericidal activity
0	homogeneous	absent	6,0	5,15	1,12	Safed
6	homogeneous	absent	5,9	5,10	1,11	Safed
12	homogeneous	absent	6,1	5,09	1,13	Safed
24	homogeneous	absent	6,0	5,02	1,14	Safed
30	homogeneous	absent	5,8	4,85	1,15	Safed

The pH level was within the range of 5.8–6.2, with changes not exceeding 0.2 units, which does not affect antimicrobial activity. Complete bactericidal activity against both test microorganisms was observed even after 30 months.

However, the meta-cresol content decreased to 4.85 after 30 months, which gives reason to recommend the product for a shelf life of 24 months.

These data indicate the high physicochemical stability of the product, which ensures a long shelf life and effectiveness in practical application.

Experiments to control activity after storage showed (Tables 4 and 5) that Streptococcus faecalis and Salmonella enteritidis are completely inactivated at a concentration of 0.5% and exposure of 20 minutes at all stages of storage (6, 12, 24, 30 months). This confirms that the active components of the preparation do not degrade during storage and that the physicochemical properties remain stable.

Table 4

Bactericidal activity of "Krezonid" against *Streptococcus faecalis* during storage

Term of storage, months	Concentration, %	Exposition, min.	Number of CFU/cm^3	Evaluation of the action
6	0,5	20	Not detected	Full
12	0,5	20	Not detected	Full
24	0,5	20	Not detected	Full
30	0,5	20	Not detected	Full

Thus, "Krezonid" meets modern requirements for veterinary biocidal products: preservation of bactericidal activity, stability of composition, and optimal operating parameters.

Table 5

Bactericidal activity of "Krezonid" against Salmonella enteritidis during storage

Term of storage, months	Concentration, %	Exposition, min	Number of CFU/cm^3	Evaluation of the action
6	0,5	20	Not detected	Full
12	0,5	20	Not detected	Full
24	0,5	20	Not detected	Full
30	0,5	20	Not detected	Full

Analysis of the experimental data presented in Tables 3–5 shows that the biocidal agent "Krezonid" retains its physicochemical homogeneity and bactericidal activity throughout the entire study period. The pH value changed by no more than 0.2 units, which is not critical for the stability of phenolic biocidal compositions.

Quantitative microbiological studies showed that after 6, 12, 24, and 30 months of storage, the preparation at a working concentration of 0.5% ensured complete inactivation of test microorganisms without the detection of viable cells during sowing. The absence of a decrease in bactericidal activity confirms the stability of the active components of the preparation and justifies the establishment of a shelf life of at least 24 months.

The results obtained are of practical importance for the biocidal agent "Krezonid", as they demonstrate the compliance of the preparation with the requirements for stability and effectiveness during storage.

Discussion. The results obtained are consistent with the literature data on the high antimicrobial activity of phenolic compounds, in particular meta-cresol, which has a bactericidal effect by disrupting the integrity of cell membranes and denaturing the protein structures of microorganisms.

An important characteristic of biocidal agents intended for veterinary use is the stability of their properties during storage. The results of the studies show that the composition of the agent "Krezonid" ensures the preservation of physicochemical parameters and bactericidal activity for at least 24 months of actual storage, which meets the requirements for drugs submitted for state registration (Rutala, 2019).

Scientific publications and regulatory documents indicate that the effectiveness of phenolic veterinary disinfectants and combined biocides is largely determined by the concentration of active ingredients, duration of exposure, and stability of the product during storage. According to Wales et al., phenolic disinfectants provide rapid bactericidal action against Gram-positive and Gram-negative bacteria in the concentration range of 0.5–1.0% and exposure time of 15–30 minutes (Wales, 2020)

The works of Russell and Boyce show that combined biocidal compositions, which combine phenolic compounds with other active components, in particular quaternary ammonium compounds, are characterized by a broader spectrum of antimicrobial activity and greater resistance to organic contaminants. Similar preparations used in the veterinary and food industries demonstrate complete inactivation of *Streptococcus faecalis* and *Salmonella enteritidis* after 20–30 minutes of exposure at concentrations of at least 0.5% (Russell, 2002; Boyce, 2023).

The results obtained in this study indicate that the biocidal agent “Krezonid” is not inferior to well-known phenolic and combined analogues described in the literature in terms of bactericidal activity and stability. The preservation of full bactericidal activity after 6, 12, 24, and 30 months of storage distinguishes “Krezonid” favorably from certain biocidal agents, for which publications note a gradual decrease in effectiveness due to the degradation of active components or changes in the acidity of the environment.

Thus, a comparative analysis with data from real scientific studies confirms the compliance of the biocidal agent “Krezonid” with modern requirements for veterinary disinfectants and justifies the expediency of its use and the establishment of a shelf life of at least 24 months for the biocidal agent “Krezonid” under regulated storage conditions.

The prospect for future work is to investigate the fungicidal properties of the biocidal agent “Krezonid”.

Conclusions. The biocidal agent “Krezonid” meets the requirements for veterinary disinfectants in terms of bactericidal activity against *Streptococcus faecalis* and *Salmonella enteritidis*. Quantitative microbiological studies confirm an effective reduction in the number of viable microorganisms to a level that cannot be determined by the sowing method, in the range of working concentrations of 0.5–1.0%.

The results of stability testing under real storage conditions for 24 months indicate that the physicochemical properties and bactericidal activity of the product are preserved.

REFERENCES

1. Addie D. D. (2018). Phenols and related compounds as antiseptics and disinfectants for use with animals. *MSD Veterinary Manual;* Retrieved from https://www.msdvetmanual.com/pharmacology/antiseptics-and disinfectants/ phenols-and-related-compounds-as-antiseptics-and-disinfectants-for-use-with animals?ruleredirectid=445

2. Britsun V. M., Simurova N. V., Popova I. V., Simurov O. V. (2021). Modern chemical disinfectants and antiseptics. Part II.; *Journal of Organic and Pharmaceutical Chemistry*; 19(4):20–32. doi.:10.24959/ophcj.21.231998.

3. CFSPH (2008–2023). Characteristics of selected disinfectants [PDF]. Center for Food Security and Public Health, Iowa State University; Retrieved from https://www.cfsph.iastate.edu/Disinfection/Assets/characteristics-of-selected-disinfectants.pdf

4. Boyce J. M. (2023). Quaternary ammonium disinfectants and antiseptics: tolerance, resistance and potential impact on antibiotic resistance*. Antimicrobial Resistance & Infection Control*; 12:Article 32. doi.:10.1186/s13756-023-01241-z

5. DIN EN 1656:2010-03 «Khimichni dezinfikuiuchi ta antyseptychni zasoby – kilkisnyi suspensiinii test dlia vyznachennia bakteritsydnoi aktyvnosti khimichnykh i antyseptychnykh zasobiv, yaki zastosovuiutsia v haluzi veterynarii – Metod vyznachennia ta vymohy (faza 2, krok 1)» [Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in the veterinary area – Test method and requirements (phase 2, step 1)]

6. EN 1040:2004 «Zasoby khimichni dezinfikuiuchi ta antyseptychni. Osnovna bakteritsydna aktyvnist. Chastyna 1. Metod vyprobovuvannia ta vymohy (stadiia 1)» [Chemical disinfectants and antiseptics. Basic bactericidal activity. Part 1. Test method and requirements (phase 1)], metodyk YeS, nyni diiuchoho standarta

7. Harkavenko T. O., Kovalenko V. L., Horbatiuk O. I., Pinchuk N. H., Kozytska T. H., Harkavenko V. M., Ordynska D. O. (2020). Metodychni rekomendatsii z vyznachennia bakteritsydnoi aktyvnosti ta kontroliu vidsutnosti bakteriostatichnoho efektu dezinfikuiuchykh zasobiv [Methodical recommendations for determining the bactericidal activity and controlling the absence of bacteriostatic effect of disinfectants]; Kyiv: DNDILDZVSE, 2020; 43 p. (in Ukrainian)

8. Gregirchak N., Lupyna T., Mordych T. (2013). Efektyvnist' diyi kombinovanykh dezyinfektantiv [PDF]. *Ukrainian Food Journal;* Retrieved from https://dspace.nuft.edu.ua/handle/123456789/21357

9. Krapež P., Lunder M., Oder M., Fink R. (2024). Evaluation of the In Vitro Disinfection Potential of the Phytochemicals Linalool and Citronellal Against Biofilms Formed by Escherichia coli and Staphylococcus aureus. Processes; 12(12):2743. doi.:10.3390/pr12122743.

10. Kovalenko V. L., Nedosiekov V. V. (2011). Metodychni pidkhody shchodo kontroliu dezinfikuiuchykh zasobiv dlia veterynarnoi medytsyny. /Methodological approaches to the control of disinfectants for veterinary medicine/ Monohrafiia. Kyiv. 224 p. *(in Ukrainian)*

11. Lineback C., Nkemngong C., Wu S., Li X., Teska P., Oliver H. (2018). Hydrogen peroxide and sodium hypochlorite disinfectants are more effective against Staphylococcus aureus and Pseudomonas aeruginosa biofilms than quaternary ammonium compounds; *Antimicrobial Resistance & Infection Control*; 7:154. doi.:10.1186/s13756-018-0447-5

12. Maillard J.-Y., Pascoe M. (2024). Disinfectants and antiseptics: mechanisms of action and resistance; *Nature Reviews Microbiology*; 22:4–17. doi.:10.1038/s41579-023-00958-3.

13. Mishra V. K. (2017). *M*icrobial degradation of phenol: A review [PDF]. *Research Gate;*https://www.researchgate.net/publication/316666855_Microbial_Degradation_of_Phenol_A_Review

14. Montagna M. T., Triggiano F., Barbuti G., Bartolomeo N., De Giglio O., Diella G., Lopuzzo M., Rutigliano S., Serio G., Caggiano G. (2019). Study on the In Vitro Activity of Five Disinfectants against Nosocomial Bacteria; *International Journal of Environmental Research and Public Health*; 16(11):1895. doi.:10.3390/ijerph16111895

16. Oulahal N., Degraeve P. (2022). Phenolic-rich plant extracts with antimicrobial activity: An alternative to food preservatives and biocides? *Frontiers in Microbiology; 13:*753518. doi:10.3389/fmicb.2021.753518

17. Palii G. K., Pavliuk S. V., Shevchenko I. P. (2018). Obgruntuvannya zastosuvannya antystepychnykh preparativ u systemi profilaktychnykh i likuval'nykh zahodiv [PDF]. *Bukovyns'kyy medychnyy visnyk; 22*(4):88–98. Retrieved from https://e-bmv.bsmu.edu.ua/article/view/2413-0737.XXII.4.88.2018.98

18. Ponomarenko G. V., Kovalenko V. L., Kukhtyn M. D., Paliy A. P., Bodnar O. O., Rebenko H. I., Kozytska T. G., Makarevich T. V., Ponomarenko O.V., Palii A. P. (2020) Evaluation of acute toxicity of the «Orgasept» disinfectant. *Ukrainian Journal of Ecology*; 10(4):273–278. Doi.:10.15421/2020_1982

19. Russell A. D. (2002). Mechanisms of antimicrobial action of antiseptics and disinfectants. *Journal of Antimicrobial Chemotherapy; 49*(4):597–599. doi.:10.1093/jac/49.4.597

20. Rutala W. A., Weber D. J. (2019). Disinfection, sterilization, and antisepsis: An overview. *American Journal of Infection Control;* 47:3–9. doi.:10.1016/j.ajic.2019.01.018

21. Takó M., Kerekes E. B, Zambrano C., Kotogán A., Papp T., Krisch J., Vágvölgyi C. (2020). Plant phenolics and phenolic-enriched extracts as antimicrobial agents. *Antioxidants; 9*(2):165. doi.:10.3390/antiox9020165

22. Tarka P., Nitsch-Osuch A. (2021). Evaluating the virucidal activity of disinfectants according to European Union standards. *Viruses*; 13(4):534. doi.:10.3390/v13040534

23. Wales A., Gosling R. J., Bare H. L., Davies R. H. (2020). Disinfectant testing for veterinary and agricultural applications. *Zoonoses and Public Health; 67*(5):435–447. doi.:10.1111/zph.12830.

