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Xenobiotic-contaminated water and its impact on animal metabolism

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Abstract. The relevance of this research arises from the fact that, despite the significant scientific and technological advancements humanity has achieved, it has not yet learned to use it carefully and rationally. Rapid industrialisation and urbanisation of the planet, a sharp increase in its

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population, intensive chemicalisation of agriculture, and the intensification of many other types of anthropogenic pressure on nature have disrupted the circulation of substances and natural energy exchange processes in the biosphere, damaged its regenerative mechanisms, and as a result, its progressive destruction has begun. This research review aimed to present some of the main impacts of water quality on metabolism in animal organisms within the concept of "One Health". The leading method for studying this problem is a review of recent literature. Typically, xenobiotics are chemical compounds that enter the body and are not formed as a result of metabolic pathway reactions. In this regard, water contaminated with xenobiotics enters the body with drinking water or food, and this does not apply to metabolic water, which is formed as a result of chemical reactions. Thus, along with the consumption of water due to hydration, other various chemical substances may enter the body, which are usually not present in drinking water. However, the type of chemical substances, as well as their quantity, are criteria that can transform water from a basic nutrient into a harmful product. Soil and water play an important role in the chemical composition of food or feed that grows in the soil, and the metabolism of plants or plant consumers can be balanced or unbalanced depending on the quality and quantity of water. The materials of the article have practical significance for elucidating the biological mechanisms of xenobiotic elimination and preserving the health of animals and humans

Keywords: pollutants; animal organisms; contaminants; fluid balance; elimination

Introduction

The impact of xenobiotics on the reproductive and developmental biology of all living organisms has become of paramount importance in the present day. As the effect of these xenobiotics on aquatic animals is an emerging area of research and development, several groups worldwide are working on these aspects, targeting various groups of fish in both marine and freshwater ecosystems (Akhila *et al.*, 2023).

Generally, xenobiotics refer to a wide range of compounds, including synthetic and industrial chemicals, drugs, pesticides, environmental pollutants, and other substances that are introduced into an organism's system through various routes, such as ingestion, inhalation, or dermal exposure, according to E.A. Noman *et al.* (2019). The presence of xenobiotics in freshwater – particularly in water used as a source of drinking water for animals and humans – can largely be attributed to sewage systems for wastewater, but also to potential leaks, especially during rainy weather and in humid atmospheric conditions.

P.F. Zabrodskii (2020) notes that when xenobiotics enter an organism, they can undergo biotransformation, which is the process of chemical modification that occurs within the body. This transformation is primarily carried out by enzymes in the liver, although other organs and tissues can also contribute. The purpose of biotransformation is to convert xenobiotics into more easily excretable forms and to neutralise or detoxify potentially harmful substances.

However, some xenobiotics may be difficult to eliminate or may be transformed into metabolites that are more toxic or reactive than the original compound. This can lead to adverse effects on the organism's health, such as organ damage, disruption of physiological processes, or the development of diseases. The study of xenobiotics and their effects on living organisms

is a significant field within toxicology and environmental science. Understanding how xenobiotics interact with biological systems and the mechanisms of their metabolism and toxicity is crucial for assessing their potential risks to human and ecological health (Oesch *et al.,* 2018; Ortiz *et al.,* 2022; Rathore *et al.,* 2022).

B. Lussenburg *et al.* (2022) indicate that understanding how exogenous chemicals (xenobiotics) are metabolised, distributed, and eliminated is critical for determining the impact of these chemicals and their metabolites on the organism. Y. Hua *et al.* (2022) point out that it is of great importance for food safety and human health to study the migration and biotransformation of chemical pollutants among agricultural elements, such as soil, water, and crops. C.H. Chen (2024) notes that household products containing chemical compounds are used daily, while industrial chemicals and environmental pollutants are prevalent in the air and water bodies. As a result, humans are constantly exposed to these foreign substances, known as xenobiotics. Many of these foreign compounds that enter the body are lipophilic, or fat-soluble. Unlike hydrophilic substances, which dissolve in water, lipophilic compounds are nonpolar and either insoluble or only slightly soluble in water. Therefore, they must be converted into water-soluble forms before being eliminated from the body. The process of removing these foreign substances involves activation and detoxification mechanisms, which serve as crucial defences for the human body.

The study aimed to analyse the influence of xenobiotics in water on metabolic processes in animals. The research methodology involved a comprehensive study and analysis of recent scientific literature. This approach aimed to review and synthesise the most up-to-date findings and theories relevant to the topic. The selection of sources was based on their scholarly significance, relevance, and contribution to the existing body of knowledge. The analysis focused on identifying key themes, patterns, and gaps in the literature, providing a robust foundation for the research. By critically evaluating recent studies, the methodology ensured that the research was grounded in current scientific understanding, allowing for a well-rounded perspective on the subject matter.

Xenobiotic water contamination and its environmental impact

In this article, it is proposed to discuss "xenobiotic water", which refers to water that is not produced within biological systems through metabolic reactions, but rather to the water ingested by humans and animals, namely drinking water or water that enters the organism along with food or feed. In this context, "xenobiotic water" does not refer to "polluted water" but to water that is not produced within the organism as an intermediate or final product of reactions.

Metabolic water is produced inside the organism in a multitude of reactions, playing a crucial role in maintaining proper hydration levels. The human body, as well as many other organisms, has evolved mechanisms to generate water as a byproduct of various metabolic processes (Nelson & Cox, 2014).

One of the primary sources of metabolic water is derived from cellular respiration (Ulrich & Zickermann, 2021; McClelland, 2022). During this process, glucose and oxygen are used to produce energy in various forms, but most commonly as adenosine triphosphate (ATP). Interestingly, as a byproduct of cellular respiration, water is generated through the combination of hydrogen ions and electrons in the electron transport chain (Bindoli & Rigobello, 2013). This metabolic water production occurs in the mitochondria, the powerhouse of the cell (Cox, 2005). Additionally, the breakdown of stored glycogen in the liver and muscles also yields metabolic water. As glycogen is

metabolised to release glucose for energy, the process produces water molecules as a byproduct. Furthermore, the oxidation of fat stores contributes to the production of metabolic water (Matkov, 2018). Fat molecules contain more hydrogen atoms compared to carbohydrates, making them a potent source for water synthesis when metabolised (Bindoli & Rigobello, 2020).

Overall, these metabolic reactions collectively contribute to the production of water within the organism. While the amount of water generated through these processes is relatively small compared to the water obtained from external sources such as drinking, it is nevertheless an important internal source of hydration, particularly in situations where access to external water is limited, such as during periods of water scarcity or in arid environments.

Water contaminated with xenobiotics refers to water that contains foreign substances, such as synthetic chemicals, pharmaceuticals, and pesticides (Gerrity *et al.,* 2011). These substances can enter water sources through direct discharge from industries, agricultural runoff, improper waste disposal, or accidental spills. They can be harmful to biological systems, including humans, animals, and plants, as they may interfere with normal cellular functions and essential physiological processes.

Water containing xenobiotics can have harmful effects on aquatic ecosystems and pose risks to human health. Many xenobiotics are persistent and do not easily degrade, leading to long-term contamination of water bodies, such as heavy metals (Ahmadi *et al.,* 2018). These substances can accumulate in the environment, potentially affecting aquatic organisms and the food chain.

Water treatment processes such as filtration, disinfection, and advanced oxidation are commonly employed to remove or reduce xenobiotics from water supplies. Additionally, regulations and monitoring programmes aim to control the release of xenobiotics into water sources and mitigate their potential impacts on both the environment and human populations.

The significance of xenobiotics present in the water composition of biological systems is that they can have detrimental effects on human health, the environment, and aquatic life. Exposure to these contaminants can lead to the development of diseases, birth defects, and other health problems (Pankaj *et al.,* 2017). Additionally, xenobiotic water can disrupt ecosystems and harm wildlife by altering their behaviour, growth, and reproduction. This is why it is essential to monitor and regulate the presence of xenobiotic substances in water sources to ensure the safety of our natural resources and public health.

Metabolic water refers to water produced as a byproduct of metabolic processes in specific organisms, particularly those adapted to survive in water-scarce environments. It serves as a supplementary source of water, but it is not the sole or primary means of meeting an organism's water requirements (Ferrier, 2014). Certain organisms, such as desert-dwelling animals, plants, and microorganisms, have metabolic adaptations that allow them to generate water as a byproduct of their metabolic processes. This metabolic water production helps these organisms survive in arid or water-scarce environments where access to external water sources is limited.

In these cases, metabolic water is derived from the breakdown or metabolism of internal energy stores, such as glycogen or fats. During the breakdown of these energy stores, metabolic reactions release water molecules as byproducts. This internally generated water can be utilised by the organism to meet its water needs, supplementing or compensating for the lack of external water sources.

The production of metabolic water varies depending on the specific organism and its

physiological adaptations. For example, some desert-dwelling animals, such as kangaroo rat (*Dipodomys phillipsii*) (Swaner, 2013) – Fig. 1a, certain reptiles (*Uma scoparia*) – Fig. 1b, and wild Bactrian camel (*Camelus ferus*) – Fig. 1c have highly efficient kidneys that concentrate their urine and minimise water loss. This conservation of water, along with metabolic water production, helps them survive in water-scarce environments and assists in regulating body temperature, humidity, evaporation, and excretion.

It is important to note that metabolic water production is often relatively limited compared to the total water needs of an organism. While it can contribute to water balance and be beneficial in water-stressed environments, additional water intake through drinking or other external sources is typically necessary for proper hydration for all living organisms.

a *b* *c*

Figure 1. Animals fully adapted to xeric environments
***Note**: a – kangaroo rat (Dipodomys phillipsii); b – reptiles (Uma scoparia); c – wild Bactrian camel (Camelus ferus)*
***Sourse**: developed by the authors*

Importance of water for metabolic pathways in animals

Water quality plays a crucial role in the health and well-being of both animal and human organisms. Here are some key influences of water quality on organisms:

Hydration – Water is essential for maintaining proper hydration in organisms. Adequate water intake is necessary for the functioning of cells, tissues, and organs (Buck *et al.,* 2017). Poor water quality, such as water contaminated with pollutants or pathogens, can lead to dehydration or waterborne diseases, negatively impacting the health of animals and humans (Hulea & Ahmadi, 2021).

Nutrient absorption – Water serves as a medium for the absorption and transportation of nutrients within organisms. Inadequate water quality can hinder nutrient absorption, leading to nutrient deficiencies and related health issues.

Contaminant exposure – Water quality affects the degree of exposure to contaminants, including pollutants, chemicals, heavy metals, pesticides, and pathogens. Consuming water with high levels of contaminants can have detrimental effects on the immune system, organ function, and overall health of organisms (Widmaier *et al.,* 2008).

Disease transmission – Poor water quality can facilitate the transmission of waterborne diseases and infections. Pathogens such as bacteria, viruses, and parasites can contaminate water sources, leading to illnesses in animals and humans when consumed or used for hygiene purposes.

Aquatic ecosystems – Water quality is vital for the health of aquatic ecosystems and the organisms that inhabit them. Contaminated water can disrupt the ecological balance, harm aquatic species, and degrade habitats, affecting the overall biodiversity and functioning of the ecosystem (Pedersen *et al.*, 2003).

Excretion – Water plays a vital role in the process of excretion, which is the removal of

waste materials from the body. The excretory system, consisting of organs such as the kidneys, helps maintain the body's internal environment by filtering waste products and excess substances from the bloodstream and eliminating them through urine.

Urine formation – The kidneys filter waste products, excess ions, and other substances from the blood to form urine. Water is a key component of urine and acts as a solvent that helps dissolve and transport these waste materials out of the body (Pollard *et al*., 2018).

Kidney function – Adequate water intake is crucial for the proper functioning of the kidneys. Water helps maintain optimal blood volume and pressure within the kidneys, ensuring efficient filtration and removal of waste products. Insufficient water intake can lead to concentrated urine, which may contribute to the formation of kidney stones or urinary tract infections.

Waste elimination – Water helps dilute waste substances in the urine, making them easier to excrete. It assists in flushing out toxins, metabolic byproducts, and excess substances like urea, uric acid, and electrolytes from the body. Without sufficient water, these waste products may accumulate and cause various health problems.

Fluid balance – Water plays a critical role in maintaining fluid balance throughout the body. It helps regulate osmotic pressure and electrolyte concentrations, which are essential for cellular function (Buck *et al.,* 2017). Adequate hydration ensures that the body can effectively eliminate waste materials and maintain overall physiological stability.

To ensure the well-being of both animals and humans, it is essential to maintain and monitor water quality through measures such as regular testing, water treatment and purification, proper waste disposal, and safeguarding water sources from pollution. Regulatory bodies, environmental agencies, and public health organisations often establish guidelines and standards to protect water quality and promote the health of organisms that depend on it (Patterson *et al*., 2010).

Water is an essential component for the proper functioning of metabolic pathways in organisms. Metabolic pathways are a series of chemical reactions that occur within cells to break down molecules, generate energy, and synthesise new molecules necessary for life processes. Here are some ways in which water is involved in metabolic pathways:

Solvent and reaction medium – Water serves as a universal solvent and provides the medium for metabolic reactions to occur. Many metabolic reactions take place in the aqueous environment inside cells, where water acts as a solvent, facilitating the transport of molecules, ions, and substrates involved in these pathways.

Hydrolysis reactions – Hydrolysis is a common metabolic reaction in which water molecules are used to break down complex molecules into simpler components. For example, during digestion, water is involved in hydrolysing food molecules such as carbohydrates, proteins, and lipids into their respective monomers (sugars, amino acids, and fatty acids).

Protein folding – Proper protein folding is essential for the functioning of enzymes, receptors, and other proteins. Water molecules interact with amino acids as proteins fold, stabilising their structures and ensuring they achieve their functional conformations.

Transport and diffusion – Water plays a crucial role in the transport and diffusion of metabolites within cells and tissues (Alberti, 2010). Metabolites produced in one part of the cell need to be transported to other cellular compartments or even different cells (Lea *et al.,* 2003). Water, along with specialised transport proteins, facilitates the movement of metabolites across cell membranes (Groot & Grubmüller, 2001). Water's role as a transport medium is vital for the movement of nutrients,

waste products, hormones, and other molecules throughout the body, being directly involved in cell size (Lloyd, 2013), volume (Sachs & Sivaselvan, 2015), and shape (Koivusalo *et al.,* 2009). Blood, for example, is primarily composed of water and acts as a medium for transporting oxygen, nutrients, and waste products between cells and organs.

Aquaporins are a family of membrane proteins that facilitate the rapid transport of water molecules across cell membranes (Abir-Awan *et al.,* 2019). They are essential for maintaining water balance in various tissues and organs of mammals. These water channels play a crucial role in regulating water movement, osmotic balance, and fluid homeostasis within cells and tissues (Lodish *et al.,* 2005). Among mammals, there are several types of aquaporins, each with specific distributions and functions, as follows:

Aquaporin-1 (AQP1) – is one of the first aquaporins discovered and is widely expressed in various tissues, including the kidneys, lungs, red blood cells, and the endothelium of blood vessels.