Експериментальна оцінка бактерицидної активності та стабільності біоцидного засобу «Крезонід»

Коваленко В.[1,2] (ORCID: 0000-0002-2416-5219), Романько М.[1] (ORCID: 0000-0003-0285-5603), Ігнатьєва Т.[2,5] (ORCID: 0000-0001-9905-4807), Лінійчук Н.[1] (ORCID: 0000-0001-6745-307X), Попов Д.[4], Мягка К.[1] (ORCID: 0000-0002-3089-4012), Ступак О.[1] (ORCID: 0000-0001-5391-3530), Пономарьова С.[3]
1 – Державний науково-дослідний інститут з лабораторної діагностики та ветеринарно-санітарної експертизи, Київ, Україна, e-mail: kovalenkodoktor@gmail.com
2 – Інститут ветеринарної медицини, м. Київ, Україна
3 – Державний науково-дослідний контрольний інститут ветеринарних препаратів та кормових добавок, м. Львів, Україна
4 – компанія SANFORT, Київ, Україна
5 – Державний біотехнологічний університет, м. Харків, Україна

Резюме. *У статті наведено результати експериментальної оцінки бактерицидної активності та стабільності біоцидного засобу «Крезонід», основним діючим компонентом якого є мета-крезол (3-метилфенол). Дослідження проведено з метою обґрунтування ефективності біоцидного препарату та підтвердження збереження його антимікробних властивостей у процесі тривалого зберігання відповідно до вимог. Бактерицидну активність оцінювали кількісним суспензійним методом із визначенням кількості життєздатних мікроорганізмів (CFU) після експозиції препарату. Як тест-культури використовували штами Streptococcus faecalis та Salmonella enteritidis. Препарат досліджували у концентраціях 0,1; 0,5 та 1,0 % за експозиції 10–30 хв. Стабільність біоцидного засобу вивчали в умовах реального зберігання протягом 30 міс шляхом контролю органолептичних показників, значення pH та збереження бактерицидної активності. Встановлено, що біоцидний засіб «Крезонід» проявляє виражену бактерицидну активність щодо обох культур тест-мікроорганізмів. Повна інактивація Streptococcus faecalis та Salmonella enteritidis досягалася при концентрації 0,5 % і експозиції 20 хв. У процесі зберігання препарат «Крезонід» зберігав фізико-хімічну однорідність, стабільне значення pH та повну бактерицидну активність протягом усього періоду спостереження. Отримані результати свідчать про високу ефективність і стабільність біоцидного засобу «Крезонід», що обґрунтовує доцільність його застосування у ветеринарній практиці та можливість встановлення терміну придатності не менше ніж 24 міс. за регламентованих умов зберігання.*

Ключові слова: біоцидні засоби, мета-крезол, бактерицидна активність, стабільність, ветеринарна дезінфекція

DOI: 10.31073/onehealthjournal2026-III-05

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

UDC 619:636.2.09:616.995.132
DOI: 10.31073/onehealthjournal2026-III-06

Diagnostic aspects of cattle sarcocystosis in Ukraine

Lytvynenko O.[1] (ORCID: 0009-0003-0682-8917), Aliekseieva G.[1] (ORCID: 0000-0001-6158-5960), Panchykhin O.[2] (ORCID: 0009-0008-5029-8024), Yanenko U.[1] (ORCID: 0000-0001-5678-3356), Galat M.[2] (ORCID: 0000-0001-8881-0865), Shevel Yu.[1], Nebeshchuk O.[1] (ORCID: 0000-0002-5838-7418)
1 – State Scientific Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise, Kyiv, Ukraine, e-mail: 2431519@ukr.net
2 – National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine

Abstract. *Sarcocystosis of cattle remains a relevant parasitological problem in the system of post-mortem veterinary and sanitary control due to the predominantly asymptomatic course of infection and the limited diagnostic value of macroscopic examination. The aim of this study was to investigate the level of sarcocystosis infestation in cattle by means of macroscopic examination of muscle tissues and compression microscopy of stained sections, to determine the degree of involvement of different anatomical muscle groups, and to study the morphological and morphometric characteristics of Sarcocystis spp.*

A total of 473 muscle samples (esophagus, myocardium, masticatory muscles, hind limb muscles, diaphragm, and intercostal muscles) collected during post-mortem veterinary inspection in December 2025 and January 2026 were examined. Diagnostic procedures included macroscopic inspection with serial incisions and compression microscopy of stained preparations, followed by morphometric analysis using digital microscopy and ImageJ software.

No macroscopic cysts of Sarcocystis spp. were detected during visual inspection. In contrast, compression microscopy revealed microsarcocysts in 105 out of 473 examined samples, corresponding to an overall prevalence of 22.2%. A pronounced anatomical unevenness of infection was established, with the highest infestation rates observed in the esophagus and hind limb muscles (up to 72%). Morphometric analysis revealed two main morphological types of microsarcocysts: oval and spindle-shaped, which differ visually.

The obtained results confirm the latent course of sarcocystosis in cattle and demonstrate the high diagnostic value of compression microscopy of stained preparations. The use of this method is essential for an objective assessment of infestation levels and for improving the effectiveness of veterinary and sanitary control of meat safety.

Keywords: sarcocystosis, cattle, compression microscopy, muscle tissues, post-slaughter diagnosis

Sarcocystosis is one of the most widespread parasitic diseases of farm animals, particularly cattle, with a pronounced zoonotic potential (Dubey, 2016). The causative agents of this disease are protozoa of the genus *Sarcocystis*, which infect the muscular tissues of animals (Fayer, 2004). According to numerous studies, the prevalence of *Sarcocystis* infection in cattle in different regions of the world may reach 70–100%, highlighting the significant epizootic importance of this parasitosis (Dubey, 2006).

The disease not only reduces animal productivity but also poses risks to public health through the consumption of contaminated meat (Moré, 2011). Zoonotic species of the pathogen, such as *S. hominis* and *S. heydorni*, can be transmitted to humans through insufficiently heat-treated meat, causing gastrointestinal disorders (Gjerde, 2013; Fayer, 2015). The parasite has a heteroxenous life cycle with alternation of definitive hosts (carnivorous animals: dogs, cats, and humans) and intermediate hosts (cattle) (Dubey, 2016, Hu, 2017). Definitive hosts excrete sporocysts with feces, contaminating the environment, feed, and water, thereby ensuring stable circulation of theparasiteintheexternal environment (Dubey, 1989). In intermediate hosts, the parasite forms sarcocysts in the muscles, which become a source of infection for definitive hosts when raw, insufficiently heat-treated, or frozen meat is consumed (Gjerde, 2014, Dubey, 2015).

The causative agents of sarcocystosis demonstrate high resistance to environmental factors (Oryan, 2010). Sporozoites and sporocysts of *Sarcocystis spp.* are able to retain invasive properties for months at low temperatures (down to −20 °C), in moist soil, or on contaminated surfaces (European Food Safety Authority (EFSA), 2015). This contributes to long-term contamination of pastures, feed, water, and animal housing facilities (Fayer R, 2019). Tissue stages of the pathogen in meat are relatively resistant to cooling, freezing, and salting: sarcocysts survive at −4 °C for several weeks but die during deep freezing (−20 °C for 3 days) or cooking (70 °C for 20 min) (World Organisation for Animal Health (WOAH, ex-OIE), 2021) Such resistance is critically important for veterinary-sanitary safety of meat products, as insufficient processing may lead to outbreaks of zoonotic infections (European Food Safety Authority (EFSA), 2015).

The pathogenic effect of *Sarcocystis spp.* is caused by mechanical damage to muscle fibers, toxic-allergic effects of parasite metabolites, and immunopathological reactions of the host organism (Lindsay, 2020). During the acute phase (schizogony), endothelial damage to blood vessels occurs, which may lead to hemorrhages, anemia, and neurological symptoms (Dubey, 2023). The chronic phase is characterized by the formation of sarcocysts in the muscles and often proceeds subclinically (Yang, 2018). In most cases, sarcocystosis in cattle does not manifest with pronounced clinical signs (latent course), complicating diagnosis during the life of animals (Araujo, 2025, European Food Safety Authority (EFSA), 2015).

The absence of specific symptoms contributes to underestimation of the invasion and preservation of infection sources within animal populations (Vangeel, 2021). Economic losses caused by sarcocystosis are complex and include reduced body weight gain (up to 20% in infected animals), deterioration of meat quality (reduced tenderness and flavor), Carcasses affected by sarcocysts are subject to technical disposal, which leads to direct losses for farms and meat processing plants (European Food Safety Authority (EFSA). 2015). The disease also requires additional costs for control and prevention, including improvement of animal husbandry hygiene and meat processing measures (Imre, 2019).

Timely laboratory diagnosis is a key aspect of sarcocystosis control, especially considering its latent course (Gjerde, 2015, Yang, 2018). Traditional post-mortem examination methods (visual inspection and macroscopic examination of muscles) detect only macroscopic forms of the parasite, missing microscopic sarcocysts (Oryan, 2010). Therefore, more sensitive diagnostic methods are commonly used, including compression microscopy, histological staining (hematoxylin–eosin), enzyme-linked immunosorbent assay (ELISA) for antibody detection, and PCR for molecular identification of species (Gjerde, 2015, Yang 2018).

Control measures involve preventive strategies such as restricting access of carnivorous animals to pastures, maintaining feed hygiene, and adequate heat treatment of meat (World Organisation for Animal Health (WOAH, ex-OIE), 2021). Further research is required to develop effective vaccines and antiparasitic drugs (Dubey, 2023, Zaib, 2024). Sarcocystosis remains a significant problem for livestock production and public health, requiring integrated approaches to diagnosis, prevention, and control (Lindsay, 2020).

Aim of the study. To determine the degree of infection of different anatomical groups of muscle tissues with *Sarcocystis spp.* using compression microscopy of stained sections for the detection of microcysts, and to investigate the morphological and morphometric characteristics of microsarcocysts.

Materials and methods. The material for the study consisted of samples taken from cattle, namely: esophagus, myocardium (heart muscle), masticatory muscles, hind limb muscles, diaphragm, and intercostal muscles. Sampling was carried out during post-mortem veterinary-sanitary inspection in accordance with generally accepted rules for the collection of biological material.

One of the first stages of diagnosis was the visual detection of macrocysts, which are larger than 2.0 cm. Examination was performed according to generally accepted methodological guidelines for the diagnosis of sarcocystosis, in compliance with standards of veterinary-sanitary meat inspection.

Each anatomical specimen was subjected to external visual inspection under sufficient lighting to evaluate the surface structure and detect pathological inclusions. Particular attention was paid to the detection of macroscopic sarcocysts in the form of whitish or grayish, elongated or oval formations located between muscle fibers or under serous membranes.

To increase diagnostic sensitivity, each examined organ was subjected to a series of longitudinal and transverse incisions with a scalpel at intervals of 0.5–1.0 cm, which allowed visualization of deeper layers of muscle tissue. The cuts were examined with the naked eye and using a magnifying glass (×2.0–5.0) to detect macrocysts.

During the macroscopic examination, no cases of macrocyst detection were recorded in the examined anatomical organs.

The second stage of the study was based on the detection of microcysts. From each anatomical sample, four sections approximately the size of a rice grain were prepared. The sections were stained for 40 minutes using methylene blue with the addition of concentrated acetic acid (1:1), which provided contrast staining of muscle fibers and intracellular parasitic structures. After staining, the sections were washed twice with distilled water.

The experimental sections were placed in the wells of the lower part of a compressorium, covered with the upper plate, and evenly fixed by tightening the screws until a thin transparent preparation suitable for microscopic examination was obtained. Microscopic examination was performed using a light microscope.

Primary examination was carried out at magnification: objective ×5, eyepiece ×10 to detect inclusions morphologically similar to sarcocysts. When suspicious forms were detected, further examination was performed at magnification: objective ×10, eyepiece ×10 for detailed assessment of the shape, size, and localization of the parasite in muscle fibers.

To establish the final diagnosis and detect developmental stages of the parasite, individual sections were transferred to a glass slide, covered with a coverslip, and examined at high magnification: objective ×40, eyepiece ×10. Diagnostic criteria included the presence of a clearly defined sarcocyst, the characteristic structure of its wall, and detection of bradyzoites.

The final stage of diagnosis involved the study of morphological and morphometric characteristics of microsarcocysts by isolating the parasite from muscle fibers through mechanical homogenization. To isolate sarcocysts, muscle samples from different anatomical locations (heart, diaphragmatic pillars, esophagus, masticatory, intercostal, and striated muscles) were used, obtained from one animal. Samples were cleaned of fat and fascia and mechanically ground twice by passing through a meat grinder with holes 3.0 mm in diameter. For examination, 10.0 g of minced muscle mass was selected, to which 40.0 ml of phosphate-buffered saline (PBS) (pH 7.4) was added, thoroughly mixed, and filtered through a medium-density sieve to remove coarse tissue fragments.

The obtained filtrate was transferred to centrifuge tubes and centrifuged at 600 × g for 5 min, after which the supernatant was removed and the sediment resuspended in 10.0–20.0 ml PBS. A portion of the suspension (1.0–2.0 ml) was transferred to a Petri dish and examined under a light microscope at low magnification (×4–5) with further clarification at ×10. Sarcocysts were identified by their characteristic elongated shape with rounded ends and absence of an internal fibrous structure; detected cysts were isolated under microscopic control and transferred into tubes with PBS for further storage in a refrigerator at 5 ± 1 °C.

Microscopic examinations were carried out using a ZEISS light microscope (Carl Zeiss, Germany) equipped with a standard digital video camera and proprietary ZEISS ZEN software. Preparations were examined in transmitted light using magnifications of ×50, ×100, and ×400, which allowed the study of microsarcocyst structure, shape, wall thickness, and the nature of internal contents, including the presence of bradyzoites.

Morphometric analysis was performed using the ImageJ software (National Institutes of Health, USA) after preliminary calibration using a micrometric scale. The length, width, and wall thickness of microsarcocysts were measured; for each morphological type, at least 20–30 measurements were performed followed by calculation of minimum, maximum, and mean values as well as standard deviation.

Results. During the examination for the presence of macrocysts, a visual inspection of anatomical organs was performed, including transverse and longitudinal incisions and the use of magnifying devices. No macrocysts were detected in the examined beef samples obtained from cattle, indicating the predominance of microscopic forms of sarcocyst invasion.

Diagnostic investigations aimed at detecting microcysts were carried out in december 2025 and january 2026. In total, 473 muscle tissue samples collected from cattle carcasses were examined. Positive results for the presence of sarcocysts were obtained during microscopic examination using the compression diagnostic method with preliminary staining of the samples.

Microscopy of the total number of samples examined (473) revealed 105 positive samples, which accounted for 22.2 % of the total number tested.

In December 2025, 376 samples were examined, of which 15 yielded positive results, representing 4 %.

In January 2026, 97 samples were examined, of which 50 yielded positive results, representing 51.5 %.

Thus, all data on the invasion of the examined cattle carcasses were obtained exclusively through compression microscopy of stained preparations. These findings confirm the low diagnostic informativeness of visual methods used during post-mortem veterinary and sanitary inspection and substantiate the necessity of mandatory microscopic diagnostics to obtain reliable information on the presence of sarcocysts in samples.

The overall detection rate of sarcocysts in 22.2% of the examined samples indicates a stable circulation of the pathogen among the cattle population, even in the absence of clinical manifestations.

The detection intensity of sarcocysts largely depends on the age of the animals, the duration of invasion, and the localization of the parasite within muscle fibers, as well as on the cyst density in the examined material. During the winter period, animals of older age groups are more frequently sent for slaughter, which potentially increases the probability of detecting sarcocystosis pathogens.

Thus, the obtained data confirm the key role of compression microscopy in the laboratory diagnostic system of sarcocystosis. The use of stained samples and stepwise microscopic analysis allowed to reveal the actual level of animal infestation, which was completely inaccessible using macroscopic examination methods.The analysis of the results suggests that the actual prevalence of sarcocystosis may be underestimated, since even the compression method has limited sensitivity in cases of low invasion intensity and uneven distribution of cysts in muscle tissue.

To determine the intensity of lesions in each anatomical group, 473 samples were examined from the esophagus, myocardium (cardiac muscle), masticatory muscles, hind limb muscles, diaphragm, cardiac muscle, and intercostal muscles.

According to the results, the intensity of muscle tissue infection ranged from 46.0% to 72.0%, indicating a significant prevalence of sarcocystosis among the examined cattle population. The obtained data made it possible to determine the percentage of infection for each organ according to the level of invasion. The highest level of invasion was observed in muscles with intensive blood supply, which creates optimal conditions for parasite migration and development. Based on the conducted studies, the following distribution of muscles according to the degree of pathogen accumulation was established:

Highly affected muscle groups:
esophagus – 72 %, myocardium (cardiac muscle) – 66 %, masticatory muscles – 61 %, hind limb muscles – 72 %

Moderately affected muscle groups: diaphragm – 52 %, intercostal muscles – 46 % (Fig. 1).

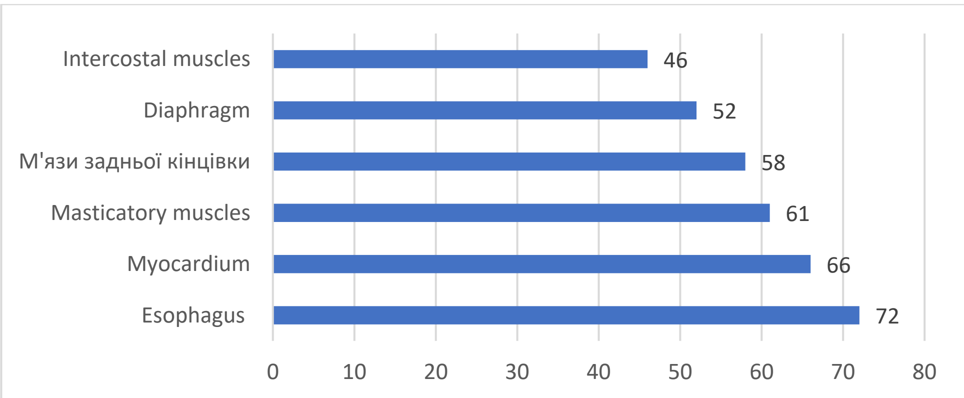


Fig. 1. Prevalence of Sarcocystis spp. infection in different muscle tissues of cattle

During microscopic examinations, microsarcocysts of *Sarcocystis spp.* were detected in the muscle tissues of cattle. These structures were predominantly localized intracellularly between muscle fibers, and less frequently arranged parallel to the direction of muscle bundles. Morphometric analysis performed using digital microscopy and measurement software (ImageJ) made it possible to classify the cysts according to their shape and size.

Based on morphological characteristics, the microsarcocysts detected in cattle muscle tissue were clearly divided into two main types, differing in shape, size, and predominant localization within the host organism.

Oval (ellipsoidal) microsarcocysts were characterized by the following parameters:

- length — 120–280 μm;
- width — 40–90 μm.

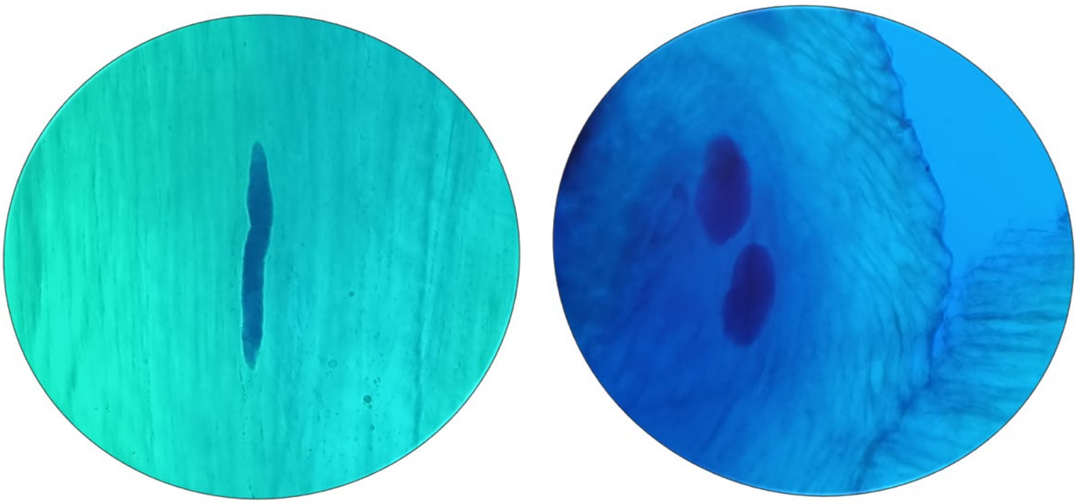
These cysts predominated in the myocardium (cardiac muscle) and esophageal muscles, which may indicate their adaptation to tissues with high functional activity and intensive blood supply.

Spindle-shaped (fusiform) microsarcocysts had the following dimensions:

- length — 180–350 μm;
- width — 30–70 μm.

They were more frequently detected in skeletal muscles, particularly in the masticatory muscles, neck muscles, limb muscles, and lumbar muscles. Their elongated shape and smaller width facilitate better integration into the long muscle fibers of skeletal musculature, which may explain their predominance in these tissues.

The sarcocyst wall was clearly delineated and thin or moderately thickened, with a thickness of 1.0–3.0 μm. The internal cavity of the cysts was filled with numerous bradyzoites, which were densely packed and oriented parallel to the longitudinal axis of the cyst.



a b

Fig. 2. Microsarcocysts of Sarcocystis spp. in cattle muscle tissues: (a) spindle-shaped (fusiform) microsarcocysts; (b) oval (ellipsoidal) microsarcocyst

Discussion. Thus, the identified morphological and morphometric heterogeneity of Sarcocystis spp. microsarcocysts indicates the presence of at least two morphotypes of the parasite, differing not only in shape and size but also in tissue tropism. The predominant localization of oval microsarcocysts in the myocardium and esophageal muscles, and spindle-shaped microsarcocysts in skeletal musculature, reflects the parasite's adaptation to the morphofunctional characteristics of different types of muscle tissue.

Conclusions

1. Macroscopic examination of cattle muscle tissues collected during post-mortem veterinary-sanitary inspection revealed no macroscopic sarcocysts (Sarcocystis spp.) in any of the examined anatomical samples, indicating the low diagnostic value of visual examination for sarcocystosis detection.
2. Compression microscopy of stained tissue sections allowed the detection of microsarcocysts of Sarcocystis spp. in 105 out of 473 examined samples, confirming the effectiveness of this method for identifying latent forms of invasion and determining the actual level of muscle tissue infection.
3. A pronounced anatomical unevenness of sarcocystosis infection was established. The highest infection rates were recorded in the esophagus and hind limb muscles (up to 72%), slightly lower in the myocardium and masticatory muscles (61–66%), while the intercostal muscles and diaphragm showed the lowest prevalence (46–52%).
4. Morphometric analysis made it possible to distinguish two main forms of microsarcocysts — oval and spindle-shaped, with lengths ranging from 120 to 350 μm and widths from 30 to 90 μm, indicating the morphological variability of tissue stages of the parasite and likely reflecting species-specific characteristics of the pathogen.
5. The obtained results confirm the latent course of sarcocystosis in cattle and justify the mandatory use of compression microscopy of stained preparations in post-mortem veterinary-sanitary control systems for ensuring the safety of meat products.

REFERENCES

1. Dubey J. P., Calero-Bernal R., Rosenthal B. M., Speer C. A., Fayer R. (2016). *Sarcocystosis of animals and humans* (2nd ed.). CRC Press; https://doi.org/10.1201/b19184
2. Fayer R. (2004). Sarcocystis spp. in human infections. *Clinical Microbiology Reviews; 17*(4), 894–902. https://doi.org/10.1128/CMR.17.4.894-902.2004
3. Dubey J. P., Lindsay D. S. (2006). Neosporosis, toxoplasmosis, and sarcocystosis in ruminants. *Veterinary Clinics of North America: Food Animal Practice; 22*(3), 645–671. https://doi.org/10.1016/j.cvfa.2006.08.001
4. Moré G., Abrahamovich P., Jurado S., Bacigalupe D., Marin J. C., Rambeaud M. (2011). Prevalence of *Sarcocystis* infection in Argentinean cattle. *Veterinary Parasitology; 177*(1–2), 162–165. https://doi.org/10.1016/j.vetpar.2010.11.036
5. Fayer R., Esposito D. H., Dubey J. P. (2015). Human infections with *Sarcocystis* species. *Clinical Microbiology Reviews; 28*(2), 295–311. https://doi.org/10.1128/CMR.00113-14
6. Gjerde B. (2013). Phylogenetic relationships among *Sarcocystis* species infecting cattle. *Parasitology Research; 112*, 3141–3150. https://doi.org/10.1016/j.ijpara.2013.02.004
7. Dubey J. P., Speer C. A., Fayer R. (1989). *Sarcocystosis of animals and man*; CRC Press.
8. Hu J. J., Huang S., Wen T., Esch G. W., Liang Y., Li H. L. (2017). Morphological and molecular characterization of *Sarcocystis* spp. in cattle. *Parasitology International; 66*(6), 818–824. https://doi.org/10.1016/j.parint.2017.05.003
9. Dubey J. P. (2015). Foodborne and waterborne zoonotic sarcocystosis. *Food and Waterborne Parasitology; 1*(1), 2–11. https://doi.org/10.1016/j.fawpar.2015.09.001
10. Tenter A. M. (1995). Current research on *Sarcocystis* species of domestic animals. *International Journal for Parasitology; 25*(11), 1311–1330. https://doi.org/10.1016/0020-7519(95)00068-d
11. Oryan A., Ahmadi N., Mousavi S. M. (2010). Prevalence, biology and distribution of *Sarcocystis* infection in cattle. *Tropical Biomedicine; 27*(2), 151–163.
12. World Organisation for Animal Health. (2021). Sarcocystosis. In *Manual of diagnostic tests and vaccines for terrestrial animals;* (pp. 1–15). WOAH.
13. Lindsay D. S., Dubey J. P. (2020). Biology of *Sarcocystis* species in livestock. *Veterinary Parasitology; 282*, 109139.
14. Dubey J. P., Rosenthal B. M. (2023). Bovine sarcocystosis: *Sarcocystis* species, diagnosis, prevalence, economic and public health considerations. *International Journal for Parasitology; 53*(9), 463–475. https://doi.org/10.1016/j.ijpara.2022.09.009