Aquaporin-2 (AQP2) – is primarily found in the kidney's collecting ducts and is regulated by the hormone vasopressin.

Aquaporin-3 (AQP3) – is present in the plasma membranes of various tissues, including the kidneys, skin, and gastrointestinal tract.

Aquaporin-4 (AQP4) – is highly expressed in the brain and spinal cord, where it plays a role in regulating brain water balance and cerebrospinal fluid dynamics.

Aquaporin-5 (AQP5) – is found in tissues with secretory and absorptive functions, such as salivary glands, the respiratory tract, and lacrimal glands.

Aquaporin-7 (AQP7) and Aquaporin-9 (AQP9) – are found in adipose tissue and liver cells, respectively.

Aquaporin-6 (AQP6) – is primarily found in intracellular vesicles of the kidney's collecting duct cells (Delgado *et al.,* 2013).

The function of aquaporins goes beyond maintaining water balance. For instance, they are involved in cell migration, cell signalling, and interactions with other molecules (Hoffmann & Pedersen, 2011).

Mammalian aquaporin water channels are fundamental for maintaining water homeostasis and osmotic balance in various tissues and organs. Their selective permeability to water allows the rapid movement of water molecules across cell membranes, ensuring the proper functioning of cells and tissues throughout the body (Roccaro *et al.*, 2013).

Enzymatic reactions – Enzymes, which are proteins that catalyse metabolic reactions, often require water as a necessary component for their activity. Water molecules can be directly involved in enzymatic reactions, either as a reactant or as a participant in the catalytic process.

Temperature regulation – Water possesses a high heat capacity, allowing it to absorb and release heat with minimal temperature changes. Since metabolic reactions produce heat as a by-product, water's ability to maintain a stable temperature is essential for regulating body heat. This characteristic ensures that metabolic processes occur within an optimal temperature range, promoting efficient enzyme activity and proper cellular function.

Metabolite synthesis – Water is involved in the synthesis of many essential metabolites, including carbohydrates, proteins, lipids, and nucleic acids. Water molecules are incorporated into these compounds during their biosynthesis, contributing to their structure and functionality.

In summary, water is integral to metabolic pathways as a solvent, participant in reactions, transport medium, and temperature regulator. It enables the efficient functioning of cellular processes and the synthesis and breakdown of molecules necessary for life (Saragovi *et al.,* 2022). Maintaining adequate hydration and

consuming high-quality water is vital for supporting optimal metabolic function in organisms. Additionally, water is involved in both catabolic (breakdown) and anabolic (synthesis) metabolic pathways. In catabolic reactions, water is often used to break chemical bonds, releasing energy. In anabolic reactions, water is often a product as molecules are built up (Khesbak *et al.,* 2011). For instance, in photosynthesis, water molecules are split to provide electrons and protons needed to convert carbon dioxide into glucose.

ATP formation – The synthesis of adenosine triphosphate (ATP), the primary energy currency of cells, occurs in metabolic pathways such as cellular respiration and photosynthesis. These processes involve the movement of protons across cell membranes, creating proton gradients that drive ATP synthesis through ATP synthase. Water molecules are released as a by-product of these reactions and help regulate the proton gradients and energy flow.

Detoxification – Water aids in the removal of waste products and toxins from the body through processes such as urination and sweating. The kidneys use water to filter waste products and excess substances from the blood, forming urine that is excreted from the body. Sweating helps regulate body temperature and eliminates waste products through the skin.

The water needs of animals can vary depending on the species, size, diet, activity level, and environmental conditions. Here are some general guidelines regarding water requirements for different types of animals:

Domestic animals – Dogs typically require about 0.3 litres of water per pound of body weight per day. However, this can vary based on factors such as size, activity level, and diet. Cats generally need about 0.1 to 0.15 litres of water per 5 pounds of body weight per day. Cats consuming wet food derive some of their water intake from their diet. Horses can between 20 and 60 litres of water per day, depending on factors such as size, climate, diet, and activity level.

Livestock animals – Cows typically require around 30 to 45 litres of water per day. However, lactating cows or those in hot climates may need more. Sheep and goats generally need about 2 to 4 litres of water per day. This requirement can increase during hot weather or lactation. Pigs typically require about 2 to 4 litres of water per day, depending on their size and environmental conditions.

Wild animals – Have varying water requirements based on their size, diet, and habitat. Desert-dwelling animals, such as camels, kangaroo rats (Longland & Dimitri, 2021), and certain reptiles, have unique adaptations to conserve water and can survive in very limited amounts.

Aquatic animals – Animals such as fish, amphibians, and marine mammals, obtain their water needs from their surrounding environment.

Common xenobiotic water pollutants that pose a risk to animals

Xenobiotics are substances that are foreign to the biological systems they enter, often originating from human activities such as pharmaceutical production, agriculture, industry, and personal care. These contaminants can enter water systems through various pathways, leading to potential environmental and human health risks.

Pharmaceuticals as xenobiotic contaminants: Pharmaceuticals are widely used for medical purposes and are commonly found in water systems due to incomplete metabolism and excretion by humans. These compounds can enter the environment through sewage discharges, landfill leachate, and runoff from areas with pharmaceutical manufacturing facilities. Once in water systems, pharmaceuticals can have diverse ecological and human health impacts. Thus, pharmaceuticals can disrupt aquatic ecosystems by affecting the behaviour, growth, and reproduction of aquatic organisms.

Hormone-disrupting pharmaceuticals, such as birth control pills, can alter the reproductive behaviours of fish, leading to changes in population dynamics (Dhaval *et al.*, 2013).

Long-term exposure to low levels of pharmaceuticals in drinking water might have cumulative effects on human health. The presence of antibiotics in water systems can contribute to the development of antibiotic-resistant bacteria, posing challenges for disease treatment.

Pesticides as xenobiotic contaminants: Pesticides are chemicals used to control pests in agriculture, and they can contaminate water systems through runoff from treated fields and improper disposal. These compounds can persist in the environment and have significant implications for both ecosystems and human health. Pesticides can harm non-target organisms, leading to declines in pollinator populations (e.g., bees) and disruptions in food chains. The bioaccumulation of pesticides in aquatic organisms can lead to increased concentrations as they move up the food chain. Pesticide-contaminated water can find its way into drinking water sources, posing potential risks to human health. Chronic exposure to pesticide residues in water has been associated with a range of health issues, including neurodevelopmental disorders and certain cancers (Johnson *et al.*, 2012).

Industrial chemicals as xenobiotic contaminants: Industrial chemicals, including heavy metals and organic pollutants, are released into water systems through industrial processes, waste disposal, and accidental spills. These contaminants can have far-reaching effects on aquatic ecosystems and human well-being. Heavy metals, such as mercury, cadmium, and lead can accumulate in aquatic organisms, causing deformities and reproductive issues (Ahmadi *et al.,* 2016).

Organic industrial pollutants, such as polychlorinated biphenyls (PCBs), can disrupt endocrine systems in aquatic animals. Consumption of fish and shellfish from contaminated water bodies can lead to human exposure to heavy metals and persistent organic pollutants.

Industrial chemicals in water systems can also contaminate agricultural produce, indirectly affecting human health.

Personal care products as xenobiotic contaminants: Personal care products, including cosmetics, fragrances, and cleaning agents, often contain chemicals that can enter water systems through domestic wastewater discharge. These compounds can contribute to emerging environmental challenges. Some compounds in personal care products can interfere with the growth and development of aquatic organisms. Synthetic fragrances and antimicrobial agents can be persistent in water systems, affecting aquatic life. Certain ingredients in personal care products, such as endocrine-disrupting phthalates, can pose risks to human health when present in water sources. The potential for exposure to a mixture of chemicals from various personal care products is a concern that requires further research (Mathew *et al.*, 2017).

Mitigating the impacts of xenobiotic contaminants on water systems requires a multi-faceted approach, such as wastewater treatment, regulation and monitoring, pharmaceutical disposal programmes, integrated pest management, green chemistry, and product design.

Upgrading wastewater treatment facilities to include advanced treatment processes, such as ozonation and activated carbon filtration, can help remove a wider range of contaminants, including pharmaceuticals and personal care products. Strengthening regulations on the discharge of pharmaceuticals, pesticides, and industrial chemicals into water systems can help minimise their entry. Regular monitoring of water bodies for contaminant levels is crucial. Implementing proper disposal programmes for unused or expired pharmaceuticals can reduce

their presence in the environment (Tahar *et al*., 2013; Embrandiri *et al*., 2016).

Promoting integrated pest management practices in agriculture can reduce reliance on synthetic pesticides, mitigating their entry into water systems. Encouraging the development of personal care products and industrial chemicals with reduced environmental persistence and toxicity can limit their impact on water systems. Xenobiotic contaminants, including pharmaceuticals, pesticides, industrial chemicals, and personal care products, pose significant challenges to water systems and the environment as a whole. Their entry into aquatic ecosystems can have far-reaching ecological and human health consequences. Effective mitigation strategies involve a combination of regulatory measures, technological advancements in wastewater treatment, and shifts in consumption patterns towards more sustainable products. Safeguarding water quality from these contaminants is crucial for the well-being of both ecosystems and human populations.

Conclusions

Water's role in metabolic pathways is multifaceted and essential for sustaining life. It acts as a solvent for reactions, participates in hydrolysis, aids in ATP production, regulates temperature, facilitates nutrient transport, supports detoxification, contributes to proper protein folding, and is intricately involved in both catabolic and anabolic processes. Water's ability to act as a medium, solvent, and participant in metabolic reactions underscores its fundamental importance to the functioning of the human body. Xenobiotic metabolism is a complex process influenced by a wide range of factors. Genetic variability, age, sex, co-exposure to other chemicals, disease states, nutritional status, and environmental factors can all impact how efficiently the body processes and eliminates foreign compounds. Understanding these factors is essential for personalised medicine approaches, risk assessment, and designing effective interventions to minimise the adverse effects of xenobiotics on human health. Enzymes such as cytochrome P450s and glucuronosyltransferases play a critical role in xenobiotic metabolism by facilitating the detoxification and elimination of foreign compounds from the body. These enzymatic processes are essential for maintaining health and preventing the accumulation of toxic substances in the body.

The perspective of the research is to obtain high-quality products from animal husbandry and, accordingly, to reduce the threat to human health.

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Conflict of Interest

None.

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Вода, забруднена ксенобіотиками, та її вплив на метаболізм у тварин

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Анотація. Актуальність дослідження зумовлена тим, що на сьогоднішній день у своєму розвитку людство досягло того рівня, коли, оволодівши величезним науково-технічним потенціалом, воно ще не навчилося дбайливо і раціонально його використовувати. Швидка індустріалізація та урбанізація планети, різке зростання її народонаселення, інтенсивна хімізація сільського господарства, посилення багатьох інших видів антропогенного тиску на природу порушили кругообіг речовин та природні обмінні енергетичні процеси в біосфері, пошкодили її регенераційні механізми, внаслідок чого почалося її прогресуюче руйнування. Цей огляд дослідження мав на меті представити деякі з основних впливів якості води на метаболізм в організмі тварин у концепції «Єдиного здоров'я». Провідним методом дослідження цієї проблеми є огляд літературних даних останніх років. Зазвичай ксенобіотики – це хімічні сполуки, що надходять в організм, які не утворюються в результаті реакцій метаболічних шляхів. З цього приводу вода, забруднена ксенобіотиками, потрапляє в організм з питвом або їжею, і це не стосується метаболічної води, яка утворюється в результаті хімічних реакцій. Таким чином, разом із споживанням води внаслідок гідратації в організм

можуть надходити інші різноманітні хімічні речовини, які зазвичай не присутні в питній воді. Але тип хімічних речовин, а також їх кількість є критеріями, які можуть перетворити воду з основної поживної речовини на шкідливий продукт. Ґрунт і вода відіграють важливу роль у хімічному складі їжі чи корму, що росте в ґрунті, а метаболізм рослин або споживачів рослин може бути збалансованим або незбалансованим залежно від якості та кількості води. Матеріали статті мають практичне значення для з'ясування біологічних механізмів елімінації ксенобіотиків та збереження здоров'я тварин і людини

Ключові слова: полютанти; тваринні організми; контамінанти; флюїдний баланс; елімінація

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Differences in the serum lipid profile of goats with varying autonomic nervous system tone

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Abstract. Goat farming is a promising branch of animal husbandry, as statistics show a rapid increase in global demand for goat milk and meat products. To meet market demand, new methodological approaches are needed to improve animal productivity while maintaining the quality of the raw

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materials. In this context, this study aims to determine the impact of autonomic nervous system tone on lipid metabolism in goats. The study involved Saanen goats, with the experimental groups formed based on the results of variation-pulsometric study and electrocardiography, followed by the determination of autonomic nervous system tone using the Baevsky method. The serum lipid profile of goats was studied using a LabLine-010 spectrophotometer. The following indicators were biochemically analysed in serum samples: content of triacylglycerols, cholesterol, high-density, low-density, and very-low-density lipoproteins, and the atherogenic index was calculated. It was established that the content of triacylglycerols was 23% lower ($P < 0.05$) in sympathotonic animals and 41% lower ($P < 0.001$) in vagotonic animals compared to normotonic animals. The level of low-density lipoproteins in the blood serum of sympathotonic animals decreased by 40% ($P < 0.05$), and in vagotonic animals – by 76% ($P < 0.01$), relative to normotonic animals. The content of high-density lipoproteins in sympathotonic animals increased by 36% ($P < 0.001$), relative to normotonic animals. The level of cholesterol in the blood serum of goats differed by 16% ($P < 0.01$) in sympathotonic animals and 15% ($P < 0.01$) in vagotonic animals compared to normotonic animals. A significant influence of autonomic nervous system tone on the processes of maintaining lipid homeostasis in goats was determined. Taking into account the individual features of the influence of the autonomic nervous system on lipid metabolism when distributing animals will contribute to the effective adjustment of diets. After all, each animal has a different tone of the autonomic nervous system, which significantly affects the features of substance transformation in its body

Keywords: lipid metabolism; cholesterol; lipoproteins of varying density; atherogenic index; autonomic regulation

Introduction

L. Wang *et al.* (2024) noted that goat farming holds a prominent position in global livestock production. This is attributed to farms producing milk and meat products with high protein content and a shorter reproductive cycle than cattle. As a result, goats are better adapted to various environmental changes and exhibit accelerated growth rates.

According to S. M'hir *et al.* (2024), the growing popularity of goat products in the consumer market can be attributed to these advantages. Furthermore, a positive aspect of goat farming is the increasing consumer preference for goat products over cow products, especially milk. Contemporary statistics reveal that a growing number of people are experiencing food allergies when consuming cow's milk. Notably, goat's milk contains a higher content of unsaturated fatty acids, making it a more desirable dietary option. This issue is being actively researched as the ratio of saturated to unsaturated fatty acids in human diets is increasing each year, playing a significant role in lipid metabolism.