15. Yang Y., Dong H., Su R., Wang Y., Wang R., Jiang Y. (2018). High prevalence of *Sarcocystis* spp. infections in cattle from central China. *Parasitology International; 67*(6), 800–804. https://doi.org/10.1016/j.parint.2018.08.006
16. Vangeel L., Houf K., Chiers K., Vercruysse J., Dorny P. (2021). *Sarcocystis* species in bovine carcasses from a Belgian abattoir: A cross-sectional study. *Parasites & Vectors; 14*, 353. https://doi.org/10.1186/s13071-021-04788-1
17. Araujo L. S., Gupta A., Papadopoulos M. D., Naguib D., Battle J., Kwok O. (2025). High but variable prevalence of *Sarcocystis cruzi* infections in farm-raised bison beef destined for human consumption. *Parasites & Vectors; 18*, 35. https://doi.org/10.1186/s13071-025-06660-y
18. Zaib E. H., Jasim G. A. (2024). Comparative analysis of *Sarcocystis* infections in cattle, sheep and camels in Thi-Qar, Iraq. *South Asian Journal of Agricultural Sciences; 4*(2), 196–201. https://doi.org/10.22271/27889289.2024.v4.i2c.161
19. Imre M., Morar A., Ilie M. S. (2019). *Sarcocystis* spp. in Romanian slaughtered cattle: Molecular characterization and epidemiological significance of the findings. *BioMed Research International; 2019*, 4123154. https://doi.org/10.1155/2019/4123154

Діагностичні аспекти саркоцистозу великої рогатої худоби в Україні

Литвиненко О.[1] (ORCID: 0009-0003-0682-8917), Алєксеєва Г.[1] (ORCID: 0000-0001-6158-5960), Панчихін О.[2] (ORCID: 0009-0008-5029-8024), Яненко У.[1] (ORCID: 0000-0001-5678-3356), Галат М.[2] (ORCID: 0000-0001-8881-0865), Шевель Ю.[1], Небещук О.[1] (ORCID: 0000-0002-5838-7418)
1 – Державний науково-дослідний інститут з лабораторної діагностики та ветеринарно-санітарної експертизи, м. Київ, Україна, м. Київ, Україна, e-mail: 2431519@ukr.net
2 – Національний університет біоресурсів та природокористування, м. Київ, Україна

***Резюме**. Саркоцистоз великої рогатої худоби залишається актуальною паразитологічною проблемою у системі післязабійного ветеринарно-санітарного контролю, з огляду на переважно безсимптомний перебіг інвазії та обмежену інформативність макроскопічних методів діагностики. Метою роботи було вивчити рівень інвазованості великої рогатої худоби саркоцистозом шляхом макроскопічного огляду м'язових тканин та компресорної мікроскопії пофарбованих зрізів, визначити ступінь ураження різних анатомічних груп м'язів, а також дослідити морфологічні й морфометричні особливості мікросаркоцист Sarcocystis spp.*

Матеріалом для дослідження слугували 473 зразки м'язових тканин (стравохід, міокард, жувальні м'язи, м'язи задньої кінцівки, діафрагма, міжреберні м'язи), відібрані під час післязабійного ветеринарно-санітарного огляду у грудні 2025 та січні 2026 років. Діагностику проводили шляхом макроскопічного огляду з виконанням серійних розрізів та компресорної мікроскопії пофарбованих препаратів із подальшим морфометричним аналізом із використанням цифрової мікроскопії та програмного забезпечення ImageJ.

У ході макроскопічного дослідження макроцисти Sarcocystis spp. не були виявлені. Водночас компресоріумна мікроскопія дозволила встановити наявність мікросаркоцист у 105 із 473 досліджених зразків, що становило 22,2 %. Встановлено виражену анатомічну нерівномірність інвазії з найвищим рівнем ураження у стравоході та м'язах задньої кінцівки (до 72 %). Морфометричний аналіз виявив дві основні морфологічні форми мікросаркоцист, овальну та веретеноподібну, які відрізнялися за розмірами та тканинною тропністю.

Отримані результати підтверджують латентний перебіг саркоцистозу у великої рогатої худоби та доводять високу діагностичну цінність компресорної мікроскопії пофарбованих зразків тканин. Застосування даного методу є обов'язковим для об'єктивної оцінки рівня інвазованості та підвищення ефективності ветеринарно-санітарного контролю безпечності м'ясної продукції.

Ключові слова: саркоцистоз, велика рогата худоба, компресоріумна мікроскопія, м'язові тканини, післязабійна діагностика.

DOI: 10.31073/onehealthjournal2026-III-06

UDC: 619:636:591.133.2:591.478
DOI: 10.31073/onehealthjournal2026-III-07

Laminitis as a cause of metabolic disorders in domestic animals (review paper)

Ligomina I. [1] **(ORCID: 0000-0001-8569-9487), Kovalchuk Y.** [1] **(ORCID: 0000-0003-3677-3411), Galatyuk O.** [1] **(ORCID: 0000-0002-9720-0660), Sokulsky I.**[1] **(ORCID: 0000-0002-6237-0328), Karpyuk V.** [1] **(ORCID: 0000-0003-3728-5698), Dubovy A.** [1] **(ORCID: 0000-0003-2341-1868), Radzikhovskyi M.** [2] (ORCID: 0000-0003-0518-8148), **Solovyova L.** [3] **(ORCID: 0000-0001-9455-8299)**
1 – Polissya National University, Zhytomyr, Ukraine, e-mail: sokulskiy_1979@ukr.net
2 – National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine,
3 – Bila Tserkva National Agrarian University, Bila Tserkva, Ukraine

Abstract. *The article presents a comprehensive analysis of modern scientific data on laminitis in domestic animals, taking into account the specific features of the course of the pathological process in horses, cattle, sheep and goats. The approaches to understanding laminitis as a systemic multifactorial syndrome, which is formed as a result of the interaction of metabolic, endocrine, infectious-toxic and mechanical factors, are summarized. It is shown that the development of the disease is accompanied by deep microcirculation disorders in the lamellar apparatus of the hoof, degradation of the dermal-epidermal junction, activation of inflammatory and ultimately leads to structural destruction of the hoof apparatus and a decrease in animal productivity.*

Particular attention is paid to the analysis of pathogenetic mechanisms taking into account the type of digestion. It has been established that in horses, as monogastric animals, endocrine and metabolic disorders play a leading role, in particular hyperinsulinemia, insulin resistance and imbalance of carbohydrate metabolism, which causes the development of endocrinopathic laminitis. In cattle, the key triggering mechanism is subclinical rumen acidosis, which is accompanied by changes in the rumen microbiota, increased blood endotoxin levels and the development of a systemic inflammatory response. In sheep and goats, laminitis is formed as a result of a combination of metabolic, infectious and mechanical factors and determines the variability of clinical and morphological manifestations of the disease.

Biochemical changes characteristic of different stages of laminitis (subclinical, acute and chronic) is systematized, including increased concentrations of insulin, glucose, triglycerides, activation of tissue damage enzymes (lactate dehydrogenase, creatine kinase), pro-inflammatory cytokines, C-reactive protein. It is shown that these indicators can be used for early diagnosis, differentiation of stages of the disease and prediction of its course. Modern approaches to the diagnosis of laminitis are also summarized, based on a combination of clinical examination, laboratory studies and instrumental methods, in particular radiography, ultrasound diagnostics, computed tomography and motor activity monitoring systems. Morphological, histological and pathoanatomical changes are characterized, which reflect the progression of the pathological process from initial microscopic lesions to pronounced hoof deformation, tissue necrosis and fibrosis.

At the same time, modern approaches to the prevention of laminitis are separately considered, which include optimization of feeding, control of metabolic status, prevention of subclinical acidosis, improvement of housing conditions and implementation of early monitoring technologies. It is emphasized that effective management of laminitis risks is possible only under the conditions of an integrated approach that takes into account the species characteristics of animals, the level of their working capacity for horses and productivity for cattle, and technological conditions of maintenance.

The results of the generalization can be used to improve the system of early diagnosis, prevention and control of laminitis and will contribute to increasing productivity and improving the welfare of domestic animals.

Keywords: hoof, inflammatory process, acidosis, endotoxemia, impaired microcirculation, metabolism, lameness

Laminitis in domestic animals is a complex multifactorial disease characterized by damage to the lamellar apparatus of the hoof, impaired microcirculation and the development of degenerative changes in the tissues. This disease is accompanied by metabolic disorders, endocrine dysfunctions, inflammatory processes and changes in the microbiota of the digestive tract (Nocek, 1997; Cook, 2004; Koziy, 2008; Enemark, 2008; Bojkovski, 2023).

Recent studies demonstrate that metabolic products of sugars, particularly methylglyoxal, can directly damage the lamellar tissue of the hoof, causing structural and histological changes characteristic of laminitis, as confirmed in ex vivo lamellar explant models in horses (Vercelli, 2021).

Laminitis is particularly relevant for working horses and high-yielding cows, where metabolic and hormonal mechanisms largely determine the severity of the disease, the level of productivity and the state of health. (Asplin, 2007; Bailey, 2013; Radzyhovskyi, 2024). Laminitis in sick animals causes lameness, reduced productivity, and a deterioration in the quality of life of animals, making it one of the key problems of modern animal husbandry (Clarkson, 1996; Cook, 2003; Greenough, 2007; Sertu, 2024).

Historically, the first scientific descriptions of laminitis in horses appeared in the second half of the 20th century, when researchers mainly associated the development of the disease with mechanical overload and circulatory disorders in the hoof. The classic works of Colles and Jeffcott laid the foundation for understanding laminitis as a vascular pathology. Subsequent studies have significantly expanded the understanding of the etiology and pathogenesis of the disease, proving that laminitis is a polyetiological process (Colles, 1977; Greenough, 2007; Vercelli, 2021). In high-yielding cows, the first detailed observations of laminitis as a factor in metabolic disorders were presented by Ukrainian researchers and emphasized the role of endocrine and cicatricial mechanisms in the development of the disease (Nocek, 1997; Koziy, 2008; Mulligan, 2008).

Laminitis is one of the most common causes of lameness in horses and cattle on all continents, although exact incidence rates vary considerably depending on the species of animal, housing conditions, type of farm and diagnostic methods (Wylie, 2011). Incidence analyses show that the incidence of laminitis in horses ranges from approximately 1.5% to 34% across populations, indicating significant geographical and technological variation (Wylie, 2011). In Finland, among horses presenting to veterinary clinics with laminitis, about 89% of cases were associated with endocrinopathies or metabolic disorders, rather than just mechanical injuries. (Karikoski, 2011).

In cattle, direct data on the incidence of laminitis on a global scale are less common, but the incidence of associated hoof lesions and lameness ranges from 10% to over 50%, depending on feeding and housing conditions (Webster, 2001; Bergsten, 2003; Oetzel, 2007; Capion, 2009). For example, in Turkey, 28.6% of cows were lame, with 82.7% of cases related to hoof lesions associated with subclinical laminitis (Belge, 2005; Bostanlık, 2025). Monitoring of dairy herds in Romania showed laminitis incidence of around 20.3% in 2021, 18.6% in 2022 and 17.4% in 2023, with a clear negative impact on productivity (Popescu, 2013; Kornienko, 2024).

In Ukraine, quantitative data on laminitis are less systematic, due to the limited number of dedicated open access studies. However, regional observations in Vinnytsia, Donetsk, Lviv and Cherkasy regions demonstrate a significant proportion of orthopedic hoof pathologies and lameness, which are often a consequence or accompany laminitis, in line with global trends (Koziy, 2008; Archer, 2010; Klymas, 2025).

Thus, laminitis is a widespread problem in livestock farming, which limits the performance of working and sports horses, the productivity of cattle, causes deterioration of the condition of animals and leads to significant costs, which provide treatment and preventive measures (Greenough, 2007; Sertu, 2024).

Analysis of recent research and publications. Laminitis in domestic animals is defined as a systemic multifactorial syndrome manifested by damage to the lamellar apparatus of the hoof and impaired microcirculation, with the subsequent development of degenerative changes in the tissues (Boosman, 1991; Greenough, 2007; Faustmann, 2025).

Modern research proves that laminitis is not only local, but also systemic in nature, affecting metabolism, the endocrine system, inflammatory processes and the microbiota of the digestive tract (Sadiq, 2020; Bezpalko, 2023).

Given the multifactorial nature of laminitis, further research focuses on its etiology, which combines systemic and local factors and determines the animal's predisposition to develop pathological changes.

Systemic ones include metabolic dysfunctions, endocrine disorders, insulin resistance and hormonal changes, which can lead to increased concentrations of insulin and glucose in the blood, and imbalance of lipid metabolism (Asplin, 2007; Bailey, 2013; de Laat, 2014; de Laat, 2019).

Local factors include mechanical overload of the limbs, hoof injuries, infectious processes and microcirculatory disorders, which contribute to ischemia and degradation of the lamellar layer (Boosman, 1991; Pollitt, 1996; Hood, 1999). The complexity of the etiology lies in the interaction of these factors: systemic disorders increase susceptibility to local damage, and injuries or inflammatory processes in the hoof exacerbate metabolic disorders.

In cows, the main risk factors are subclinical cicatricial acidosis, impaired mineral metabolism, a highly productive lactation period, and irrational feeding (Bergsten, 2003; Oetzel, 2007; Enemark, 2008). Local factors include mechanical overload of the hooves, injuries and improper care of the hooves (Greenough, 1997; Sadiq, 2020).

The mechanisms of laminitis development vary depending on the form of the disease. Acute laminitis in horses and cows is often associated with impaired microcirculation and inflammatory reactions in the lamellar tissue, leading to detachment of the stratum corneum of the hoof (Pollitt, 1996; Hood, 1999; Ding, 2020). Metabolic mechanisms include the effects of hyperinsulinemia on endothelial cells and a reduction in the ability of lamellar tissue to repair or regenerate (Asplin, 2007; de Laat, 2019).

In cattle, endotoxins and acid-base imbalances are of additional importance in subclinical cicatricial acidosis (Boosman, 1991; Nocek, 1997).

Clinical signs. Laminitis is characterized by variability of signs and depends on the form of the disease. Acute laminitis is manifested by sudden lameness, hoof pain, change in posture and refusal to move (Hood, 1999; Vercelli, 2021).

Chronic forms are characterized by progressive destruction of lamellar tissue, hoof deformation, and persistent lameness (Greenough, 2007).

Subclinical laminitis often has no obvious external symptoms, making it difficult to diagnose without the use of special research methods (Mudron, 1996; Koziy, 2008).

The inflammatory component of the disease is manifested by the activation of pro-inflammatory cytokines, endothelial dysfunction and tissue edema, and increased activity of matrix metalloproteinases contributes to the destruction of intercellular connections in the lamellar tissue (Capion, 2009; Ding, 2020).

Biochemical damage to the hoof is accompanied by increased activity of tissue damage enzymes and an imbalance of antioxidant defense.

Experimental studies in horses have confirmed the key role of metabolic factors in the development of laminitis: prolonged hyperinsulinemia itself is capable of inducing laminitis in clinically healthy animals, which emphasizes the importance of endocrine mechanisms in the pathogenesis of the disease (Asplin, 2007; de Laat, 2019).

Metabolic responses to high glycemic index diets in horses and ponies also suggest a link between feeding and the risk of laminitis (Bailey, 2013).

In this regard, timely and accurate diagnosis of the disease is of particular importance. Diagnosis of the disease is based on a comprehensive approach and includes clinical examination, assessment of gait and posture, laboratory studies of metabolic and inflammatory markers, as well as instrumental methods (radiography, ultrasound, thermography) (Greenough, 2007; Danscher, 2009; Danscher, 2010). For example, telemetric monitoring of movement allows you to detect early changes in gait that precede severe lameness, and behavioral changes can be a significant marker of the initial stages of laminitis (Danscher, 2009; Danscher, 2010).

Based on the identified risk factors and early changes, a comprehensive prevention of laminitis is formed, it is multi-component and includes feeding control with avoidance of excess of easily digestible carbohydrates, balancing the diet, prevention of subclinical cicatricial acidosis

(Oetzel, 2007; Enemark, 2008), monitoring of insulin resistance (Harris, 2006), optimization of housing conditions, timely hoof trimming (Sadiq, 2020) and other management measures (Bergsten, 2003). Veterinary monitoring of productivity, reliability and motor activity of animals allows for timely identification of risks for the development of laminitis and adjustment of feeding and housing.

Despite the implementation of preventive measures, laminitis and related forms of lameness have been shown to significantly reduce milk yields, reduce live weight gain and reduce reproductive performance (Archer, 2010; Sertu, 2024). Additional costs to the farm arise from the need for treatment, specialized care and hoof correction, as well as from the loss of working and sporting horses, which emphasizes the importance of an integrated approach to prevention and monitoring of animal health.

Therefore, current scientific publications emphasize and emphasize the need to consider and study the features of the development of laminitis as a systemic syndrome, including metabolic, hormonal and inflammatory components, and not only as a local pathology of the hoof (Eades, 2010; de Laat, 2019). This opens new perspectives for the development of comprehensive preventive programs that take into account both nutritional and hormonal metabolic risk factors.

That is why the purpose of the study is to determine the prevalence of laminitis, assess its impact as a factor in metabolic dysfunctions, and develop approaches to diagnosis and prevention to increase productivity and maintain the health of domestic animals.

Materials and methods. To prepare the review article, a systematic analysis of the scientific literature on the topic of laminitis in horses and cattle was conducted. The PubMed, Scopus, Web of Science and Google Scholar databases were used, in particular, studies related to the etiology, pathogenesis, risk factors, clinical manifestations and prevention of laminitis. Only works with reliable methodological data were selected, and information from different sources was subjected to comparative and analytical synthesis. This approach allowed us to systematize modern scientific data and identify the main patterns of the development of laminitis and its prevention.

Results. *Pathogenesis of laminitis in domestic animals.* Laminitis in domestic animals is the result of a complex interaction of pathogenetic mechanisms, leading to damage to the lamellar apparatus of the hoof and disruption of its structural integrity. The pathogenesis of the disease differs significantly depending on the species of animal, which is due to the characteristics of the digestive system, metabolism, endocrine regulation and conditions of detention.

In horses, which are monogastric animals, fiber fermentation occurs mainly in the cecum and colon. Excessive consumption of concentrated feeds, a high content of easily digestible carbohydrates and abrupt changes in the diet lead to a rapid increase in blood glucose levels, stimulating insulin secretion and the development of insulin resistance (Table 1).

Table 1

Risk factors for the development of laminitis in domestic animals

Group of factors	Examples	Animal species
Metabolic	Insulin resistance	Horses
Nutritional	Concentrates	Cattle
Mechanical	Hard floors	All
Infectious	Endotoxins	Cattle

Chronic hyperinsulinemia and insulin regulation imbalance disrupt the function of the endothelial microvessels of the lamellar apparatus, reduce vasodilation and increase capillary permeability. This leads to local edema, impaired blood supply and oxygen metabolism in the epidermal and dermal layers of the hoof. At the cellular level, pro-inflammatory signaling pathways are activated, the expression of cytokines (TNF-α, IL-1β, IL-6) and inducible oxide synthase (iNOS) increases, which causes the accumulation of free radicals and oxidative stress, with the destruction of the basement membrane of the epidermis and the degradation of lamellar structures (Belknap et al., 2007).

Increased pressure on the hoof apparatus due to physical exertion, insufficient trimming or hard surfaces increases mechanical damage and contributes to the progression of degenerative changes (van Oldruitenborgh Oosterbaan, 1999; Hood, 1999; Asplin, 2007; Bailey, 2013).

Pathogenetic mechanisms of laminitis in cattle, which are polygastric animals, are mainly associated with impaired rumen digestion and endotoxin intoxication. Thus, in subclinical rumen acidosis (SARA), the rumen microbiota changes, the level of lipopolysaccharides (endotoxins) increases, which are absorbed into the bloodstream and activate a systemic inflammatory response. At the level of the lamellar apparatus, endotoxins cause endothelial dysfunction, increase leukocyte adhesion, enhance the expression of intercellular adhesion molecules (ICAM-1, VCAM-1) and activate the synthesis of pro-inflammatory cytokines. This leads to impaired capillary hemodynamics, local edema, ischemia and oxidative tissue damage. At the same time, high-yielding cows that stand on hard surfaces for long periods of time are exposed to mechanical stress, which increases stratum corneum degradation and the formation of chronic laminitis (Boosman, 1991; Oetzel, 2007; Enemark, 2008; Ding, 2020).

In sheep and goats, the pathogenesis of laminitis is often combined and includes metabolic, nutritional, mechanical and infectious factors. Excessive introduction of concentrates into the diet and a deficiency of coarse fibers can cause carbohydrate metabolism disorders and microcirculatory changes in the hoof, while prolonged stay on wet, dirty or hard litter increases the mechanical load on the lamellar apparatus. The lesion is accompanied by activation of the local inflammatory reaction, degradation of horny structures and disruption of the integrity of the basal membrane of the epidermis. In this group of animals, a combination of factors plays a particularly important role: for example, endotoxinemia due to infectious-toxic processes in the gastrointestinal tract increases mechanical damage and degradation of the stratum corneum (Table 2) (Boosman, 1991; Lischer, 1994).

Table 2

Comparison of the pathogenesis of laminitis by animal species

Animal type	Key mechanism	Trigger	Main pathogenetic links
Cattle	Metabolic-toxic	Subclinical cicatricial acidosis (SARA)	Endotoxinemia, inflammation
Horse	Endocrinopathic	Hyperinsulinemia	Microcirculation disorders, basement membrane degradation
Sheep/Goat	Combined	Feeding + conditions	Inflammation + mechanical stress

Thus, the pathogenesis of laminitis in domestic animals is multifactorial and specific for each species: in horses, endocrine and metabolic disorders play a leading role, in cattle – feed, infectious-toxic and hemodynamic changes, and in sheep and goats - a complex of metabolic, feed and mechanical factors. These processes determine local changes in lamellae, microcirculation disorders, stratum corneum degradation and systemic biochemical imbalances, which directly determine the formation of clinical manifestations and severity of laminitis.

Clinical picture of laminitis in domestic animals. In this regard, the clinical picture of the disease in domestic animals is characterized by species-specific characteristics, variability of symptoms, and different dynamics of the development of the pathological process (Table 3).

Table 3

Clinical symptoms (systematized)

Symptom	Horses	Cattle	Sheep/Goats
Pain	Pronounced	Moderate/Extreme	Variable
Position	"Backward deviation"	Cautious gait	Constrained gait
Hoof Temperature	↑	↑	±
Performance	↓ impaired motor activity	↓ milk	↓ increments

In horses, the course of laminitis is closely related to endocrine and metabolic disorders, and in the early, subclinical stages may manifest only with minor changes in behavior and motor activity. Animals become less active, there is periodic weight transfer from one limb to the other, a slight increase in local hoof temperature and pulsation of the digital arteries, and often remains unnoticed without specialized control (Asplin, 2007; Belknap, 2007; Danscher, 2010; Bailey, 2013).

As the acute stage progresses, horses develop a characteristic clinical picture: pronounced lameness, reluctance to move, a typical "laminitis posture", which is associated with local inflammation and increased expression of cytokines in the lamellae (Belknap, 2007) with weight transfer to the hind limbs and forelimbs forward, which is caused by pain in the hoof area. There is increased pulsation of the digital arteries, local hyperthermia, pain on palpation and testing of the hoof, and in severe cases, rotation or lowering of the third phalanx, which is confirmed by instrumental methods (Hood, 1999; Vercelli, 2021). The chronic course in horses is characterized by persistent morphofunctional changes: hoof deformation, formation of so-called "laminite rings", expansion of the white line, decreased elasticity of the stratum corneum, and prolonged lameness, which significantly limits the working capacity and sporting use of animals. (O'Grady, 2008; Wylie, 2011).

In cattle, clinical signs of the disease are often subclinical or chronic, making timely diagnosis difficult. In the initial stages, decreased activity, changes in posture, cautious gait, increased lying time, and reduced feed intake are noted (Cook, 2003; Danscher, 2009). Subclinical laminitis in cows often manifests as hemorrhages in the sole of the hoof, yellow spots of the stratum corneum, and minor abnormalities in the growth of the hoof horn, which are detected only when examining or trimming the hoof (Mudron, 1996; Zhao, 2020). In clinically pronounced cases, lameness of varying degrees, gait asymmetry, pain when loading the limbs, and the development of complications in the form of plantar ulcers, double soles, and white lines are observed (Bergsten, 2003; Greenough, 2007). The chronic course is accompanied by persistent lesions of the ungulates, which leads to a decrease in milk yield, deterioration of reproductive performance and the general condition of the animals (Capion, 2009; Archer, 2010).

In sheep and goats, the clinical manifestations of laminitis are less specific, but are important in intensive housing conditions. In the early stages, lameness, limited mobility, frequent lying down, and changes in the shape and structure of the hoof are noted. Later, deformities of the hoof horn, its delamination, increased fragility and susceptibility to secondary infections develop, which can complicate the course of the disease (Lischer, 1994). In these species, housing conditions play a significant role, in particular, the humidity of the litter and the density of the animals, which contribute to the chronicity of the process.

Thus, the clinical course of laminitis in domestic animals is characterized by significant variability and depends on the type of animal, conditions of detention and stage of the disease. In the end, acute and endocrinopathic forms with pronounced pain syndrome and local inflammatory process in the lamellae predominate, which is confirmed by increased activity of IL-1β, IL-6, TNF-α (Belknap, 2007), in cattle - subclinical and infectious course with gradual development of affected hooves, while in sheep and goats clinical manifestations are often associated with conditions of detention and tend to become chronic. This necessitates a differentiated approach to the diagnosis, treatment and prevention of laminitis in different types of domestic animals.