S.A. Allaoua *et al.* (2021) demonstrated that society is increasingly interested in evaluating what they consume and their preferences, particularly regarding fatty acids such as omega-3 and omega-6. Scientists are paying growing attention to lipid metabolism and methods for its regulation. The main challenge lies in increasing the fat content of milk while enriching it specifically with omega-3 and omega-6 fatty acids. It is also important to note that when altering the concentration of these fatty acids, it is essential to maintain their balanced ratio, as failing to do so can disrupt lipid metabolism.

H.T. Faisal *et al.* (2022) found that increasing the unsaturated fatty acid content in goats is achieved through the use of various dietary supplements, which positively affect fatty acid content. However, changes in diet do not always yield the desired improvements in indicators. The range of results often shows minimal differences or only slight variations. As a result, when these figures are consolidated, they lead to significant errors in the average values.

Z. Antunović *et al.* (2020) demonstrated that this trend persists primarily when attempts are made to adjust baseline lipid metabolism indicators without considering the factors that regulate metabolism in the body. It should also be noted that each animal has individual characteristics, which are reflected in its overall metabolism. As a result, the effectiveness of certain dietary supplements will vary, and when deriving an overall numerical outcome, there can be significant discrepancies. To address this issue, attention should be paid to the body systems that directly regulate homeostasis. Among the complex mechanisms of neurohumoral regulation, there is a framework for understanding individual characteristics that can improve approaches to regulating lipid metabolism. According to T. LeBouef *et al.* (2023), the autonomic nervous system is a key factor in analysing the body's metabolism. Its primary role is to maintain homeostasis and to provide an active response to external influences that may negatively affect metabolic processes.

Y. Huang *et al.* (2023a) argued that the study of lipid metabolism is a crucial issue in the development of goat farming. According to him, these animals, during foetal development, early lactation, and periods of intensive milk production, expend a significant amount of energy-rich substances. Such intense metabolism, without certain adjustments in the diet, can lead to an imbalance in energy, which, as a consequence, will result in economic losses. An example of this, according to Y. Huang (2023b), is the low birth weight of newborn kids. A deficiency of high-energy compounds during the period of active foetal growth often leads to the birth of newborn kids with reduced weight. In the future, these animals will have a lower survival rate at a young age and, as adults, will be prone to metabolic disorders.

D. Wang *et al.* (2023) noted that the active growth of young animals and their productivity largely depend on sufficient levels of amino acids and lipids in the body. Based on their own research, the authors assert that the rates of development vary depending on the individual characteristics of goats, as evidenced by the metabolic profile of their blood. Analysis of lipid metabolism is crucial as it can facilitate effective modulation and improve the quality of milk and meat. J. Wu *et al.* (2023a; 2023b) in their research highlighted the significant importance of goat milk as a source of bioactive components in the form of polyunsaturated fatty acids and emphasised the importance of studying lipid metabolism to better regulate the mechanisms of synthesis of these same fatty acids. Given this, the value of goat products is increasing daily, prompting scientists to seek methods to increase the productivity of these animals and improve the quality of goat products.

To enhance productivity and balance lipid metabolism, researchers have employed various methods. For instance, Y. Zhang *et al.* (2023) supplemented goat diets with copper to improve weight gain in meat breeds by influencing lipid metabolism. According to their hypothesis, copper supplementation in goat diets reduces the content of mRNA for enzymes involved in the oxidation of long-chain fatty acids. F. Zamuner *et al.* (2020) utilised infusions of glucose, insulin, and adrenocorticotropin to stimulate milk production and improve metabolic transformations. S. Liu *et al.* (2021) in-

vestigated the effects of soybean oil as a supplement to enhance milk production in goats. Specifically, the use of this supplement provided an increase in lipid content in goats while simultaneously increasing the fat percentage in milk, and evaluated the animals' ability to counteract heat stress by compensating for energy expenditure under its influence. The application of plant-based lipid supplements with a high content of polyunsaturated fatty acids to improve goat productivity by stimulating fat accumulation in the mammary gland, leading to an increase in its percentage and the consumer value of dairy products, has been explored.

This study aimed to investigate the sympathovagal balance in goats to better understand metabolic processes, particularly lipid metabolism. This understanding is essential for the formation of technological groups of animals based on their individual characteristics.

Materials and Methods

The biological material for this study was obtained from Saanen goats at a farm in the village of Knyahynynok, Lutsk District, Volyn Region, during the years 2023-2024. Samples were taken from animals in experimental groups, with 5 animals in each group. The formation of these groups was based on the results of the variation-pulsometric study conducted using the methodology of R.M. Baevsky *et al.* (2001). Based on the results obtained, three experimental groups of animals were formed: normotonics, sympathotonics, and vagotonics. During the experimental studies, all manipulations with the goats involved in the study were carried out following the basic principles of bioethics, in compliance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986) and the Procedure for Conducting Research and Experiments on Animals by Scientific Institutions (2012).

For the biochemical analysis, blood serum was obtained by allowing native blood samples to stand and incubating them for one hour in a thermostat at 37°C.

A LabLine-010 spectrophotometer (Austria) was used to determine cholesterol content. A test system from LLC "Granum Laboratory", Kharkiv, was used to determine total protein content. Measurement conditions: wavelength 505 (500-550) nm, cuvette with an optical path length of 1 cm, temperature 15-25°C.

The obtained results were calculated using the formula:

$$C_{exp} = \frac{E_{exp}}{E_{std}} \times C_{std}, \quad (1)$$

where C_{exp} is the cholesterol concentration in the experimental sample, mmol/L; E_{exp} is the optical density of the experimental sample, ODU; E_{std} is the optical density of the standard, ODU; C_{std} is the cholesterol content in the standard, 5.16 mmol/L.

Determination of triacylglycerol content in blood serum using a LabLine-010 (Austria) spectrophotometer. Measurements were taken at a wavelength of 490-550 nm in 1 cm cuvettes.

The obtained results were calculated using the formula:

$$C_{exp} = \frac{E_{exp}}{E_{std}} \times C_{std}, \quad (2)$$

where C_{exp} is the triglycerides concentration in the experimental sample, mmol/L; E_{exp} is the optical density of the experimental sample, ODU; E_{std} is the optical density of the standard, ODU; C_{std} is the triglycerides content in the standard, 2.25 mmol/L.

The content of low-density lipoproteins (LDL) was determined using the LabLine-010 (Austria). Measurements were taken at a wavelength of 590-700 nm in 1 cm cuvettes.

The obtained results were calculated using the formula:

$$C_{exp} = \frac{E_{exp}}{E_{std}} \times C_{std}, \quad (3)$$

where C_{exp} is the low-density lipoproteins concentration in the experimental sample, mmol/L; E_{exp} is the optical density of the experimental sample, ODU; E_{std} is the optical density of the standard, ODU; C_{std} is the low-density lipoproteins content in the standard, 5.17 mmol/L.

The determination of high-density lipoproteins (HDL) was carried out using the LabLine-010 (Austria). Measurements were taken at a wavelength of 550-650 nm in 1 cm cuvettes.

$$C_{exp} = \frac{E_{exp}}{E_{std}} \times C_{std}, \quad (4)$$

where C_{exp} is the high-density lipoproteins concentration in the experimental sample, mmol/L; E_{exp} is the optical density of the experimental sample, ODU; E_{std} is the optical density of the standard, ODU; C_{std} is the high-density lipoproteins content in the standard, 2.585 mmol/L.

The content of very low-density lipoproteins (VLDL) was determined using Friedewald's formula:

$$VLDL = TotalChol - (HDL + LDL). \quad (5)$$

The atherogenic index was calculated using the formula:

$$AI = (TotalChol - HDL)/LDL. \quad (6)$$

Reagents from LLC "Granum Laboratory", Kharkiv, were used to determine the above-mentioned lipid metabolism parameters.

Statistical analysis of the obtained results was carried out using Microsoft Excel software. The probability of the difference between the obtained indicators was calculated using Student's t-test. Differences between the compared indicators were considered significant at a significance level of $P < 0.05$, $P < 0.01$, $P < 0.001$.

Results and Discussion

According to the results of the biochemical analysis of the blood serum of goats, the lipid profile indicators for the vagotonic experimental group were established (Table 1).

Table 1. Lipid profile indicators of blood serum of vagotonic goats

Indicator	NVO	SE	Mdn.	SD	Asym.	Min.	Max.
Triglycerides, mmol/L	5	0.01	0.26	0.02	-0.08	0.23	0.28
VLDL, mmol/L	5	0.01	0.13	0.02	-0.58	0.10	0.14
LDL, mmol/L	5	0.02	0.44	0.03	-0.04	0.4	0.47
HDL, mmol/L	5	0.02	1.22	0.04	-0.41	1.16	1.27
Cholesterol, mmol/L	5	0.02	1.79	0.04	0.27	1.76	1.84
Atherogenic index	5	0.02	0.47	0.04	-1.34	0.39	0.50

Note: *NVO – number of valid observations, SE – standard error, Mdn. – median, SD – standard deviation, Asym. – asymmetry, Min. – minimum value, Max. – maximum value*
Source*: authors' development*

According to the study results, the triglyceride levels in the blood serum of vagotonic goats displayed minimal variation between the minimum and maximum values, amounting to 0.05 mmol/L, which ensured low standard deviation and standard error values. The concentration of very low-density lipoproteins in animals with a predominance of the parasympathetic nervous system showed a difference of 0.04 mmol/L between the minimum and maximum values, accompanied by low standard deviation and standard error.

The low-density lipoproteins in this group of animals exhibited a range difference of 0.07 mmol/L, with similarly low standard deviation and standard error values. The concentration of high-density lipoproteins in the goats characterised by a predominance of the parasympathetic nervous system indicated a range difference of 0.11 mmol/L between minimum and maximum values, with low standard deviation and standard error. The cholesterol level in the blood serum of vagotonic goats fluctuated between minimum and maximum values by 0.08 mmol/L, also accompanied by low standard deviation and standard error. The atherogenic index in the vagotonic animals revealed a difference of 0.11 mmol/L between the minimum and maximum values, with low standard deviation and standard error.

According to the results of the biochemical analysis of the blood serum of goats, the lipid profile indicators for the normotonic experimental group were established (Table 2).

Table 2. Lipid profile indicators of blood serum of normotonic goats

Indicator	NVO	SE	Mdn.	SD	Asym.	Min.	Max.
Triglycerides, mmol/L	5	0.01	0.42	0.02	0.60	0.40	0.47
VLDL, mmol/L	5	0.01	0.16	0.02	-0.08	0.14	0.19
LDL, mmol/L	5	0.01	0.26	0.03	-1.67	0.21	0.27
HDL, mmol/L	5	0.01	1.15	0.03	-1.34	1.10	1.17
Cholesterol, mmol/L	5	0.01	1.56	0.03	-0.04	1.52	1.59
Atherogenic index	5	0.02	0.36	0.04	2.00	0.34	0.44

Note: *NVO – number of valid observations, SE – standard error, Mdn. – median, SD – standard deviation, Asym. – asymmetry, Min. – minimum value, Max. – maximum value*
Source*: authors' development*

According to the results of the lipid profile analysis of the serum in normotonic goats, the concentration of triacylglycerols ranged between the minimum and maximum values of 0.07 mmol/L, which indicated low values of standard deviation and standard error. The concentration of very low-density lipoproteins in animals with a balanced influence of the sympathetic and parasympathetic nervous systems demonstrated a difference in the range of values between the minimum and maximum indicators of 0.05 mmol/L, also ensuring low parameters of standard deviation and standard error. The levels of low-density lipoproteins in normotonic animals showed a difference between the minimum and maximum values of 0.06 mmol/L, which again reflected low values of standard deviation and standard error. The concentration of high-density lipoproteins in goats with a balanced influence of the sympathetic and parasympathetic nervous systems was characterised by a difference between the minimum and maximum values of 0.07 mmol/L, ensuring low values of standard deviation and standard error. The cholesterol level in the serum of normotonic animals revealed a difference between the minimum and maximum values of 0.07 mmol/L, which also indicated low values of standard deviation and standard error. The atherogenic index exhibited a difference between the minimum and maximum values of 0.10 mmol/L, confirming low values of standard deviation and standard error.

According to the results of the biochemical analysis of the blood serum of goats, the lipid profile indicators for the sympathotonic experimental group were established (Table 3).

Table 3. Lipid profile indicators of blood serum of sympathotonic goats

Indicator	NVO	SE	Mdn.	SD	Asym.	Min.	Max.
Triglycerides, mmol/L	5	0.01	0.34	0.02	0.24	0.32	0.37
VLDL, mmol/L	5	0.01	0.15	0.02	0.59	0.13	0.18
LDL, mmol/L	5	0.01	0.35	0.02	-0.01	0.33	0.37
HDL, mmol/L	5	0.01	0.84	0.02	-0.55	0.82	0.86
Cholesterol, mmol/L	5	0.02	1.35	0.04	-1.53	1.27	1.38
Atherogenic index	5	0.02	0.59	0.04	0.17	0.54	0.63

Note: *NVO – number of valid observations, SE – standard error, Mdn. – median, SD – standard deviation, Asym. – asymmetry, Min. – minimum value, Max. – maximum value*
Source*: authors' development*

According to the results of the lipid profile analysis of serum in sympathotonic goats, the concentration of triacylglycerols showed a difference between the minimum and maximum values of 0.05 mmol/L, which resulted in low values of standard deviation and standard error. The concentration of very low-density lipoproteins in animals with a predominance of the sympathetic nervous system exhibited discrepancies between the minimum and maximum values of 0.05 mmol/L, which accounted for low values of standard deviation and standard error. The levels of low-density lipoproteins in sympathotonic goats displayed differences between the minimum and maximum values of 0.05 mmol/L, again ensuring low values of standard deviation and standard error. The concentration of high-density lipoproteins in animals with a predominance of the sympathetic nervous system corresponded to a difference between the minimum and maximum values of 0.04 mmol/L, which provided low parameters of standard deviation and standard error. The cholesterol concentration in the serum of sympathotonic goats was characterised by differences between the minimum and maximum values of 0.11 mmol/L, reflecting low values of standard deviation and standard error. The atherogenic index in this group of animals exhibited differences between the minimum and maximum values of 0.09 mmol/L, ensuring low values of standard deviation and standard error.

When comparing the concentration of triacylglycerols in the serum of goats from different experimental groups with varying autonomic nervous system tones, some differences were identified. In comparison with the normotonic experimental group, it was found that the concentration of triacylglycerols in sympathotonic goats was 23% lower ($P < 0.05$), while in the vagotonic experimental group, it was 41% lower ($P < 0.001$). Consequently, the normotonic experimental group had higher triacylglycerol levels, reflecting the intensity of fatty acid metabolism in the goats (Fig. 1).

When analysing the concentration of low-density lipoproteins, it was established that, in relation to the normotonic experimental group, characterised by a balanced sympathovagal balance, sympathotonic goats exhibited values that were 40% higher ($P < 0.05$), while the animals in the vagotonic experimental group had values that were 76% higher ($P < 0.01$) (Fig. 2).