Biochemical changes in laminitis. Taking into account the stages of the pathological process, the development of laminitis in domestic animals is characterized by specific morphofunctional and biochemical changes. Laboratory methods include the determination of biochemical indicators that allow differentiating the stages of laminitis. At the subclinical stage, clinical manifestations are often absent, but biochemical changes already allow detecting the pathological process. Among them, a moderate increase in plasma insulin by 15–25% is observed, which reflects early metabolic control disorders, as well as an increase in glucose levels by 10–20% (Table 4) (Asplin, 2007; Bailey, 2013; Delarocque, 2021).

The concentration of triglycerides and free fatty acids in the blood increases by 5–15%, which signals initial disorders of lipid metabolism (Zhao, 2020). At the same time, markers of oxidative stress are noted - malondialdehyde (MDA) increases by 15–20%, and the activity of antioxidant enzymes, such as superoxide dismutase (SOD) and catalase, remains at the lower level of the norm (Treiber, 2009). In the blood plasma, a moderate increase in the pro-inflammatory cytokines TNF-α and IL-6 by 10–20% is observed, which indicates an early systemic inflammatory response (Belknap, 2007; Zhao, 2020).

Locally in the lamellar apparatus, edema, hyperemia, and initial degenerative changes in the basal membrane of the epidermis are noted (Treiber, 2009).

Moving on to acute (clinical) laminitis in cattle, the manifestations become more pronounced: animals demonstrate lameness, pain when supporting the limbs, the affected hooves become hot and sensitive to the touch. Biochemically, at this stage, a significant increase in insulin and glucose is noted - 50–70% above normal, which reflects a violation of endocrine control. The activity of the enzymes lactate dehydrogenase (LDH) and creatine kinase (CK) increases by 1.5–2 times, which indicates damage to the muscles and tissues of the hoof (Belknap, 2007; Delarocque, 2021). At the same time, markers of systemic inflammation increase – C-reactive protein (CRP) by 2–3 times, TNF-α and IL-6 by 50–70%, and the level of MDA increases by 40–50%, which indicates pronounced oxidative stress (Harris, 2006; Treiber, 2009). Local microcirculatory disorders lead to ischemia and necrosis of the stratum corneum. These changes confirm an active systemic inflammatory response, combined with local ischemia of the lamellar apparatus (Belknap, 2007; Harris, 2006).

Table 4

Clinical course and main biochemical changes in domestic animals during the development of laminitis

Animal type	Stage	Main biochemical changes	Indicators
Horses	Subclinical	↑ insulin, ↑ glucose (slightly), ↑ MDA, ↑ TNF-α, IL-6	Insulin 15–25 μIU/mL, glucose 5.5–6.5 mmol/L
Horses	Acute	Significant ↑ insulin, ↑ LDH, ↑ CK, ↑ CRP, ↑ free radicals	LDH 400–600 U/L, CK 350–500 U/L
Horses	Chronic	Insulin resistance, fatty acid imbalance, low antioxidant activity	MDA ↑ 20–30%, SOD ↓ 10–15%
Cattle	Subclinical	↑ LPS, moderate ↑ TNF-α	LPS 0.5–1.0 EU/mL
Cattle	Acute	Significant ↑ lactate, ↑ cytokines, ↑ CRP	Lactate 2–4 mmol/L
Cattle	Chronic	Moderate ↑ inflammatory markers	TNF-α 5–10 pg/mL
Sheep, goats	Subclinical	Moderate changes in insulin and inflammatory markers	Insulin 12–20 μIU/mL
Sheep, goats	Clinical	↑ inflammatory cytokines, metabolite imbalance	TNF-α 8–12 pg/mL

Chronic laminitis in cows and bulls is characterized by persistent hoof deformity, lamellar fibrosis, and gait mechanics disorders. Biochemically, persistently elevated insulin (60–90% above normal), prolonged lipid profile imbalance, and low antioxidant system activity (SOD, catalase 30–40% below normal) are noted (Zhao, 2020). Proinflammatory cytokines TNF-α and IL-6 remain moderately elevated by 30–50%, CRP by 20–40%. Locally, dystrophy of horny spines, degeneration of the basement membrane, and chronic infiltration by lympho- and neutrophils are noted in the lamellae (Treiber, 2009).

In sheep and goats, the pathogenesis of laminitis has specific features due to the characteristics of the digestive system and housing conditions. In the subclinical stage, even in the absence of obvious lameness, a moderate increase in insulin (10–20%), glucose (5–15%), and triglycerides (5–10%) is noted (Zhao, 2020). There is activation of oxidative stress (MDA +10–15%, SOD – decrease by 5–10%) and a moderate increase in TNF-α and IL-6 (+10–15%). Locally, edema, hyperemia, and initial degenerative changes of the basement membrane are observed in the lamellae (Treiber, 2009).

In the acute stage, clinical signs become noticeable: lameness, soreness, increased hoof temperature. Biochemical indicators reflect pronounced disorders: insulin +40–60%, glucose +20–35%, LDH and CK are increased by 1.5–2 times, and the MDA level increases by 30–50% (Delarocque, 2021). Pro-inflammatory cytokines TNF-α and IL-6 increase by 40–60%, CRP – 2 times (Zhao, 2020).

In chronic laminitis in sheep and goats, hoof deformities, lamellar fibrosis and chronic inflammation are accompanied by biochemical disorders: constantly high insulin (50–80% above normal), lipid profile disorders, reduction of antioxidant activity by 30–40%, TNF-α and IL-6 +30–50%, CRP +20–40%. Locally – degeneration of the basement membrane, dystrophy of the horny spines, infiltration by lympho- and neutrophils and fibrosis of the lamellar apparatus (Treiber, 2009; Zhao, 2020).

Thus, biochemical markers in different species of domestic animals allow not only to diagnose laminitis in the early stages, but also to determine the stage of the disease and predict its course. In horses, the key is the control of insulin and markers of oxidative stress (Delarocque, 2021), in cattle - monitoring of endotoxemia, LDH, CK and inflammatory cytokines (Zhao, 2020), in sheep and goats - a combination of metabolic and inflammatory indicators together with an assessment of housing conditions and mechanical load on the limbs (Treiber, 2009; Zhao, 2020). A comprehensive analysis of these indicators allows for a differential assessment of subclinical, acute and chronic forms of laminitis and to direct preventive and therapeutic measures.

Diagnosis of laminitis. Given the variety of biochemical, morphological and functional changes, timely detection of laminitis requires the use of a comprehensive diagnostic approach. Diagnosis of laminitis in domestic animals includes clinical assessment, laboratory studies and modern instrumental methods that provide accurate identification of the stage of the disease and justification for the choice of therapeutic and preventive measures (Table 5) (O'Grady, 2008; Vercelli, 2021).

Table 5

Differential diagnosis of laminitis in domestic animals

Animal type	Method	What reveals	Morphological/histological changes	Pathological and anatomical changes
Horses	Ultrasound	Swelling of the lamellar layer, displacement of the 3rd phalanx	Swelling, hyperemia, degeneration of the basement membrane	Initial edema of the subcutaneous tissue, weak lymphocyte infiltration, minimal necrosis of the stratum corneum
Horses	X-ray / CT	Rotation or lowering of the 3rd phalanx	Necrotic changes of the lamellar layer	Deformation of the sole, bone erosions, displacement of the phalanx
Cattle	Laboratory (LPS, cytokines)	Subclinical inflammation	Plantar hemorrhages, edema	Initial hemorrhages in the dermis, mild infiltration by neutrophils
Cattle	X-ray	Extent of hoof damage	Ulcers, subendothelial edema	Degenerative changes in the dermis and epidermis, thickening of the stratum corneum
Sheep, goats	X-ray	Lameness, inflammation	Hoof deformity, initial fibrosis	Local necrosis of the stratum corneum, initial fibrous changes in the lamellar layer

In cattle, subclinical laminitis is often associated with subclinical cicatricial acidosis and elevated endotoxin levels, which trigger a systemic inflammatory response. Biochemically, moderate elevations in proinflammatory cytokines, markers of oxidative stress, and impaired microcirculation are observed (Boosman, 1991; Cook, 2004; Harris, 2006; Belknap, 2007; Zhao, 2020; Ding, 2020). The acute stage is accompanied by pronounced inflammatory changes, increased lipase, protease, C-reactive protein, and impaired hoof hemodynamics. The chronic stage shows long-term structural changes in the stratum corneum, rotation and lowering of the phalanx, impaired microcirculation, and decreased performance (Greenough, 2007; Koziy, 2008; Archer, 2010).

Instrumental diagnostics allows to detect pathological changes in the early stages.

Radiography is used to assess the angle of rotation and descent of the third phalanx in horses, the thickness of the stratum corneum and the presence of plantar defects in cattle (Cook, 2004; O'Grady, 2008; Vercelli, 2021).

Ultrasound (US) allows assessment of soft tissue edema, lamellar blood flow, and early degenerative changes, which is particularly useful in subclinical laminitis and is confirmed by increased expression of pro-inflammatory cytokines in lamellae (Hood, 1999; Asplin, 2007; Belknap, 2007; Vercelli, 2021).

Computed tomography (CT) provides three-dimensional assessment of bone and horn structures, accurate determination of phalanx rotation, and surgical planning (O'Grady, 2008; Vercelli, 2021).

Magnetic resonance imaging (MRI) provides high-resolution soft tissue detail, early detection of degenerative changes in lamellae, and impaired blood flow (Pollitt, 1996; Vercelli, 2021). Additionally, thermography and laser Doppler are used to assess local temperature and microcirculation, which allows differentiation of early stages of laminitis (Bailey, 2013; Vercelli, 2021).

Comprehensive diagnostics of laminitis, including clinical assessment, laboratory markers and instrumental methods, allows to accurately determine the stage of the pathological process, predict the risk of progression and choose the optimal therapeutic strategy. In horses, it is especially important for the early detection of endocrinopathic forms of laminitis, in cattle - for the assessment of the consequences of SARA and systemic endotoxemia, and in sheep and goats - for the control of combined nutritional, mechanical and infectious factors (Boosman, 1991; Lischer, 1994; Cook, 2004; Asplin, 2007).

Morphological changes in laminitis in domestic animals are complex, multi-level and reflect both local lesions of the lamellar apparatus of the hoof and systemic disorders that underlie the pathogenesis of the disease. At the macroscopic level, horses, cattle, sheep and goats exhibit characteristic changes in the hoof horn and underlying structures, the severity of which depends on the stage and duration of the pathological process. In the initial stages, especially in the subclinical course, macroscopic changes may be barely noticeable, but already at this stage, there are disturbances in the color of the stratum corneum in the form of yellowish or grayish areas corresponding to areas of impaired blood supply and microhemorrhages (Mudron, 1996; Enemark, 2008; Zhao, 2020).

In cattle, a typical sign is saline hemorrhages, localized mainly in the sole and white line, indicating chronic microcirculation disorders and increased pressure on the hoof dermis (Greenough, 2007; Enemark, 2008; Zhao, 2020).

In horses, in the acute stage, there is an increase in hoof temperature, soreness, and as the process progresses, rotation or distal displacement of the third phalanx, accompanied by deformation of the hoof capsule and a change in its configuration (Hood, 1999; Vercelli, 2021). Chronic laminitis is characterized by the formation of so-called "laminitis rings", thickening and deformation of the hoof wall, widening of the white line, development of a double sole, and in severe cases, stratum corneum detachment and the formation of chronic ulcers (Weaver, 2005; Greenough, 2007). In sheep and goats, macroscopic changes are often combined with signs of hoof rot and secondary infections, which complicates the course of laminitis and contributes to the development of necrotic processes (Lischer, 1994).

At the histological level, the key manifestation of laminitis is damage to the dermal-epidermal junction of the lamellar apparatus. Early changes include lamellar edema, capillary dilation, disruption of the basement membrane, and epidermal cell disorganization (Pollitt, 1996; Hood, 1999; Belknap, 2007).

In horses, early changes include lamellar edema, capillary dilation, disruption of basement membrane integrity, and epidermal cell disorganization (Pollitt, 1996; Hood, 1999). An important pathomorphological mechanism is the activation of matrix metalloproteinases, which leads to degradation of basement membrane components and loss of dermis-epidermal junction, clinically manifested by a weakening of the attachment of the hoof wall to the underlying tissues (Pollitt, 1996; van Oldruitenborgh Oosterbaan, 1999).

In cattle, the histological changes are somewhat different and include hyperemia, blood stasis, microthromboses in the dermal vessels, perivascular infiltration by neutrophils and lymphocytes, and degeneration of keratinocytes (Boosman, 1991; Ding, 2020; Zhao, 2020). In subclinical laminitis in cattle, microscopic hemorrhages, impaired keratinization, and a decrease in the quality of the stratum corneum are observed, which creates the prerequisites for the development of secondary lesions, such as plantar ulcers or white line disease (Mudron, 1996; Greenough, 2007).