The levels of high-density lipoproteins in the normotonic experimental group of goats were found to be 36% higher ($P < 0.001$) when compared with the sympathotonic goats, which exhibited a predominance of the sympathetic nervous system. In relation to the vagotonic experimental group, characterised by a predominance of the parasympathetic nervous system, the levels of high-density lipoproteins showed minor differences compared to the normotonic group (Fig. 3).

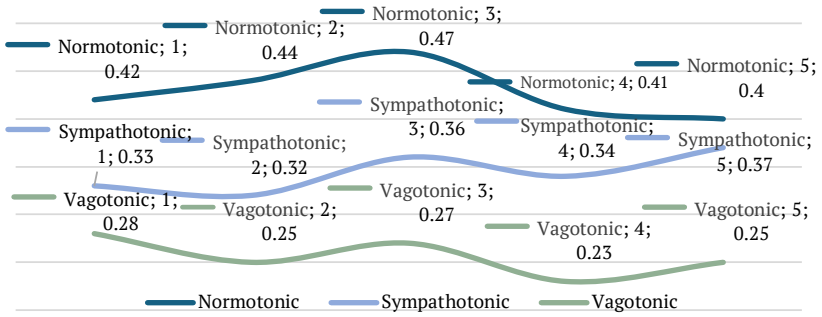

Figure 1. The concentration of triacylglycerols in the serum of goats with different autonomic nervous system tones, mmol/L

***Note**: colours indicate different tones of the autonomic nervous system, respectively*

***Sourse**: authors' development*

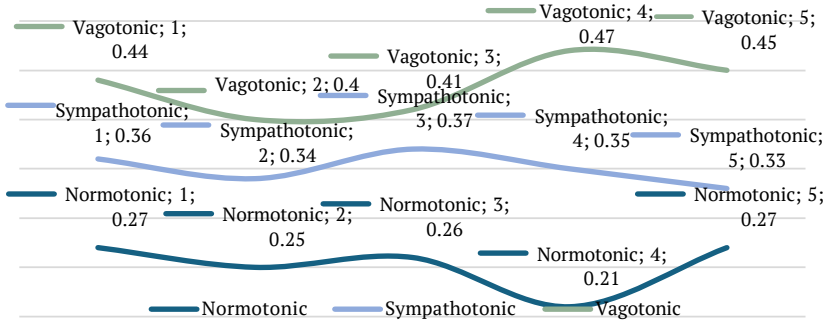

Figure 2. The concentration of low-density lipoproteins in the serum of goats with different autonomic nervous system tones, mmol/L

***Note**: colours indicate different tones of the autonomic nervous system, respectively*

***Sourse**: authors' development*

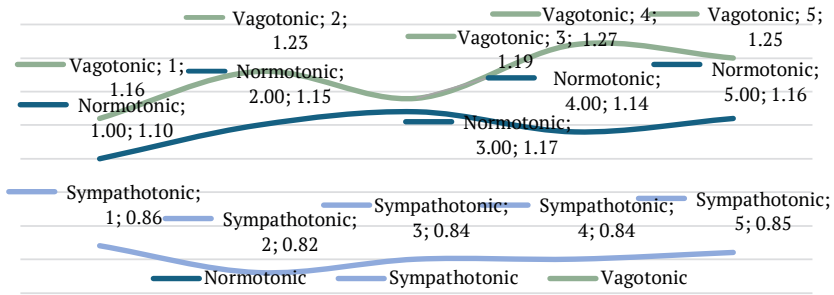

Figure 3. The concentration of high-density lipoproteins in the serum of goats with different autonomic nervous system tones, mmol/L

***Note**: colours indicate different tones of the autonomic nervous system, respectively*

***Sourse**: authors' development*

The total cholesterol concentration in the serum of normotonic goats was 16% higher (P < 0.01) compared to that of sympathotonic goats. When comparing the cholesterol levels between the normotonic animals and the vagotonic experimental group, it was established that the concentration in vagotonic goats was 15% higher compared to the normotonic goats (P < 0.01) (Fig. 4).

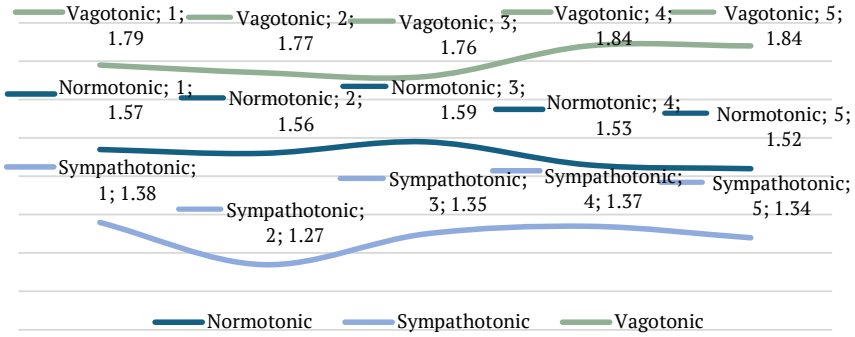

Figure 4. The concentration of cholesterol in the serum of goats with different autonomic nervous system tones, mmol/L
***Note**: colours indicate different tones of the autonomic nervous system, respectively*
***Sourse**: authors' development*

Analysis of the atherogenic index revealed that in normotonic goats, compared to sympathotonic goats with a predominance of the sympathetic nervous system, the atherogenic index was 38% lower (P < 0.01). In relation to the vagotonic experimental group, the values of the atherogenic index showed minor differences when compared with the normotonic group (Fig. 5).

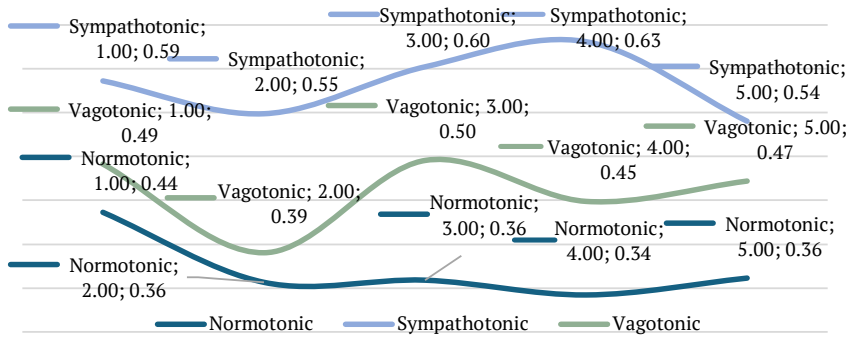

Figure 5. Atherogenic index values in goats with different autonomic regulation tones
***Note**: colours indicate different tones of the autonomic nervous system, respectively*
***Sourse**: authors' development*

The atherogenic index values in the sympathotonic experimental group were characterised by high values, indicating a low level of high-density lipoproteins relative to total cholesterol parameters. The normotonic goats had the lowest atherogenic index values, reflecting a higher concentration of high-density lipoproteins relative to total cholesterol concentration.

Analysis of the lipid composition in goats is crucial. For high-yielding animals, a deficiency in energy-rich substances can become a key factor in the further development of their organism, which will be reflected in future productivity. In addition, it is worth conducting a risk assessment for these ruminants. The issue of the stress factor is always relevant due to its diversity and frequency of occurrence. Even though, at first glance, the threat of a stressful state of an animal carries insignificant shifts in the body, over time this problem can grow, which will be reflected in lipid metabolism and, as a consequence, in productivity. The question of improving metabolism in this case is quite relevant since with an increase in the content of energy-rich substances, an acceleration of the growth and development of goats will be noted, which will subsequently be reflected in productivity (Fougère *et al.*, 2021).

To improve lipid metabolism, researchers have developed various approaches primarily focused on adjusting the levels of fats and fatty acids in the body through different dietary supplements. The aim of introducing additional nutrients into the diet is to influence the enzymatic pathway, provide an additional source of lipids, and correct the antioxidant system, among other factors. Q. Li *et al.* (2024) analysed the biochemical composition of goat serum at different stages of their development. Their study found that from the age of 180 days, the intensity of lipid metabolism in goats was significantly lower compared to other periods of young animal development. It is important to note that the aforementioned authors emphasised the significance of lipid metabolism for ensuring adequate growth and development in goats. Analysing the data obtained by these researchers, it can be noted that the lipid profile indicators are similar, but due to the distribution of animals according to the tone of the autonomic nervous system, the error of the data in the presented material is significantly reduced. This will substantially enhance the analysis of lipid metabolism research results for scientists, as the obtained indicators have a smaller margin of error and take individual characteristics into account.

A. Ianni *et al*. (2021) conducted a study investigating the effects of an olive leaf dietary supplement on the lipid profile of goats. The experimental group of goats fed a diet supplemented with olive leaves, had a 9% lower cholesterol content in their milk compared to the control group. More significantly, the blood cholesterol content was 20% lower in the experimental group compared to the control. However, these results had a considerable margin of error, which adversely affected the values of the variation error. Such findings indicated that the animals were not categorised according to their individual characteristics, resulting in disparate indicators with significant errors. The results of this study highlight the necessity of distributing animals according to their individual traits, which would facilitate a more accurate analysis of lipid metabolic transformations.

Y. Shen *et al.* (2022) conducted a study involving the addition of resveratrol supplements to the diet of goats to enhance animal growth and improve lipid metabolism. The biochemical analysis of the blood serum revealed the impact of the resveratrol dietary supplement on the levels of triglycerides, as well as high-density and low-density lipoproteins. Compared to the control group, it was found that the triglyceride content significantly decreased by 55% in goats receiving a high concentration of resveratrol, with a dosage of 600 mg per kilogram of body weight. The levels of high-density lipoproteins increased by 1.5 times in animals receiving this dietary supplement relative to the control group, while the low-density lipoprotein levels decreased by 2.5 times compared to the control

group. There was also an increase in the atherogenic index. Despite these significant differences, it is worth noting that during the study, the methodology for correcting lipid metabolism and improving lipid levels exhibited considerable discrepancies in the results. Consequently, there was a lack of variation in the triglyceride levels and LDL ($P > 0.05$), as well as a weak correlation when comparing the total cholesterol and HDL ($P < 0.05$). In comparing these results, it can be concluded that distinguishing the experimental groups of animals according to the activity of the autonomic nervous system would enhance the effectiveness of the biochemical analysis of lipid metabolism and reduce the initial error in the statistical data.

M. Zheng *et al.* (2021) investigated the impact of zinc supplements, including zinc sulphate, zinc methionine, and zinc glycinate, on lipid metabolism. The aim of their study was to determine the effects of these dietary supplements on the performance indicators of goats during the growth period. The highest lipid content in blood serum was observed in animals fed zinc sulphate and zinc glycinate supplements. The lowest lipid content was noted in goats that consumed zinc methionine. It is worth noting that although differences in indicators were found in the three experimental groups of animals, not all the results obtained showed a correlation between lipid metabolism indicators and the additional administration of zinc supplements to these animals. This indicates a significant discrepancy in the obtained results, which confirms the need to divide the experimental groups of animals according to their individual characteristics. This can be ensured by taking into account the tone of the autonomic nervous system, as confirmed by the results described in this material.

L. Min *et al*. (2023) investigated the impact of dietary supplements consisting of *Aquilaria sinensis* leaves on lipid metabolism in goats. The aim of their research was to improve productivity, specifically meat quality and weight gain. Their research suggests that diets supplemented with *Aquilaria sinensis* leaves offer a viable alternative for improving lipid metabolism and meat quality. This affordable ingredient promotes balanced weight gain in goats and enriches their bodies with polyunsaturated fatty acids, thereby enhancing the quality of the meat. The use of this dietary supplement also contributed to a decrease in blood cholesterol levels and an increase in high-density lipoprotein levels in goats. An analysis of the data from the study suggests the need to categorise animals based on the influence of the divisions of the autonomic nervous system. This is because not all lipid metabolism indicators showed significant results when comparing animals from the experimental group with the control group. It is worth noting that, according to the results obtained, animals have different intensities of lipid metabolism under different tones of the autonomic nervous system, which provides new information for studying the improvement of organic substance metabolism.

S. Liu *et al*. (2021) used dietary supplements in the form of flaxseed oil and heated flaxseed. This resulted in a decrease in total levels of triacylglycerols, cholesterol, and low-density lipoproteins in goats, which was significantly lower compared to diets using flaxseed oil alone. However, when assessing the reliability of the results, it was found that although the obtained indicators showed some changes in plasma lipids, the difference between the values in the experimental and control groups was quite insignificant. Comparing with the results described in this research, it should be noted that the informativeness of such indicators significantly depends on the body systems that control and directly influence metabolism. To obtain reliable results, it is recommended that animals be divided into experimental groups according

to their sympathovagal balance, which would simplify the analysis of the results for scientists.

Having analysed various studies on improving lipid metabolism in goats, it is evident that the data showing changes in serum lipid profiles exhibit significant numerical discrepancies. This is corroborated by statistical analysis results, which lie at the lower boundary of acceptability (Rawat *et al.,* 2021). Several factors can account for these discrepancies. Firstly, the formation of experimental and control groups can be a significant issue. Farmers do not always consider the individual characteristics of each animal, which is crucial as metabolic processes vary from one organism to another. These oversights can lead to significant discrepancies in biochemical analysis results. Secondly, different animals may react differently to changes in feeding strategies. The introduction of various dietary supplements can have both positive and negative effects, as alterations in diet composition can modify metabolic processes. This once again highlights the need to consider individual animal characteristics (Singh *et al.,* 2023).

An analysis of serum lipid profiles in goats with varying autonomic nervous system tones revealed differences between animals in different experimental groups. These differences reflect the metabolic processes occurring at different intensities. The activity of the sympathetic and parasympathetic nervous systems is a key factor in this study. Each animal has a specific sympathovagal balance, characterised by varying activity of the divisions of the autonomic nervous system (Metz *et al.,* 2022). By classifying goats based on individual characteristics, three groups were formed: normotonics, vagotonics, and sympathotonics. Each group of animals was characterised by the different activity of the sympathetic and parasympathetic nervous systems, and consequently different intensities of metabolic processes. This is confirmed by the research results, which showed high levels of triacylglycerols in normotonic animals, high levels of low-density lipoproteins in vagotonic animals, and low levels of high-density lipoproteins in sympathotonic animals. Considering this, it becomes possible to better understand the picture of lipid metabolism in goats.

By incorporating these characteristics in methods to improve lipid metabolism in goats, scientists will gain an additional reference point. This is because, according to the obtained data, there are already differences in the content of cholesterol, lipoproteins of various densities, and triacylglycerols without the addition of dietary supplements. These indicators are quite important in assessing not only lipid metabolism but also overall metabolic processes and goat productivity. Therefore, considering this criterion when forming experimental animal groups will help scientists better evaluate the obtained results. This can elevate the value of dietary supplements used to improve lipid metabolism to a new level.