In sheep and goats, the histological changes are characterized by a combination of lamellar damage with pronounced inflammatory processes, often complicated by bacterial infection, which leads to tissue necrosis and destruction of the stratum corneum (Lischer, 1994).

Pathological and anatomical changes in laminitis reflect hoof damage, changes in internal organs associated with metabolic and inflammatory disorders.

In horses with acute laminitis, pronounced circulatory disorders in the lamellar apparatus are noted, including ischemia, venous stasis and edema, which leads to tissue necrosis and hoof wall detachment (Hood, 1999; Vercelli, 2021). In chronic cases, fibrous changes, tissue remodeling and persistent hoof deformation are formed.

In cattle, pathoanatomical changes are often associated with the sequelae of subclinical laminitis and manifest as plantar hemorrhages, ulcers, abscesses, and hoof deformities, accompanied by changes in the scar, including signs of acidosis, mucosal damage, and the presence of high levels of volatile fatty acids (Nocek, 1997; Enemark, 2008; Mulligan, 2008). Cattle may also exhibit systemic changes, including hepatodystrophy, inflammation, and signs of intoxication associated with endotoxemia (Boosman, 1991; Mulligan, 2008). In sheep and goats, pathological and anatomical changes are characterized by a combination of laminitis with infectious lesions of the hoof, which leads to the development of purulent-necrotic processes, the spread of inflammation to deeper tissues and, in severe cases, to systemic intoxication (Lischer, 1994).

Thus, morphological, histological and pathological and anatomical changes in laminitis in domestic animals form a single pathogenetic complex, in which local damage to the lamellar apparatus is inextricably linked with systemic metabolic, vascular and inflammatory disorders. The specific features of anatomy, physiology and digestion determine the nature and severity of these changes, which must be taken into account in the diagnosis, treatment and prevention of laminitis in horses, cattle, sheep and goats.

Laminitis prevention. Modern approaches to the prevention of laminitis in domestic animals are based on the understanding of it as a systemic multifactorial syndrome, requiring a comprehensive impact on metabolic, nutritional, mechanical and management risk factors.

In horses, control of endocrine status and carbohydrate metabolism is of key importance, since hyperinsulinemia and insulin resistance are considered to be the leading mechanisms for the development of endocrinopathic laminitis. Limiting the intake of non-structural carbohydrates with feed, using diets with a low glycemic index, gradual transition between feeding types and body weight control are basic preventive measures, the effectiveness of which has been confirmed by experimental and clinical studies (Harris, 2006; Asplin, 2007; Bailey, 2013). An important element is regular monitoring of insulin, glucose and lipid profile levels, which allows for timely identification of animals at increased risk of laminitis and early correction of metabolic disorders (Karikoski, 2011; de Laat, 2019). In addition to feeding, conditions of maintenance and operation play a significant role: a sufficient level of physical activity, avoiding prolonged standing on hard surfaces, proper forging and regular correction of hooves ensure optimal load distribution on the limbs and reduce the risk of damage to the lamellar apparatus (O'Grady, 2008; Sadiq, 2020).

In cattle, the prevention of laminitis is closely linked to the control of feeding and the prevention of subclinical ruminal acidosis (SARA), which is one of the key factors in the development of the pathology. Diets should be balanced in terms of fiber and concentrates, with sufficient structured fibers to stimulate the rumen and stabilize the pH of the rumen. The gradual introduction of concentrates, the use of buffering additives and control of the structure of the diet can reduce the risk of acidosis and the associated endotoxemia (Nocek, 1997; Oetzel, 2007; Enemark, 2008).

Herd management is also essential: optimizing housing conditions, providing comfortable flooring, sufficient resting space and regular hoof trimming are critical factors in preventing laminitis and associated hoof horn lesions (Webster, 2001; Bergsten, 2003; Sadiq, 2020).

In high-producing cows, special attention is paid to the transition period, when metabolic stress and dietary changes can contribute to the development of laminitis, which justifies the need for metabolic monitoring and early intervention (Mulligan, 2008; Koziy, 2008). Preventive measures in cattle also have a significant economic effect, as a decrease in the incidence of laminitis is directly related to increased productivity and reduced milk losses (Archer, 2010; Sertu, 2024).

In sheep and goats, the prevention of laminitis has its own characteristics, associated with a combination of metabolic, infectious and mechanical factors. The diet should be adapted to the physiological needs of the animals, limiting the excess intake of concentrates and providing a sufficient amount of roughage, which helps stabilize digestion and reduce the risk of metabolic disorders. Housing conditions play a significant role: dry, clean bedding, regular disinfection of premises and humidity control reduce the risk of infectious lesions of the hoof and the development of secondary laminitis (Lischer, 1994). Regular trimming of hooves, control of the load on the limbs and prevention of injury are necessary components of prevention, especially in conditions of intensive livestock farming. An important modern direction in the prevention of laminitis in all types of domestic animals is the introduction of early monitoring and diagnostic systems. The use of telemetric systems for monitoring activity, gait and behaviour allows for the detection of early changes characteristic of subclinical laminitis, even before the appearance of pronounced clinical signs (Danscher, 2009; Danscher, 2010). The combination of such technologies with laboratory monitoring of biochemical parameters, including insulin, glucose, markers of inflammation and oxidative stress, creates opportunities for an individualized approach to prevention and risk management (Bailey, 2013; de Laat, 2019).

Thus, modern prevention of laminitis in domestic animals is based on an integrated approach that takes into account the species-specific characteristics of digestion, metabolism, housing and exploitation. In horses, the key is the control of endocrine and metabolic factors, in cattle - the prevention of acidosis and the optimization of herd management, while in sheep and goats - a combination of measures regarding feeding, hygiene and infection control. The implementation of these measures allows not only to reduce the incidence of laminitis, but also to improve the overall health of animals and increase the efficiency of livestock farming.

Discussion. Laminitis is a serious disease that affects the performance and health of livestock, particularly horses and cattle. Studies show that the prevalence of lameness in dairy cows can range from 10–40%, depending on housing conditions and feeding systems (Clarkson, 1996; Cook, 2003; Capion, 2009). Laminitis is often associated with metabolic disorders, including insulin resistance and impaired carbohydrate metabolism, as supported by studies in horses and ponies (Asplin, 2007; Bailey, 2013; Stefaniuk, 2023).

One of the key mechanisms of laminitis development is increased prolonged hyperinsulinemia, which stimulates laminar inflammation and destabilization of the hoof basement membrane (Pollitt, 1996; de Laat, 2014; Vercelli, 2021). In cattle, acute and subclinical laminitis often occurs against the background of cicatricial digestive disorders, in particular subclinical cicatricial acidosis (SARA), which causes endotoxin release and inflammatory reactions (Boosman, 1991; Nocek, 1997; Enemark, 2008). Oligofructose overload has also been shown to have a provocative effect on the development of lameness in cattle (Danscher, 2009; Ding, 2020).

Clinical signs of laminitis include behavioral changes, lameness, increased sensitivity to hoof pressure, and loss of performance (Danscher, 2010; Archer, 2010). In addition, there is evidence of an association between infectious processes such as mastitis and the development of acute laminitis in cows (Zhao, 2020; Faustmann, 2025).

Prevention and control of the disease are based on a comprehensive approach: diet optimization, body weight control, restriction of high-glycemic feed intake, correction of metabolic disorders, regular hoof examination and shoeing (Webster, 2001; Harris, 2006; O'Grady, 2008). In addition, early diagnosis is an important aspect, which reduces the risk of chronic laminitis and loss of productivity (Greenough, 2007; Koziy, 2008; Bojkovski, 2023).

Current research highlights that laminitis is a multifactorial disease where genetic, metabolic, inflammatory and management factors interact. Further research should focus on the molecular mechanisms of inflammation in laminar tissue, interactions with metabolic disorders and the development of new prevention and treatment strategies (de Laat, 2019; Delarocque, 2021; Menzies-Gow, 2025).

Thus, timely diagnosis, control of metabolic disorders and comprehensive preventive measures allow not only to reduce the risk of laminitis, but also to increase productivity and ensure the well-being of domestic animals.

Conclusions. Laminitis in domestic animals is a complex systemic multifactorial syndrome, the development of which is due to the interaction of metabolic, endocrine, infectious-toxic and mechanical factors. The key role in the pathogenesis is played by impaired microcirculation of the lamellar apparatus, degradation of the dermal-epidermal junction and activation of inflammatory and oxidative processes, which leads to the destruction of the hoof apparatus and reflects systemic changes in the body. The disease has a progressive nature: from subclinical metabolic and biochemical changes (increased insulin, glucose, markers of inflammation and oxidative stress) to clinically pronounced forms with deep structural changes, hoof deformation, tissue necrosis and chronic destruction of lamellae. Clear species-specific features of the pathogenesis of laminitis have been established: in horses, endocrine and metabolic disorders (hyperinsulinemia, insulin resistance) prevail, in cattle - subclinical cicatricial acidosis with endotoxemia and systemic inflammatory response, in sheep and goats - the combined effect of metabolic, infectious and mechanical factors, which determines the variability of manifestations. Differential diagnostics of laminitis should be based on a comprehensive approach, including the assessment of clinical signs and stage of the course, analysis of biochemical indicators, as well as morphological, histological and pathological-anatomical studies, which allows for timely detection of the disease and distinguishing it from other hoof pathologies. Effective prevention and control of laminitis are possible only under the conditions of a comprehensive approach, which involves optimizing feeding, controlling metabolic status, improving housing conditions and using modern monitoring methods. Early diagnosis of subclinical forms is key to preventing the development of severe and chronic stages and reducing economic losses.

REFERENCES

1. Archer S. C., Green M. J., Huxley J. N. (2010). Association between lameness and milk yield. *Journal of Dairy Science*; 93(4), 151–160. https://doi.org/10.3168/jds.2010-3062

2. Asplin K. E., Sillence M. N., Pollitt C. C., McGowan C. M. (2007). Induction of laminitis by prolonged hyperinsulinaemia in clinically normal ponies. *The Veterinary Journal*; 174(3), 530–535. https://doi.org/10.1016/j.tvjl.2007.07.003

3. Bailey S. R., Bamford N. J. (2013). Metabolic responses of horses and ponies to high and low glycaemic feeds. *Animal Production Science*; 53(11), 1182–1187. https://doi.org/10.1071/AN13266

4. Belge A., Bakır B., Gönenci R., Ormancı S. (2005). Subclinical laminitis in dairy cattle: 205 selected cases. *Turkish Journal of Veterinary and Animal Sciences*; 29(1), 9–15

5. Belknap J. K., Giguère S., Pettigrew A., Cochran A. M., Van Eps A. W., & Pollitt C. C. (2007). Lamellar pro-inflammatory cytokine expression patterns in laminitis. *Equine Veterinary Journal*; 39(1), 42–47. https://doi.org/10.2746/042516407X155406

6. Bergsten C. (2003). Causes, risk factors, and prevention of laminitis and related claw lesions. *Acta Veterinaria Scandinavica*; 44(Suppl 1), S157–S166. https://doi.org/10.1186/1751-0147-44-S1-S157

7. Bezpalko O., Machuskyi O., Vygovska L., Ushkalov V., Radzihovsky M., Ushkalov A., Danchuk V. (2023). Determination of antagonistic properties and biofilm formation in Bacillus spp.