Considering environmental factors, an animal can be subjected to a stressor at any given time. Primarily, under these circumstances, the stability of the body's homeostasis will suffer, as it will significantly mobilise energy reserves to counteract these changes. One of the main systems that respond to stress is the autonomic nervous system, which, depending on individual characteristics, will have a different tone (Durosaro *et al.,* 2023). As a result, metabolic processes in each goat will shift differently. Consequences of this, if left unaddressed, may include decreased productivity and economic losses.

Conclusions

The influence of autonomic nervous system tone on serum lipid profiles in goats has been established. Differences in lipid metabolism indicators in these animals have been determined depending on the different tones of

the autonomic nervous system: normotonics, vagotonics, or sympathotonics. In particular, in the serum lipid profile of goats, differences have been found in indicators such as cholesterol content, high-density and low-density lipoproteins, triacylglycerols, and atherogenic index values with different autonomic nervous system tones.

It was determined that the content of triacylglycerols in the blood serum of normotonic goats was the highest compared to the experimental groups of sympathotonic animals ($P < 0.05$) and vagotonic animals ($P < 0.001$). It was established that the level of low-density lipoproteins was lowest in normotonic animals compared to the experimental groups of sympathotonic ($P < 0.05$) and vagotonic ($P < 0.01$) animals. It was determined that the content of high-density lipoproteins was the lowest in goats of the sympathotonic experimental group compared to the normotonic experimental group ($P < 0.001$). It was established that the values of total cholesterol in the blood serum of normotonic animals were higher compared to the experimental group of sympathotonic animals ($P < 0.01$) and lower when compared to the experimental group of vagotonic animals ($P < 0.01$). The value of the atherogenic index in the experimental group of normotonic animals was the lowest compared to the experimental group of sympathotonic animals ($P < 0.01$) and animals of the vagotonic experimental group ($P < 0.05$). It was established that depending on the individual characteristics of the goat's organism, reflected in the form of sympathovagal balance, in which the influence of the sympathetic or parasympathetic nervous system predominates or there is a balanced interaction of the sympathetic and parasympathetic nervous systems, lipid metabolism processes differ.

The prospect of further research involves the use of dietary supplements in goat feed, formulated based on the tone of the autonomic nervous system. This approach is expected to significantly enhance the effectiveness of these supplements and contribute to improving lipid metabolism in goats, ultimately leading to increased productivity.

Acknowledgements

None.

Conflict of Interest

None.

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Відмінності ліпідограми сироватки крові кіз із різним тонусом автономної нервової системи

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Анотація. Козівництво є перспективною галуззю тваринництва, оскільки за статистикою попит на споживання молочної і м'ясної продукції кіз на світових ринках стрімко зростає. Для насичення ринку необхідною кількістю продукції потрібні нові методичні підходи з покращення продуктивності тварин зі збереженням якості вихідної сировини. У зв'язку з цим, метою дослідження було визначення впливу тонусу автономної нервової системи на обмін ліпідів в організмі кіз. Для дослідження використовували тварин породи Зааненська, формування дослідних груп проводилось з урахуванням результатів варіаційно-пульсометричного дослідження і даних електрокардіографії з подальшим визначенням тонусу автономної нервової системи за методикою Баєвського. Дослідження ліпідограми сироватки крові кіз здійснювали за допомогою спектрофотометра LabLine-010. У зразках сироватки крові здійснювали біохімічний аналіз наступних показників: вмісту триацилгліцеролів, холестеролу, ліпопротеїнів високої, низької і дуже низької щільності та розраховували величину індексу атерогенності. Встановлено, що вміст триацилгліцеролів набував значень менших на 23% ($P < 0,05$) у симпатотоніків та на 41% ($P < 0,001$) в тварин

ваготоніків порівняно з нормотоніками. Рівень ліпопротеїнів низької щільності у сироватці крові симпатотоніків зменшувався на 40% (P < 0,05), а у ваготоніків – на 76% (P < 0,01), відносно нормотоніків. Уміст ліпопротеїнів високої щільності у симпатотоніків збільшувався на 36% (P < 0,001), відносно нормотоніків. Рівень холестеролу в сироватці крові кіз відрізнявся значеннями вищими на 16% (P < 0,01) у симпатотоніків та на 15% (P < 0,01) у ваготоніків порівняно з нормотоніками. Визначено істотний вплив тонусу автономної нервової системи на процеси підтримання ліпідного гомеостазу в організмі кіз. Врахування індивідуальних особливостей впливу автономної нервової системи на метаболізм ліпідів при розподілі тварин сприятиме ефективному коригуванню раціонів. Адже кожна тварина має різний тонус автономної нервової системи, що істотно впливає на особливості перетворення речовин в її організмі

Ключові слова: обмін ліпідів; холестерол; ліпопротеїни різної щільності; коефіцієнт атерогенності; автономна регуляція

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Key aspects of biosafety in modern animal husbandry

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Abstract. The relevance of this research is driven by the need to enhance biosecurity in animal husbandry to improve the health and productivity of livestock, as well as to safeguard human health. Biosecurity is a critical component that prevents the spread of infectious diseases, mitigates the risks of epidemics, reduces treatment costs, and increases productivity and economic efficiency within agricultural enterprises. In this regard, this study aimed to reveal the role of biosafety in preventive veterinary medicine. The leading approach to investigating this issue involved analysing existing practices and methods, which allows for a comprehensive examination of various aspects of biosecurity. The conducted analysis substantiated that preventive veterinary medicine is founded on the principle of disease prevention through risk reduction and the implementation of healthy practices. It has been established that this approach encompasses not only the treatment of sick animals but also the provision of advice to farmers on enhancing the health and productivity of their livestock. It has been established that biosecurity also helps prevent the emergence and spread of infectious diseases, reducing the need for antimicrobial drugs and lowering the risk of antimicrobial resistance. The research highlighted that in today's rapidly changing world, biosecurity is becoming even more important due to population growth, urbanisation, climate change, and human health threats such as antimicrobial resistance. The concept of "One Health" is encompassed, emphasising the close connection and interdependence among the health of humans, domestic and wild animals, plants, and the environment. The core

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principles of biosecurity included segregation, cleaning and disinfection, and considering the behaviour and attitudes of people working with animals. The material presented in this article is of practical value to veterinarians, farmers, and livestock farms, assisting them in developing and implementing effective biosecurity plans adapted to changing conditions, thereby contributing to reducing the risks of infectious diseases and improving livestock productivity

Keywords: preventive veterinary medicine; infectious diseases; antimicrobial resistance; cleaning and disinfection; human health protection; climate change

Introduction

Biosecurity, as highlighted by A. Diana *et al.* (2020), is a pivotal aspect of modern livestock farming, playing a crucial role in ensuring animal and human health, as well as enhancing the productivity of farms. In the context of growing populations, urbanisation, and climate change, the need for implementing effective biosecurity measures is becoming increasingly urgent. Biosecurity not only prevents the spread of infectious diseases among animals but also contributes to reducing the use of antimicrobial drugs, which in turn lowers the risk of developing antimicrobial resistance.

The relevance of this research is driven by the need to prevent the spread of infections that negatively impact animal and human health, as well as industry productivity. With the expansion of animal husbandry, the risk of epidemics also increases, necessitating the implementation of effective biosecurity measures. This is critically important for ensuring food security, economic stability, and reducing environmental impact, particularly through the fight against antibiotic resistance. V. Renault *et al.* (2022) argued that adhering to these principles helps to reduce the risks of infectious diseases and increase livestock productivity.

Biosecurity in modern animal husbandry is a subject of research for many scientists who have analysed various aspects of this field. V. Renault *et al.* (2022) investigated the importance of the biosecurity concept as a key element of the "One Health" approach. Using the concept of "One biosecurity", they emphasised the close link between public health components and the need to consider them as an integrated structure.

Adherence to biosecurity procedures is a key aspect of preventing the spread of infectious diseases among animals, as confirmed in the scientific research of E. Butucel *et al.* (2022). This can be achieved through an implemented strategy that involves preventing direct or indirect contact with pathogenic agents, vaccination, disinfection, rational water management, and appropriate disposal procedures, among others. Non-compliance with biosecurity protocols can lead to a loss of disease control on farms, resulting in animal mortality and significant economic losses for farmers.

As highlighted in the study by S. Sarrazin *et al.* (2019), the transmission routes of epidemiological diseases can be highly diverse, necessitating the implementation of comprehensive principles. The development of biosecurity plans and the analysis of the effectiveness of preventive measures can reduce risks and improve the control of disease spread. T. Zhang *et al.* (2024) have demonstrated that modern animal husbandry faces numerous challenges that require the implementation of effective biosecurity measures. One of the primary concerns is the risk of infectious disease spread among animals, which can lead to significant economic

losses and pose a threat to human health. The intensification of animal husbandry, globalisation of trade, and climate change create favourable conditions for the spread of pathogens, making biosecurity issues even more pressing.

One of the primary threats is antimicrobial resistance, which arises from the excessive and improper use of antimicrobial agents in animal husbandry. N.I. Azman *et al.* (2021) indicated that this leads to a decrease in the effectiveness of treating infectious diseases in both animals and humans, which can have serious consequences for public health. In particular, the rise of antimicrobial resistance threatens a return to an era when numerous infectious diseases became untreatable. Another issue is the inadequate understanding and implementation of biosecurity measures on farms. J.W. Aleri and M. Laurence (2020) noted that some farmers do not recognise the importance of biosecurity or lack sufficient knowledge for its effective implementation. The absence of appropriate education and training for farmers and livestock workers results in even the best biosecurity measures being ineffective due to human factors. For instance, as mentioned by E. Butucel *et al.* (2022), non-compliance with hygiene protocols, improper storage of feed and water, and insufficient isolation of new or sick animals can undermine all efforts to prevent the spread of diseases.

Furthermore, the globalisation of trade in animals and animal products creates additional biosecurity risks. The international movement of animals, feed, and equipment can provide a pathway for the introduction of new pathogens into different regions, making it more difficult to control infectious diseases. This is particularly important in the context of zoonotic diseases, which can be transmitted from animals to humans. According to the World Health Organization (WHO), approximately 60% of all human infectious diseases have a zoonotic origin, highlighting the importance of a comprehensive approach to biosecurity. This statistic is also supported by data from the Food and Agriculture Organization of the UN (FAO, n.d.), which indicates that 75% of new human infectious diseases also originate from animals. This data demonstrates the importance of coordinating efforts between the human and animal sectors to prevent the spread of diseases and improve biosecurity levels. (World Health Organization, 2020; World Health Organization, 2023).

Another aspect of the problem is the impact of climate change on animal health. Changes in temperature regimes, increased humidity, and other climatic factors can create favourable conditions for the development and spread of pathogens. For instance, rising temperatures can lead to increased populations of disease-carrying insects, posing additional risks to animal husbandry. Thus, as argued by D. Black *et al.* (2023), biosecurity measures must consider changing climatic conditions and the need to adapt to them.

Finally, the implementation of biosecurity measures often faces economic barriers. Some farms may lack sufficient financial resources to implement and maintain effective biosecurity measures. This includes the costs of constructing and maintaining isolation zones, purchasing disinfectants, ensuring proper hygiene, and training staff. Without financial support and incentives from governments or international organisations, farms cannot afford to implement the necessary biosecurity measures. Thus, to address biosecurity challenges in modern animal husbandry, a comprehensive approach is needed, including the development of educational programmes for farmers and farm workers, financial support, research, and international cooperation. Only then can sustainable and safe production of animal products be ensured, meeting the demands of the contemporary world.

This study aims to highlight the importance of biosecurity in animal husbandry and to provide recommendations for its improvement. The research employed methods of analysis, synthesis, and the summarisation of scientific and methodological literature on biosecurity in modern animal husbandry. The primary sources for analysis were scientific articles published by leading researchers abroad. These articles included reviews and original studies addressing various aspects of biosecurity, from risk management to the application of cutting-edge technologies in the field of animal husbandry. During the research, a substantial number of scientific sources were analysed, including articles published in peer-reviewed journals that explored diverse aspects of biosecurity in animal husbandry. Particular attention was given to research describing innovative approaches to ensuring biosecurity, as well as studies evaluating the effectiveness of various methods for protecting animals from infectious diseases. For example, some researchers proposed the use of biosensors for the early detection of diseases, which can reduce the risks of infection spread. Other scientists focused on developing new vaccines and antimicrobial agents that may be more effective and safer for animals.

Based on the analysis of the literature, the main trends and approaches to ensuring biosecurity in modern animal husbandry have been summarised. In particular, key aspects have been identified, such as risk management, sanitary measures, disease control, and the use of modern technologies for monitoring animal health and husbandry conditions. Risk management involves assessing and minimising potential threats that may arise during animal husbandry. Sanitary measures encompass a wide range of practices aimed at maintaining a high level of hygiene on farms, including regular cleaning and disinfection of animal housing.

Importance of biosecurity in modern animal husbandry: Key aspects

Biosecurity is a vital component of modern animal husbandry, helping to improve animal health and productivity while also protecting human health. This article explores the key aspects of biosecurity and its significance in preventive veterinary medicine. It aims to enhance the knowledge and skills of veterinarians in developing and implementing effective biosecurity plans in collaboration with farmers and farms.

J. Dewulf *et al*. (2018) demonstrated that preventive veterinary medicine is based on the principle of disease prevention through risk reduction and the implementation of healthy practices. This approach involves not only treating sick animals but also providing ongoing advice to farmers on improving the health and productivity of their animals. As A. Diana *et al.* (2020) found, biosecurity is a key component of this approach, as it helps prevent infectious diseases, reducing the need for the use of antimicrobials and lowering the risk of developing antimicrobial resistance.

In today's rapidly changing world, biosecurity is becoming even more important. Population growth, urbanisation, climate change, and threats to human health, such as antimicrobial resistance, demand effective solutions. According to the FAO, the world's population is projected to reach 9.15 billion people by 2050, placing significant pressure on food production systems (FAO, n.d.). Furthermore, as H.W.F. Waldeck *et al.* (2021) note, approximately 60% of all human infectious diseases have a zoonotic origin, highlighting the risks associated with close interactions between humans and animals.

Biosecurity is not limited to animal health; it also considers the health of humans, plants, and the environment. V. Renault *et al.* (2022) noted that the concept of "One Health" emphasises that the health of humans, domestic and wild animals, plants, and the environment

are closely linked and interdependent. This approach mobilises various sectors and disciplines to work together to ensure well-being and combat threats to health and ecosystems.

For example, brucellosis is a zoonotic disease that has serious consequences for livestock productivity and human health. This disease is spread through contact with aborted materials or birth fluids, as well as through the consumption of contaminated milk. In humans, brucellosis causes flu-like symptoms, but in some cases, chronic complications can develop, significantly impacting health and work capacity.

Control of brucellosis in animals involves vaccination and the implementation of biosecurity measures, such as maintaining hygiene during milking, as well as boiling and pasteurising milk. I. Garcia *et al.* (2023) highlight that veterinarians play a crucial role in educating farmers about effective preventive measures, thereby safeguarding both animal and human health.

In summary, veterinary professionals have a significant role in improving the resilience and efficiency of livestock farming. Implementing biosecurity measures as part of preventive veterinary medicine not only increases farm productivity but also contributes to protecting human health from zoonotic diseases and antimicrobial resistance. Preventive veterinary medicine helps ensure the stability and prosperity of both farmers and veterinarians, creating strong mutually beneficial relationships.

Basic principles of biosecurity in animal husbandry

Biosecurity is a key aspect of maintaining animal health and preventing the spread of infectious diseases. This article focuses on the fundamental principles of biosecurity, aiming to assist farms in developing effective measures to mitigate disease risks.

A. Diana *et al*. (2020) demonstrated that biosecurity is a strategic and integrated approach that encompasses policies and regulatory frameworks for analysing and managing risks in the domains of food safety, animal and plant health, as well as associated environmental risks. It covers a wide range of pathogens, including viruses, bacteria, fungi, endo- and ectoparasites, as well as protozoa. The foundation of effective biosecurity is a risk-based approach. In their study, M. Rahman *et al.* (2020) noted that it is impossible to completely eliminate the possibility of animals being infected by pathogens; however, it is possible to reduce this risk as much as practically possible. Prioritising high-risk factors allows for the optimal use of available resources.

Understanding the transmission pathways of pathogens is the first step towards effective biosecurity. The primary routes include aerosol, direct contact, oral, vector-borne, and fomite transmission. For instance, some diseases are transmitted through inhalation of respiratory droplets expelled by another animal, or through direct contact with infected bodily fluids. Effective biosecurity measures are typically categorised into three main components: segregation, cleaning, and disinfection. Segregation involves preventing contact between healthy and infected animals, as well as between potentially contaminated objects and healthy animals. Cleaning refers to the removal of all organic material from surfaces, significantly reducing the number of pathogens. J.S. Ferreira *et al.* (2024) emphasise that disinfection involves the use of chemicals to kill pathogens but its effectiveness depends on correct application.

Physical biosecurity measures, such as buildings and fencing, are important, but equally critical are the attitudes and behaviours of those working with animals. Even the best biosecurity protocols will be ineffective if people fail to recognise the risks and do not adhere to the guidelines.

Disease risks can fluctuate due to a variety of factors, including seasonality, weather conditions, and changes in farming practices. Therefore, biosecurity measures must be adapted to the current risk level. For instance, during an outbreak in a neighbouring area, a farm may temporarily change its feed sources to reduce potential exposure.

A combination of different biosecurity measures is often more effective than a single approach. Research by J. Denis-Robichaud *et al.* (2019) has shown that combining multiple layers of protection reduces the likelihood of a pathogen penetrating all barriers.

In conclusion, biosecurity is an indispensable component of successful livestock farming, serving to mitigate the risks of infectious diseases and enhance animal productivity. A risk-based approach, understanding pathogen transmission pathways, and implementing principles of segregation, cleaning, and disinfection are fundamental to effective biosecurity measures. Additionally, it is crucial to consider the behaviour and attitudes of individuals working with animals and to adapt measures to changing conditions.

An integrated approach to biosafety: Preventing pathogens from the environment

Biosecurity is a critical aspect of modern animal husbandry, playing a pivotal role in preventing the spread of infectious diseases among animals. This section of the article discusses biosecurity as outlined by K. Koutsoumanis *et al.* (2022), specifically about the movement of animals and vehicles visiting the farm.

One of the most crucial aspects of biosecurity is controlling the movement of animals. Introducing new animals to a farm can become a source of pathogens, making it essential to follow specific measures. Before purchasing new animals, a list of pathogens to avoid should be compiled. This will help assess the risks and implement the necessary measures to prevent them. Practices before purchasing animals include reducing the number of sources of new animals and conducting health assessments of the source herd through testing for undesirable pathogens. New animals should be isolated from the main herd for a period of three to four weeks, allowing for the detection of potential diseases and preventing their spread.

Vehicles visiting a farm can also act as a source of infection. These include vehicles for transporting animals, collecting milk, delivering feed, and others. As noted by F.J. Villaamil *et al.* (2020), to reduce this risk, the following measures should be adhered to: vehicles transporting animals should be thoroughly cleaned and disinfected after each journey. Where possible, access to areas where animals are housed should be restricted to vehicles. It is recommended to create separate parking areas outside the farm boundaries for certain types of vehicles. Loading and unloading points for animals should be located as far as possible from the main animal housing areas (Center for disease control ..., n.d.).

It is crucial to understand that all types of animal movements, not just the acquisition of new individuals, can introduce pathogens, so implementing appropriate biosecurity measures essential. Isolating new animals is a vital step to prevent the introduction of diseases onto the farm, with the quarantine period needing to be long enough to detect potential illnesses, while also conducting diagnostic tests. Vehicles in contact with animals present a high risk of spreading pathogens, making cleaning and disinfection mandatory measures. Recommendations for vehicle movement on farms include using separate vehicles for feed transport and the removal of manure or dead animals. It is also advisable to minimise the number of vehicles allowed access to animal

housing areas and to carefully plan their routes on the farm to prevent cross-contamination (Denis-Robichaud *et al.,* 2019).

Thus, implementing effective biosecurity measures when moving animals and vehicles on the farm is essential for maintaining animal health and improving farm productivity. Adhering to these principles will help reduce the risk of introducing and spreading infectious diseases, which is critical for the sustainable development of livestock farming. Visitors and farm workers can also be sources of pathogens, which may be carried on clothing, footwear, or equipment (National Association of State Public Health, 2023). Depending on the frequency of visits and contact with animals, the level of risk for pathogen introduction varies. High-risk groups include veterinarians, hoof trimmers, and workers employed on other farms or with their own animals. Medium-risk groups consist of product vendors, consultants, and milk truck drivers, while visitors who rarely visit farms, such as local school children, pose a low risk. To reduce the likelihood of pathogen introduction, hygiene zones with showers and toilets for visitors and workers should be established, separate clothing and footwear for each farm should be used, and regular hand washing and disinfectant use should be practised.

Farm equipment can also serve as a source of pathogen transmission between animals and farms. To mitigate this risk, it is recommended to use single-use needles and syringes for each animal, disinfect reusable equipment such as thermometers and ultrasound sensors, and clean and disinfect instruments after each use. Contact with animals from neighbouring farms can introduce pathogens, so it is essential to install robust fences to prevent contact between animals from different farms. These fences should be regularly maintained to prevent damage, and manure and other waste should be stored in airtight containers to prevent leaks.

Improper storage of feed and water can lead to contamination with pathogens. To ensure the safety of feed and water, they should be stored in closed and dry premises, protected from the weather and animals. The quality of water should be regularly checked and treated, such as by chlorination, and water should be stored in closed reservoirs to prevent access by animals and pests (Black *et al.,* 2020; Waldeck *et al.,* 2021).

Implementing effective measures for visitors, equipment, neighbouring farms, feed, and water is crucial for reducing the risks of introducing and spreading pathogens, thereby contributing to sustainable animal husbandry development.

Interaction with dogs, cats and pests as a component of biosecurity in animal husbandry

Dogs and cats can carry a variety of pathogens that are capable of infecting livestock and humans. Major biological threats include helminths such as *Taenia hydatigena*, which causes liver lesions in sheep, *Taenia ovis*, which affects the heart muscle of sheep, *Taenia multiceps*, which causes brain lesions in sheep, and *Echinococcus granulosus*, which causes the formation of hydatid cysts in the liver. Among protozoa, *Toxoplasma gondii* is particularly dangerous, as it can infect domestic animals and humans, with cats as the primary transmitters of this parasite, which can cause abortions in livestock. *Neospora caninum* can also cause abortions in cattle (Alocilla & Monti, 2022).

To prevent the spread of these pathogens, it is essential to restrict access of dogs and cats to feed storage areas, water sources, calving areas, and places of storage of animal corpses. This can be achieved by closing doors and gates to prevent access to feed storage areas, installing fences to restrict animal movement using physical barriers, and ensuring proper management

and training of dogs, which should have designated areas for resting, feeding, and drinking.

Pests such as rodents, insects, and birds can also carry pathogens that pose a threat to livestock. For instance, rodents can transmit leptospirosis, toxoplasmosis, and campylobacteriosis through their faeces. Birds are capable of spreading *Salmonella* and *Cryptosporidium*, contaminating feed and water with their faeces. Insects may carry various viruses, including the bluetongue virus and the nodular dermatitis virus. To manage pest populations, it is crucial to prevent conditions that favour their development, which includes ensuring proper storage of feed and water, adhering to hygiene practices to prevent the accumulation of waste and litter, and employing mechanical traps and insecticides for insect control. Additionally, rodenticides should be used to manage rodent populations, while physical barriers, such as nets, can help restrict bird access. As a biological control method, birds of prey can be used to deter other birds from the area (Ouchene *et al.,* 2023).

Products designed to control pests should be stored in designated areas, according to the storage conditions specified by the manufacturer. O. Alocilla & G. Monti (2022) pointed out that these areas should be accessible only to authorised personnel to avoid accidental ingestion or the inclusion of toxic products in animal feed.

In conclusion, adherence to biosecurity principles in interactions with dogs, cats, and pests is crucial for preventing the spread of pathogens on farms. Proper storage of feed and water, restricting animal access to storage areas, regular disinfection, and pest control will help to ensure animal health and increase the productivity of farms.

Security at livestock markets: Key aspects and measures

Although the popularity of buying and selling live animals at markets has significantly decreased over the past decade, according to S. Sarrazin *et al.* (2019), this practice still exists and therefore requires special attention and control from a biosecurity perspective. The movement of animals is one of the primary routes for the transmission of pathogens between herds. Research shows that livestock markets can play a central role in the spread of diseases. The mixing of animals of different age and sex groups and from farms with different epidemiological statuses plays a significant role in this process. As scientists have noted, this creates a high potential for the spread of pathogens both between farms and between regions.

Visiting livestock markets can introduce pathogens onto a farm through infected animals or fomites such as footwear or clothing that have been used at the market. For instance, if a farmer returns from a market without purchasing any animals, the risk is minimal. However, if a farmer brings new animals onto their farm, the risk increases significantly due to potential contact with other animals at the market. A medium-risk arises when a farmer returns with unsold animals, which may also have had contact with other animals.

To prevent the introduction of new pathogens onto a farm, farmers should adhere to specific biosecurity measures. Firstly, it is essential to conduct a visual inspection and avoid purchasing animals that appear ill. Before buying, it is necessary to consult with a veterinarian regarding the required vaccinations and treatments for new animals. All purchased or returned animals from the market should undergo a quarantine period. Additionally, it is necessary to plan in advance the number of animals to be purchased to avoid unforeseen situations, and to thoroughly clean and disinfect transport vehicles before and after transporting animals.

Livestock markets should also provide appropriate conditions to prevent the spread of

pathogens. This includes adequate infrastructure for animal housing, ventilation, lighting, and feeding, systems for waste removal and disposal, and facilities for maintaining hygiene for both workers and visitors. It is also important to have animal identification systems to easily track their movements and ensure the presence of official veterinary control to check the health status of animals before they are brought to the market. Particular attention should be paid to an isolation zone where animals requiring veterinary examination can be segregated (Avalos *et al.,* 2022).

In conclusion, livestock markets serve as significant hubs for the dissemination of pathogens; therefore, implementing effective biosecurity measures is crucial to preventing disease outbreaks. Adherence to appropriate biosecurity measures by both visitors and market operators will help reduce the risks of introducing and spreading pathogens, ensuring animal health and enhancing livestock productivity.

Biosafety assessment and development of a biosafety plan

Evaluating the level of biosecurity is a crucial step in identifying potential risks and developing effective measures to protect farms from threats while minimising their impact on animal health and productivity. A. Kucuk & Y. Yildirim (2024) argued that the first step in developing a realistic and effective biosecurity plan is to assess the existing biosecurity measures on the farm. According to W.R. Ashmawy *et al.* (2022), biosecurity assessment enables the identification of the strengths and weaknesses of current measures, allowing for recommendations for their improvement. This requires gathering accurate data on the biosecurity measures that have already been implemented. The assessment should be structured to collect all necessary information regarding the current biosecurity practices. The most common method for conducting the assessment is through surveys, which evaluate how risks are prevented or mitigated on the farm. It is essential to carry out the assessment directly on the farm rather than relying solely on interviews.

It is important to note that obtaining accurate responses from a farm can be challenging. Often, what is stated to be done differs significantly from what is actually being practised. This is why, as B. Benavides *et al.* (2021) pointed out, it is necessary to carefully plan the assessment structure to obtain a realistic picture of the biosecurity measures implemented. Conducting a biosecurity assessment requires a specific approach to ensure accuracy and effectiveness. It is important to listen attentively and not to perceive the assessment process as an audit but as an opportunity for improvement. To obtain complete information, it is advisable to use open-ended questions that allow farmers to explain in detail how biosecurity measures are implemented. The assessment procedure requires sufficient time to avoid hasty conclusions. Additionally, as M. Leite *et al.* (2023) noted, that visiting different parts of the farm is necessary to observe how biosecurity measures are implemented in practice on-site.

Following the completion of the biosecurity assessment, the next step is to develop a plan that should cover all possible routes of pathogen entry into the farm and their subsequent spread. When developing the plan, it is essential to carefully assess the areas that require improvement, based on the level of risk of pathogen transmission. This also includes implementing effective biosecurity measures that address the identified threats. It is important to consider the sociological and psychological factors that may influence the implementation of biosecurity measures. In addition, it is necessary to carefully discuss the advantages and disadvantages of the proposed measures. It is important to anticipate systematic monitoring,

regular review, and adaptation of the plan to ensure its long-term effectiveness. Furthermore, it is necessary to immediately identify the most important measures for improvement, taking into account the limited resources of the farms. The plan should be developed in partnership with the farms to ensure the acceptability, feasibility, and sustainability of the proposed changes.

Implementing the changes outlined in the biosecurity plan is a critical step. It is important to periodically assess and monitor the implemented measures to improve them. Farms and their workers must understand and be convinced of the necessity and benefits of these measures. As V. Sri Lestari *et al.* (2022) noted, the implementation of biosecurity measures can face various barriers, such as a lack of infrastructure or socio-economic factors. It is important to focus on measures that are feasible and sustainable over time.

Veterinary professionals play a key role in promoting biosecurity plans on farms. The use of good communication skills is essential to influence the behaviour of farm workers regarding biosecurity. In conclusion, it should be noted that developing an effective biosecurity plan requires a detailed assessment of existing practices, taking into account the specific risks for each farm, and cooperation with them. The continuous implementation and maintenance of biosecurity measures are key to protecting animal health and improving farm productivity.