and Lactobacillus spp. *Scientific Reports of the National University of Life and Environmental Sciences of Ukraine*; 19(4). https://doi.org/10.31548/dopovidi4(104).2023.007
8. Bojkovski J. A., Nedić S., Arsić S., Vujanac I., Prodanović R., Mitrović A., Đurić M., Bugarski D., Panousis N. K., Kalaitzakis E., Ninković M. (2023). Pathogenesis of laminitis in dairy cows. *Veterinary Journal of Republic of Srpska*; XXIII(1–2), 307–317. https://doi.org/10.7251/VETJEN2301307B
9. Boosman R., Mutsaers C. W., Klarenbeek A. (1991). The role of endotoxin in the pathogenesis of acute bovine laminitis. *Veterinary Quarterly*; 13(3), 155–162. https://doi.org/10.1080/01652176.1991.9694301
10. Boosman R., Németh F., Gruys E. (1991). Bovine laminitis: Clinical aspects, pathology and pathogenesis. *Veterinary Quarterly*; 13(3), 163–171. https://doi.org/10.1080/01652176.1991.9694302
11. Bostanlık M., Yönez M. K., Aslan N. E. (2025). Determination of the prevalence of foot diseases in Holstein dairy cattle. *Journal of the Faculty of Veterinary Medicine Erciyes University*; 22(3), 199–211. https://doi.org/10.32707/ercivet.1740655
12. Clarkson M. J., Downham D. Y., Faull W. B., Hughes J. W., Manson F. J., Merritt J. B., Murray R. D., Russell W. B., Sutherst J. E., Ward W. R. (1996). Incidence and prevalence of lameness in dairy cattle. *Veterinary Record*; 138(23), 563–567. https://doi.org/10.1136/vr.138.23.563
13. Colles C. M., Jeffcott L. B. (1977). Laminitis in the horse. *Veterinary Record*; 100(13), 262–264. https://doi.org/10.1136/vr.100.13.262
14. Cook N. B. (2003). Prevalence of lameness among dairy cattle. *Journal of the American Veterinary Medical Association*; 223(9), 1324–1328. https://doi.org/10.2460/javma.2003.223.1324
15. Danscher A. M., Enemark J. M. D., Telezhenko E., Capion N., Ekstrøm C. T., Thoefner M. B. (2009). Oligofructose overload induces lameness in cattle. *Journal of Dairy Science*; 92(2), 607–616. https://doi.org/10.3168/jds.2008-1271
16. de Laat M. A., Clement C. K., McGowan C. M., Sillence M. N., Pollitt C. C., Lacombe V. A. (2014). Cytokine expression during hyperinsulinaemia. *Veterinary Immunology and Immunopathology*; https://doi.org/10.1016/j.vetimm.2013.10.010
17. de Laat M. A., Reiche D. B., Sillence M. N., McGree J. M. (2019). Incidence and risk factors for laminitis. *Journal of Veterinary Internal Medicine*; 33(3), 1473–1482. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6524073
18. Delarocque J., Reiche D. B., Meier A. D., Warnken T., Feige K., Sillence M. N. (2021). Metabolic profile distinguishes laminitis-susceptible ponies. *BMC Veterinary Research*; 17, 56. https://doi.org/10.1186/s12917-021-02763-7
19. Ding J., Li S., Jiang L., Li Y., Zhang X., Song Q., Hayat M. A., Zhang J.-T., Wang H. (2020). Laminar inflammation responses in bovine laminitis. *Frontiers in Veterinary Science*; 7, 351. https://doi.org/10.3389/fvets.2020.00351
20. Eades S. C. (2010). Overview of current laminitis research. *Clinical Techniques in Equine Practice*; 26(1), 51–63. https://pubmed.ncbi.nlm.nih.gov/20381735
21. Enemark J. M. D. (2008). Subacute ruminal acidosis (SARA). *The Veterinary Journal*; 176, 32–43. https://doi.org/10.1016/j.tvjl.2007.12.021
22. Faustmann F., Baumgartner M., Piechl S., Fuerst-Waltl B., Kofler J. (2025). Mastitis and acute laminitis association. *Animals*; 15(12), 1709. https://doi.org/10.3390/ani15121709
23. Harris P., Bailey S., Elliott J., Longland A. (2006). Countermeasures for laminitis in horses. *Journal of Nutrition*; 136, 2094S–2097S. https://doi.org/10.1093/jn/136.7.2114s
24. Karikoski N. P., Horn I., McGowan T. W., McGowan C. M. (2011). Prevalence of endocrinopathic laminitis. *Domestic Animal Endocrinology*; 41(3), 111–117. https://pubmed.ncbi.nlm.nih.gov/21696910
25. Klymas A., Syrlyk M. (2025). Prevalence of hoof diseases in cows. *Ukrainian Journal of Veterinary Sciences*; 16(1), 88–103. https://doi.org/10.31548/veterinary1.2025.88
26. Kornienko L. Y., Ukhovskyi V. V., Karpulenko M. S., Moroz O. A., Tsarenko T. M., Radzyhovskyi M. L., Ruda M. Y. (2024). Epizootic situation on transboundary diseases. *One Health Journal*; 2(II), 41–58. https://doi.org/10.31073/onehealthjournal2024-II-06

27. Koziy V. I. (2008). Prevalence of laminitis in cows. *Scientific Bulletin of Lviv National University of Veterinary Medicine and Biotechnologies*; 10(2), 128–133
28. Lischer C. J., Ossent P. (1994). Laminitis in cattle: A literature review. *Tierärztliche Praxis*; 22(5), 424–432
29. Menzies-Gow N. J., Knowles E. J. (2025). SGLT2 inhibitors in laminitis management. *Journal of Veterinary Pharmacology and Therapeutics*; 48(Suppl 1), 31–40. https://doi.org/10.1111/jvp.13470
30. Mulligan F. J., & Doherty M. L. (2008). Production diseases of the transition cow. *The Veterinary Journal*; https://doi.org/10.1016/j.tvjl.2007.12.018
31. Nocek J. E. (1997). Bovine acidosis and laminitis. *Journal of Dairy Science*; 80, 1005–1028. https://doi.org/10.3168/jds.S0022-0302(97)76026-0
32. Oetzel G. R. (2007). Subacute ruminal acidosis in dairy herds. *Veterinary Clinics of North America: Food Animal Practice*; https://doi.org/10.1016/j.cvfa.2017.06.004
33. Pollitt C. C. (1996). Basement membrane pathology in laminitis. *Equine Veterinary Journal*; 28(1), 38–46. https://doi.org/10.1111/j.2042-3306.1996.tb01588.x
34. Popescu S., Borda C., Diugan E. A., Spinu M., Groza I. S., Sandru C. D. (2013). Welfare in dairy cows. *Acta Veterinaria Scandinavica*; 55(1), 43. https://doi.org/10.1186/1751-0147-55-43
35. Radzyhovskyi M. L., Sachuk R. M., Koshevoy V. I., Dyshkant O. V., Sokulskyi I. M., Katsaraba O. A., Kulishenko O. M., Davydenko P. O., Ruda M. E. (2024). Infectious abortions in cows. *Scientific and Technical Bulletin of SSCIVP*; 25(1), 133–139. https://doi.org/10.36359/scivp.2024-25-1.18
36. Sadiq M. B., Ramanoon S. Z., Mansor R., Syed-Hussain S. S., Mossadeq W. M. S. (2020). Claw trimming and lameness. *Animals*; 10(9), 1515. https://doi.org/10.3390/ani10091515
37. Sertu S. (2024). Economic impact of laminitis. *Scientific Papers: Animal Science and Biotechnologies*; 57(2)
38. Sloet van Oldruitenborgh Oosterbaan M. M. S. (1999). Laminitis in the horse: A review. *Veterinary Quarterly*; 21(4), 121–127
39. Stefaniuk-Szmukier M., Piórkowska K., Ropka-Molik K. (2023). Equine metabolic syndrome. *Genes*; 14(8), 1544. https://doi.org/10.3390/genes14081544
40. Thoefner M. B., Pollitt C. C., Van Eps A. W., Milinovich G. J., Trott D. J., Wattle O., Andersen P. H. (2004). Acute bovine laminitis model. *Journal of Dairy Science*; 87(9), 2932–2940. https://doi.org/10.3168/jds.S0022-0302(04)73424-4
41. Treiber K., Carter R., Gay L., Williams C., Geor R. (2009). Inflammatory status in ponies. *Veterinary Immunology and Immunopathology*; 129(3–4), 216–220. https://doi.org/10.1016/j.vetimm.2008.11.004
42. Vercelli C., Tursi M., Miretti S., Giusto G., Gandini M., Re G., Valle E. (2021). Methylglyoxal effects in laminitis. *PLoS ONE*; 16(7), e0253840. https://doi.org/10.1371/journal.pone.0253840
43. Weaver A. D., St John G., Steiner A. (2005). *Bovine surgery and lameness*. Oxford: Blackwell
44. Wylie C. E., Collins S. N., Verheyen K. L. P., Newton J. R. (2011). Frequency of equine laminitis. *The Veterinary Journal*; 189(3), 248–256
45. Zhao Y., Xia C., Wang J., Li Y., Xu D., Zhou Y. (2020). Inflammatory mediators in laminitis. *BMC Veterinary Research*; 16, 119. https://doi.org/10.1186/s12917-020-02319-1

Ламініт як чинник метаболічних порушень у свійських тварин (оглядова стаття)

Лігоміна І.[1] (ORCID: 0000-0001-8569-9487), mailto:ligominairina@ukr.net**Ковальчук Ю.[1] (ORCID: 0000-0003-3677-3411), Галатюк О.[1] (ORCID: 0000-0002-9720-0660), Сокульський І.[1] (ORCID: 0000-0002-6237-0328), Карпюк В.[1] (ORCID: 0000-0003-3728-5698), Дубовий А.[1] (ORCID: 0000-0003-2341-1868), Радзиховський М.[2] (ORCID: 0000-0003-0518-8148), Соловйова Л.[3] (ORCID: 0000-0001-9455-8299)**
1. – Поліський національний університет, м. Житомир, Україна, * e-mail: sokulskiy_1979@ukr.net
2. – Національний університет біоресурсів і природокористування України, м. Київ, Україна
3. – Білоцерківський національний аграрний університет, м. Біла Церква, Україна

Резюме. *У статті проведено комплексний аналіз сучасних наукових даних щодо ламініту у свійських тварин із урахуванням видових особливостей перебігу патологічного процесу у коней, великої рогатої худоби, овець і кіз. Узагальнено підходи до розуміння ламініту як системного мультифакторного синдрому, який формується внаслідок взаємодії метаболічних, ендокринних, інфекційно-токсичних та механічних чинників. Показано, що розвиток захворювання супроводжується глибокими порушеннями мікроциркуляції у ламелярному апараті копита, деградацією дермально-епідермального з'єднання, активацією запальних і в кінцевому результаті призводить до структурної деструкції копитного апарату та зниження продуктивності тварин.*

Особливу увагу приділено аналізу патогенетичних механізмів із урахуванням типу травлення. Встановлено, що у коней, як моногастричних тварин, провідну роль відіграють ендокринно-метаболічні порушення, зокрема гіперінсулінемія, інсулінорезистентність і дисбаланс вуглеводного обміну, що обумовлює розвиток ендокринопатичного ламініту. У великої рогатої худоби ключовим пусковим механізмом є субклінічний рубцевий ацидоз, що супроводжується змінами мікробіоти рубця, підвищенням рівня ендотоксинів у крові та розвитком системної запальної відповіді. У овець і кіз ламініт формується внаслідок поєднання метаболічних, інфекційних і механічних чинників та визначає варіабельність клінічних і морфологічних проявів захворювання.

Систематизовано біохімічні зміни, характерні для різних стадій ламініту (субклінічної, гострої та хронічної), включаючи підвищення концентрації інсуліну, глюкози, тригліцеридів, активацію ферментів тканинного ушкодження (лактатдегідрогенази, креатинкінази), прозапальних цитокінів, С-реактивного білку. Показано, що ці показники можуть використовуватися для ранньої діагностики, диференціації стадій захворювання та прогнозування його перебігу. Також узагальнено сучасні підходи до діагностики ламініту, що базуються на поєднанні клінічного обстеження, лабораторних досліджень і інструментальних методів, зокрема рентгенографії, ультразвукової діагностики, комп'ютерної томографії та систем моніторингу рухової активності. Наведено характеристику морфологічних, гістологічних і патолого-анатомічних змін, які відображають прогресування патологічного процесу від початкових мікроскопічних ушкоджень до вираженої деформації копита, некрозу тканин і фіброзу.

Водночас, окремо розглянуто сучасні підходи до профілактики ламініту, які включають оптимізацію годівлі, контроль метаболічного статусу, запобігання субклінічному ацидозу, покращення умов утримання та впровадження технологій раннього моніторингу. Підкреслено, що ефективне управління ризиками ламініту можливе лише за умов інтегрованого підходу, який враховує видові особливості тварин, рівень їх працездатності для коней і продуктивності для великої рогатої худоби та технологічні умови утримання.

Результати узагальнення можуть бути використані для удосконалення системи ранньої діагностики, профілактики та контролю ламініту і сприятиме підвищенню продуктивності і покращенню добробуту свійських тварин.

Ключові слова: копито, запальний процес, морфологічні зміни, ацидоз, ендотоксинемія, порушення мікроциркуляції, обмін речовин, кульгавість.

DOI: 10.31073/onehealthjournal2026-III-07

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 (наказом МОН України № 768 від 20 червня 2023 р.). Матеріали номера схвалено редколегією до друку 09.05.2026 р.
Видається чотири рази на рік, щоквартально.

НАУКОВЕ ВИДАННЯ

УДК 636.09:614:606

ISSN (print)2786-7420
ISSN (online) 2786-7439

One Health Journal. Збірник наукових праць. Том 4. Номер 3. **– Харків, Державний науково-дослідний інститут з лабораторної діагностики та ветеринарно-санітарної експертизи та ГО “Інститут Єдиного Здоров’я”, 2026 – 70 стор.**

SCIENTIFIC EDITION

УДК 636.09:614:606

ISSN (print) 2786-7420
ISSN (online) 2786-7439

One Health Journal. Collected papers. Volume 4. Number 3. **- Kharkiv, State Scientific and Research Institute for Laboratory Diagnostics and Veterinary and Sanitary Expertize and One Health Institute, NGO, 2026 – 70 pages.**