Conclusions

Biosecurity is an integral part of modern animal husbandry, contributing to the preservation of animal, human, and environmental health. Implementing biosecurity principles prevents the spread of infectious diseases, reduces the use of antimicrobial drugs, and helps to reduce the risk of developing antimicrobial resistance. This article examines the key aspects of biosecurity, its importance, and the main principles that can be applied on farms to improve animal health and productivity. Biosecurity is a vital tool in modern animal husbandry, ensuring not only animal health but also the protection of human health. This is achieved through the implementation of preventive measures that reduce the risk of pathogen spread. Preventive veterinary medicine is based on the principle of disease prevention by reducing risks and implementing healthy practices. This includes not only treating sick animals but also providing advice to farms on improving the health and productivity of their animals. The growing population, urbanisation, and climate change create additional challenges for biosecurity. Effective measures that are implemented must take into account these changes and adapt to new conditions. The “One Health” concept emphasises the interconnectedness of human, animal, and environmental health. It mobilises diverse sectors and disciplines to work together to ensure well-being and combat threats to health and ecosystems. The main principles of biosecurity include segregation, cleaning, and disinfection. It is also important to consider the behaviour and attitudes of people working with animals. A combination of different biosecurity methods increases the effectiveness of measures. Developing an effective biosecurity plan requires a detailed assessment of existing practices and the specific risks for each farm. Cooperation with farmers is key to ensuring the acceptability and sustainability of proposed changes. The regular development of educational programs and training for farmers and farm workers is critical to ensure understanding and effective implementation of biosecurity measures. Investments in infrastructure, such as isolation zones, cleaning, and disinfection systems, will help reduce the risks of pathogen spread. Continuous monitoring and adaptation

of biosecurity measures to changing conditions will help maintain their effectiveness. Providing financial support to farms for implementing biosecurity measures is a crucial step in ensuring their sustainability and efficiency. Overall, implementing effective biosecurity measures is an integral part of sustainable livestock production. Considering global challenges such as climate change and population growth requires a comprehensive approach to biosecurity. Cooperation between farms, veterinarians, and scientists, as well as financial support and educational programs, will help ensure animal health and increase the productivity of livestock farms, creating strong mutually beneficial relationships between all stakeholders.

Future research will focus on developing effective biosecurity plans as preventative measures against the emergence of infectious diseases, to increase livestock productivity.

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Conflict of Interest

The authors of this article declare that there are no commercial or financial conflicts of interest that could influence the objectivity of the research or the interpretation of its findings. All data used in this article were obtained from open sources, and the analysis was based solely on scientific principles, without any influence from external organisations.

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Ключові аспекти біобезпеки у сучасному тваринництві

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Анотація. Актуальність дослідження зумовлена необхідністю покращення біобезпеки у тваринництві для покращення здоров'я та продуктивності худоби, а також захисту здоров'я людей. Біобезпека є ключовим елементом, що запобігає поширенню інфекційних захворювань, сприяє зниженню ризиків епідемій, зменшенню витрат на лікування й підвищенню продуктивності і економічної ефективності сільськогосподарських підприємств, сприяючи покращенню якості продукції та зміцненню продовольчої безпеки, що підвищує конкурентоспроможність господарств на ринку. У зв'язку з цим, мета цього дослідження спрямована на розкриття ролі біобезпеки у превентивній ветеринарній медицині. Провідним підходом до дослідження цієї проблеми є аналіз існуючих практик та методів, що дозволяє комплексно розглянути різні аспекти біобезпеки. В результаті проведеного аналізу обґрунтовано, що превентивна ветеринарна медицина базується на принципі попередження хвороб через зниження ризиків та впровадження здорових практик. З'ясовано, що цей підхід включає не лише лікування хворих тварин, але й надання консультацій фермерам щодо покращення здоров'я та продуктивності їхніх тварин. Встановлено, що біобезпека також допомагає запобігати виникненню і поширенню інфекційних захворювань, зменшуючи необхідність у використанні антимікробних препаратів та знижуючи ризик розвитку антимікробної резистентності. Дослідженням підкреслено, що в сучасному світі, що швидко змінюється, біобезпека стає ще більш важливою через зростання населення, урбанізацію, зміни клімату та загрози для здоров'я людини, такі як антимікробна резистентність. Охоплено концепцію «Єдиного здоров'я», яка підкреслила тісний зв'язок і взаємозалежність здоров'я людей, свійських та диких тварин, рослин і довкілля. Основні принципи біобезпеки включають сегрегацію, очищення та дезінфекцію, а також врахування поведінки та ставлення людей, що працюють із тваринами. Матеріали статті становлять практичну цінність для ветеринарних лікарів, фермерів та тваринницьких господарств, допомагаючи їм розробляти та впроваджувати ефективні біобезпекові плани, адаптовані до змінних умов, що сприяє зменшенню ризиків інфекційних захворювань та підвищенню продуктивності тваринництва

Ключові слова: превентивна ветеринарна медицина; інфекційні захворювання; антимікробна резистентність; очищення та дезінфекція; захист здоров'я людей; зміни клімату

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Modern methods of raw meat processing to reduce microbial contamination

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Abstract. There are various methods to preserve the quality and extend the expiration date of food products. Raw meat from different animal species is a highly perishable product. The relevance of this research is driven by the need to summarise contemporary knowledge on approaches and

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techniques aimed at extending the storage duration of safe and high-quality meat, alongside their scientific justification. This article focused on finding means to reduce bacterial contamination, as microorganisms are the primary cause of meat spoilage. This study aimed to identify the most promising and optimal methods for extending the freshness of raw meat. To conduct the analysis, information from scientific primary sources, and electronic resources was used, and the results of experimental data on the application of scientifically based safe approaches were studied. A review of the main methods and measures aimed at extending the storage period of raw meat was carried out. The advantages and disadvantages of various methods of raw meat processing that have a bactericidal or bacteriostatic effect on various, including pathogenic, microorganisms that not only cause meat spoilage but can also be a danger to the potential consumer were established. Various traditional, novel, and innovative methods are currently employed in meat processing. Given the advancements in science and technology, a comprehensive approach to raw meat processing is emerging as the most promising. After analysing the research findings of numerous scientists, it has been concluded that biological technologies utilising lactic acid bacteria, combined with traditional physical methods such as refrigeration, offer the most optimal solution. This approach preserves the freshness of meat while minimising the impact on organoleptic properties, provided that temperature regimes and processing technologies involving cultures of lactic acid microorganisms are adhered to. The practical significance of this study lies in identifying the most promising directions for further research and improving existing safe antimicrobial treatments, particularly through the use of various strains of lactic acid starters. Their practical application will enable the preservation of meat freshness and extend its storage life

Keywords: slaughter products; lactic acid bacteria; disinfection with antimicrobial additives; quality and safety control; antimicrobial chemical substances

Introduction

Producing safe, high-quality food products is one of the primary and most important tasks of the agro-industrial sector in Ukraine and globally. Today, the issue of food safety is critically pressing, and there is a need to further research the justification and application of modern approaches used by the European Union. Modern retail trends and developments, driven by the demands of the food industry, are focused on extending the storage life of meat. Slaughter facilities play a pivotal role in supplying the population with high-quality, fresh, and safe meat products. This is achieved through the use of appropriate equipment, stunning methods, hygienic primary processing, and the implementation of specific measures designed to minimise bacterial contamination of half-carcasses and facilitate the subsequent storage of raw meat.

F. Leroy *et al.* (2023) highlighted in their study that raw meat is a complex, biologically active product undergoing numerous physicochemical and biochemical processes. Simultaneously, it is a valuable food source, rich in essential amino acids, fatty acids, and various bioactive compounds with health-promoting properties (e.g., taurine, carnosine), as well as easily absorbable micronutrients, often providing adequate nutrition for a wide range of consumers. According to European legislation, meat refers to muscle tissue and associated tissues obtained from the carcasses of domestic ungulates, including pigs, cattle, sheep, goats,

domestic one-hoofed, as well as poultry, lagomorphs, and wild game animals (Regulation EC No. 853/2004, 2004).

Ukrainian researchers have investigated a promising method of treating meat with lactic acid bacteria cultures. They have demonstrated a reduction in microbial contamination of meat, allowing for an extended shelf life of pork half-carcasses under refrigeration (Vovkotrub *et al.,* 2023).

Meat quality refers to the degree of properties that characterise its nutritional, biological, and energy value, as well as its organoleptic, physico-chemical, biochemical, and other attributes that meet consumer needs. While meat quality can be assessed using various methods, safety and sensory attributes are paramount for consumers. These components are influenced by a range of internal and external factors, including animal genetics, production systems, feeding, pre-slaughter preparation, stress during slaughter, and post-slaughter carcass handling. Moreover, M. Gagaoua *et al.* (2022) and V.Z. Trokhymenko *et al.* (2023) emphasised the importance of the economic aspect of food production, which is ensured by rational primary processing methods and the subsequent utilisation of raw materials.

V. Kurcubic *et al.* (2022) highlighted that meat processing facilities employ a significant number of technologies aimed at enhancing the quality of the final product. Particular attention is paid to the method and speed of animal slaughter, as well as subsequent carcass chilling and storage. Furthermore, there has been a growing demand in recent years for safe and high-quality meat that contains only natural ingredients to extend its shelf life. Products should include natural food preservatives, such as organic acids and antimicrobial agents, which do not have adverse effects on human health.

Despite significant scientific advancements, there remains a need for more detailed research and in-depth study of the impact of primary processing of carcasses from livestock and poultry on the safety, quality, and marketability of meat. This research aimed to review modern methods and analyse the most promising approaches used to extend the shelf life of raw meat and to formulate recommendations for their practical implementation in production. The literature search utilised scientific databases such as Scopus, Web of Science, and other electronic resources, which provided information on meat processing methods, references to legal frameworks, and food legislation. Both Ukrainian and international research data were consulted. A thorough analysis of existing findings and studies in this field was conducted, followed by the systematisation of the available information. Traditional as well as new and innovative processing methods were examined. Given the importance of ensuring safe food products and the development of modern technologies, preference was given to the most up-to-date sources of information.

Cooling and freezing of farm animal carcasses

Cooling and freezing of agricultural animal carcasses are the most common traditional methods of meat preservation. Cooling extends the shelf life of meat by slowing down the growth of microorganisms and the chemical reactions that lead to spoilage. Another technique, freezing, primarily employs temperatures of -8ºC and below.

H.E. Rojas *et al.* (2022) pointed out that, as global trade expands and the distance between producer and consumer increases, the demand for cooled and frozen meat is rising. This is due to the need to satisfy consumer expectations for longer product freshness, while also facilitating logistics and enhancing marketing. In recent years, research on the cooling of pork half-carcasses has focused on accelerated

cooling to minimise microbial contamination and weight loss through evaporation. Additionally, accelerated cooling can also improve the physicochemical and biochemical properties of meat by reducing the post-slaughter metabolic rate.

Pork displaying signs of PSE (pale, soft, exudative) is frequently encountered. Typically, such animals have been raised using intensive feeding practices and restricted movement during their confinement. PSE characteristics are also observed in stress-sensitive animals, which can be caused by improperly conducted stunning, prolonged transportation, exposure to high temperatures, lack of oxygen, and so forth. A delay in the onset of cooling and insufficiently low cooling temperatures contribute to a decrease in pH levels. This defect is particularly pronounced in light-coloured muscle fibres. The meat produced from these animals has lower technological and commercial value. During freezing and thawing, a significant amount of fluid is released, and substantial mass losses occur during storage, boiling, or frying; moreover, when salted, the meat retains its pale colour. It is important to note that the light colour of the meat is attributed to a sharp decrease in pH levels following slaughter, facilitated by enzymes that more rapidly break down glycogen into lactic acid. This results in earlier post-mortem rigour, while the temperature of the carcass remains high, promoting the rapid denaturation of muscle proteins due to the action of proteases (Trevisan & Brum, 2020). Consequently, as noted by A. Rybarczyk *et al.* (2015), the meat assumes a lighter colour.

The DCB (dark cutting beef) defect primarily occurs in steers and rarely in heifers. The discolouration arises from prolonged pre-slaughter stress and the depletion of glycogen reserves. Additionally, a stress factor can lead to another meat defect, characterised by the presence of DFD (dark, firm, dry) attributes, most commonly observed in the carcasses of cattle and sheep, and less frequently in pigs and turkeys. This defect occurs when, as a result of stress, strenuous physical exertion, fatigue, or exhaustion, the glycogen content in muscle tissue decreases before slaughter, leading to an increase in pH due to reduced post-mortem glycolysis and resulting in rapid post-mortem rigour. H.E. Rojas *et al.* (2022) noted that the pH of such meat is typically greater than 6.2. These characteristics indicate that the carcass originated from an animal that experienced pre-slaughter stress, possibly due to injury or illness. Such meat has high water-binding capacity, a dark red colour, coarse fibrousness, a tough texture, increased stickiness, and poor storage stability.

Consequently, in animals that have experienced acute and short-term stress, the carcass may exhibit PSE characteristics due to protein denaturation, which contributes to a decrease in water-holding capacity, displacement of intracellular water and myoglobin into interfibrillar spaces, and softening of the muscle structure. If the animal has undergone prolonged pre-slaughter stress, the pH value increases as a result of low energy reserves in the muscles, the muscle tissue structure becomes denser and firmer, and the meat acquires DFD characteristics.

Therefore, the post-mortem changes that occur during the process of transforming the muscles of slaughtered animals into meat play a crucial role in the development of quality attributes and the overall perception of the fresh product. S.K. Matarneh *et al.* (2017) emphasised that after an animal is slaughtered, the supply of oxygen ceases, and glycogen with phosphate compounds is mobilised to maintain homeostasis through the synthesis of adenosine triphosphate. As a result of lactate formation, the pH level decreases. Subsequently, post-mortem rigour develops. With the inactivation of enzymes, metabolism ceases, and ATP, which provides the elasticity of muscle fibres, is depleted. This is

followed by the proteolytic breakdown of structural proteins in the muscles, resulting in an enhancement of the meat's flavour properties.

H.E. Rojas *et al.* (2022) noted that the cooling process does not improve meat quality but merely maintains it, and therefore the meat must be of a satisfactory sanitary and hygienic standard. A safe cooling temperature is considered to be 4°C or below to inhibit the growth of microorganisms. This method is one of the most common in industrial settings. The biggest drawback of this method is the weight loss of the carcass due to the evaporation of water from the meat surface, which depends on the rate of temperature decrease. Consequently, when the cooling rate is lower, the weight loss increases.

Freezing is also a widely used method for preserving meat from various types of animals. This method is economically significant for the industry as it facilitates the trade of meat and logistical connections through extended storage periods. The primary objective of freezing is to maximise the retention of the meat's original properties, which requires careful control over the reduction of temperature and the crystallisation of liquids during the storage of carcasses in a frozen state, as well as during their thawing process. When meat is frozen, most of the water solidifies and forms ice crystals, which is accompanied by the separation of dissolved solids. Rapid freezing results in the formation of small ice crystals that generally remain within the muscle tissue, thereby preserving its microstructure. During the thawing process, the liquid is released and bound to proteins. If freezing occurs quickly while thawing is slow, moisture loss is minimised. However, during the storage of meat in a frozen state, ice crystals grow and damage muscle cells, which, in turn, leads to the release of enzymes that contribute to the oxidation of proteins and lipids. This process can, as noted by V. Lebret & M. Candek-Potokar (2022), lead to a deterioration of flavour, changes in colour, and alterations in texture.

Rapidly reducing the temperature of muscles before they reach the appropriate level of acidification can lead to carcass shrinkage (weight loss). Shrinkage is particularly evident in carcasses from animals with lower levels of subcutaneous fat, which facilitates faster heat loss. Shock cooling also contributes to the freezing of the skin and muscles located directly beneath the skin. During thawing, this leads to the leaching of adjacent tissue and undesirable meat colour. In studies by A. Rybarczyk *et al.* (2015), it was shown that shock cooling of meat had a negative impact on organoleptic characteristics such as tenderness and flavour. However, the results of their study did not reveal any significant differences between the effects of shock cooling and conventional cooling systems on meat quality between pig exterior classes.

The well-known Rinse&Chill (RCT) technology involves washing the vascular system of various types of animals after slaughter using a chilled isotonic solution at a temperature of +3°C. This solution contains 98.5% water along with a mixture of dextrose, maltose, and sodium phosphate (Hwang *et al.,* 2022). Additionally, an isotonic solution at +14°C is used, which comprises 98.5% water and a mixture of glucose, polyphosphates, and maltose (Fowler *et al.,* 2017).

According to research by S.V. Erazo-Castrejon *et al.* (2019), which determined the haemoglobin content in muscle extracts as a measure of blood removal, the application of the RCT method ensures the removal of 40% more blood from the muscles compared to conventional methods, which leave approximately 60% of blood in the muscles of pigs. This technology effectively removes residual blood from the carcass, rapidly lowers the internal temperature of the muscles, and optimises the reduction of pH by delivering glycolytic substrates in the wash solution.

Research on the Rinse&Chill (RCT) technology has shown that early post-mortem washing of the blood vessels in beef carcasses positively impacts the quality and safety of the meat, as well as its shelf life and the safety of meat products (Hwang *et al.,* 2022). This procedure facilitates the washing away of residual blood from both beef and pork carcasses. The use of pre-chilled isotonic solutions for intact animal carcasses ensures a rapid reduction in the internal temperature of the muscles and accelerates the cooling of the entire carcass. This technology, in particular, contributes to a decrease in pH and ensures effective cooling of the carcass, thereby improving the quality and safety of the meat. The isotonic RCT solution contains substrates that permeate the muscles, which remain physiologically active during the early post-mortem vascular washing. As a result, they are fully metabolised by the muscles and leave no residues. This technology has been commercially approved and has been in continuous use in the United States since 2000 and in Australia since 1997. As of January 2022, 23 meat processing enterprises in five countries (Australia, the USA, Canada, New Zealand, and Japan) have implemented this technology, all of which have assessed and approved the RCT. K. Hwang *et al.* (2022) noted that these enterprises operate under standard sanitation operating procedures (SSOP) and a Hazard Analysis Critical Control Point (HACCP) system.

Therefore, to effectively cool pork carcasses in meat processing plants, various methods are employed, often in combination with pre-slaughter preparation techniques and animal stunning methods.

Chemical technologies for meat processing

M. Lulietto *et al.* (2015) noted that meat is a unique nutrient-rich food but can pose a risk when contaminated with and reproduction of pathogenic microorganisms in it. To reduce microbial contamination of meat, particularly animal carcasses post-slaughter, chemical treatments are employed. The European Food Safety Authority (EFSA) has issued guidelines outlining the essential components and prerequisites that studies must include demonstrating that a substance intended for use to remove microbial surface contamination from food of animal origin will not pose any significant risk to consumer health and will contribute to a significant reduction in the prevalence and number of pathogenic bacteria, thereby reducing the microbiological risk. Given such regulations, an increasing number of new products are seeking to enter the European Union market. These are primarily foods produced using new technologies, derived from novel sources, or containing novel substances. Additionally, traditional foods consumed in non-EU countries and not consumed in the EU before 15 May 1997 are also considered "novel foods" according to Regulation (EU) No. 2015/2283, 2015. E. Ververis *et al.* (2020) emphasised that food business operators wishing to market such products in the European Union must obtain authorisation from the European Commission.

Scientific research conducted by Z.A. Ellatif *et al.* (2020) and Z. Kaplan *et al.* (2021) has demonstrated that a significant number of chemical components, including organic acids, have been developed and used to reduce contamination of slaughtered animal meat and extend its shelf life. At the same time, D.N.N. Madushanka *et al.* (2018) and O.B. Braiek & S. Smaoui (2021) noted that treatment with solutions of organic acids, such as lactic and acetic acid at concentrations of 1-2%, is also effective in reducing bacterial contamination, but higher concentrations of these acids cause the carcasses to whiten.

H. Van Ba *et al.* (2019) conducted a study using 3% acetic and lactic acid solutions to

reduce bacterial contamination on beef carcasses. Live animals were sprayed with the acid solution on their skin, and carcasses were sprayed immediately after slaughter. Identified microorganisms were identified through sequencing. Various microorganisms, such as *Staphylococcus, Shigella, Bacillus, Escherichia,* and *Salmonella*, were localised on the outer skin surface of live animals and the surface of their carcasses. Disinfectant sprays significantly reduced the number of all the aforementioned bacteria on the carcass skin surface compared to the control group without spraying. Thus, the spraying method can be recommended to reduce cross-contamination of beef carcasses without negatively affecting meat quality.

A similar study was conducted to detect and identify microbial populations on pork carcasses at various stages of slaughter and on pork cuts 24 hours post-slaughter, as well as to evaluate the effectiveness of sprays containing different concentrations (2% and 4%) of lactic acid. The spray was applied to the carcass surface at the end of the slaughter line. Bacterial genus identification, including *Staphylococcus, Salmonella, Shigella*, *Enterococcus, Escherichia, Acinetobacter* and *Corynebacterium* spp., was carried out. Their counts ranged from 2.70 to 4.91 log10 CFU/100 cm^2 on carcasses during slaughter. Most of these genera were also found on carcasses after 24-hour cooling. *Staphylococcus, Acinetobacter* and *Corynebacterium* were identified on cuts from untreated carcasses but were absent on treated ones. The bacterial count significantly decreased on carcasses and cuts treated with a lactic acid solution, particularly at the 4% concentration. Thus, according to H. Van Ba *et al.* (2019), spraying pig carcasses with a 4% lactic acid solution could be an effective method for reducing bacterial contamination, and enhancing the microbiological safety of pork.

In Brazil, the HACCP (Hazard Analysis and Critical Control Points) program was established by Circular No. 369 of June 2, 2003, issued by DCI/DIPOA (Control Division of International Trade/Department of Inspection Animal Products, Brazil). Common critical control points (CCPs) for slaughter are faeces, intestinal, and milk contamination of carcasses, with no tolerance limits for their presence. The same program recommends corrective actions for faecal contamination removal through trimming the contaminated part of the carcass and visual inspection. Under appropriate slaughter conditions, despite swine carcasses having satisfactory visual characteristics, they can be heavily contaminated, as their skin is a primary source of contamination. S. Bonardi (2017) noted that the stress factor associated with grouping, transportation, and holding animals at the slaughterhouse can provoke the excretion of *Salmonella* and other pathogens in latent carriers. This highlights the importance of adhering to good slaughter hygiene practices.

The study found that washing pig carcasses with water at a pressure of 8 bars can replace the method of cutting away contaminated parts without significantly affecting microbial contamination, while also reducing the risk of cross-contamination. J. Brustolin *et al.* (2014) found a decrease in the number of psychrophilic and mesophilic microorganisms on the surface of pig carcasses that were treated with steam and then irrigated with a 2% lactic acid solution.

For the treatment of pig carcasses, V. Silano *et al.* (2018) conducted a study using a 2-5% lactic acid solution in the form of an aerosol at temperatures up to 80°C. In this process, meat cuts pre-chilled by cold water showering were immersed in solutions at temperatures up to 40°C, with an optimal exposure time of up to 30 seconds. It was noted that exceeding the established concentration could, at least temporarily, change the appearance of meat cuts. The treatment of carcasses with organic acid solutions was allowed at any stage: both after

bleeding the carcasses and immediately before packaging. Regarding the amount of solution, a sufficient amount to cover the entire surface of the carcass was used. Additional rinsing after treatment with lactic acid was not provided. On the contrary, the remaining organic acid suppressed the growth of microorganisms in case of repeated bacterial contamination.

Therefore, organic acids are the safest chemicals used to treat meat to extend the product's shelf life by killing or inhibiting the growth of pathogenic microorganisms. A. Morey *et al.* (2014) indicated that acids accumulate in the cytoplasm of microorganisms. This rapidly decreases the pH, disrupts optimal enzymatic activity, and negatively affects the synthesis of proteins, DNA, and RNA. Currently, lactic and acetic acids are the most widely used in the food industry. G.A.M. Rossi *et al.* (2023) highlighted the potential use of other acids, such as benzoic, ascorbic, citric, propionic, formic, succinic, and tartaric acids as food preservatives to increase the shelf life of meat.

M. Ciriaco *et al.* (2021) investigated the bacteriostatic effects of acetic and citric acids on *Salmonella*. To treat pig carcasses, 2% and 5% solutions of these acids were used. Both acids at the specified concentrations exhibited bacteriostatic activity. However, better results were obtained when treating pig carcasses with 5% lactic acid. In another study, S. Dan *et al.* (2017) investigated the effects of lactic, acetic, and citric acids at various concentrations (1%, 2%, 3%) on bacterial strains of *Salmonella enteritidis, Escherichia coli* and *Listeria monocytogenes* when treating beef. The researchers noted that among these organic acids, a 3% solution of lactic acid was the most effective, while acetic and citric acids showed slightly lower effectiveness. In the food industry, malic and tartaric acids are also allowed to be used, but these acids are less effective when treating carcasses.

It is important to remember that the effectiveness of carcass surface treatment depends not only on the choice of a specific organic acid but also on the water pressure, its temperature, and the duration of the treatment. J.H. Park *et al.* (2005) recommended using low-pressure hot water washing or immersion, as well as high-pressure spraying, with an average treatment time of 10 to 20 seconds. Thus, there is currently a wide range of antimicrobial measures, such as carcass washing, treatment with organic acids, trimming, and deburring with a knife, etc. However, intestinal pathogens, especially *Escherichia coli* and *Salmonella*, remain a major problem for the safety of meat products, including beef. Currently, none of the methods is sufficiently effective for these pathogens when treating carcasses. C. Kocharunchitt *et al.* (2019) noted that during the study of antimicrobial agents, chlorine dioxide and peracetic acid showed good results when treating beef carcasses by spraying during cooling.

Chlorine is the most popular and widely used antimicrobial agent in poultry processing in the USA but is not permitted for use in the European Union (EU). M. Sohaib *et al.* (2016) noted that adding 18-25 mg/L to cold water could significantly reduce *Salmonella* counts. The effectiveness of chlorine increases proportionally with its concentration in the disinfectant solution. It should be noted that higher concentrations can affect the carcass colour and also contribute to the development of a specific odour.

The disinfection of animal carcasses with chlorine also has its advantages and disadvantages. Chlorine is widely used for the disinfection of animal products, particularly poultry. It is a relatively inexpensive chemical compound that is easy to apply to carcasses. It has high antimicrobial activity against both gram-positive and gram-negative microorganisms. The mechanism of action of chlorine involves the destruction of the bacterial cell wall due to its

high oxidative potential. As noted by S. Sheen *et al.* (2011), chlorine solutions are effective even at low concentrations. M. Kumari & S.K. Gupta (2022) expressed concerns about the limitations of using chlorine, which is neutralised by organic matter, and recommended using it only after skinning to prevent neutralisation by organic matter on the carcass surface. In addition, chlorine is a toxic substance, its concentration must be carefully controlled, and when reacting with organic matter, it can form carcinogenic compounds – trihalomethanes, which pose a serious threat to human health.

It is important to note that the use of various chemicals, including chlorine, depends on geographic location and the subsequent marketing of finished products. K. Chousalkar *et al.* (2019) noted that in Australia and many Asian countries, chlorine remains the most common disinfectant for poultry meat. Compared to other disinfectants, it is cheaper and easier to use. A.R. McWhorter *et al.* (2023) indicated that Australia adheres to recommendations for the safe use of chlorine in the food industry, particularly the meat industry, where the concentration of this compound is not allowed to exceed 10 mg/L (ppm). In the USA, regulations allow a higher concentration – up to 20 mg/L for washing or spraying poultry carcasses, and up to 50 mg/L in cooling tanks.

S S.M.E. Rahman *et al.* (2013) noted that chemical treatment also includes ozone treatment, which is a water-soluble gas of natural origin that is a strong oxidant. This gas can attack the cell membrane of bacterial cells and also has antiviral effects. However, ozone also accelerates the oxidative spoilage of fats and affects muscle tissue, causing discolouration. Therefore, ozone is mainly used to treat empty freezers for meat storage.

Thus, the significant number of antimicrobial chemical agents designed to reduce contamination of animal carcasses after slaughter indicate their effectiveness but have several drawbacks, including deterioration of the commercial appearance of meat, toxicity to humans, and geographical restrictions. The search for safe and effective agents capable of ensuring long-term storage of meat and meat products continues.

Use of biological approaches to extend the shelf life of meat

In the food industry, the use of biological methods to extend the shelf life of raw meat is considered promising. Two main categories of biological agents have been used recently: starter cultures and protective cultures. Starter cultures are a type of microorganism used to initiate fermentation and produce specific chemicals that give fermented products their characteristic texture and flavour. V.P. Singh (2018) noted that protective probiotics are primarily used to manage antibacterial properties and minimise the persistence and growth of harmful microorganisms in food. Due to their non-toxic, non-immunogenic characteristics, thermal stability, and broad bactericidal activity, lactic acid bacteria serve as excellent biopreservatives. In addition to lactic acid bacteria, bacteriocins, bacteriophages, and bacteriophage-encoded enzymes are also used to ensure the quality and safety of food products.

Biological methods are gaining popularity due to their ecological approach. T. Bintsis (2018) established the safety of lactic acid bacteria (*LAB*) for food products, specifically *Lactococcus, Lactobacillus, Pediococcus, Leuconostoc, Streptococcus,* and *Enterococcus*, which can be added as starters to meat products. E.E. Imade *et al.* (2021) indicated that these microbial groups can inhibit the growth and activity of bacteria that spoil meat, providing an alternative to the use of chemical compounds. As the demand for high-quality food products free from synthetic chemicals increases –

driven by changes in lifestyle and consumer eating habits – lactic acid bacteria are increasingly utilised to extend the shelf life of fermented products. Their antimicrobial activity is attributed to metabolites such as lactic acid, hydrogen peroxide, and bacteriocins. S. Kaveh *et al.* (2022) demonstrated that *LAB* can inhibit pathogenic bacteria during the processing of raw meat and meat products. However, there are limitations to their use related to regulatory frameworks, the technology for applying different types of lactic acid microorganisms, and the need to consider factors such as storage time, temperature, and pH levels. Improper application can lead to spoilage of meat. The positive influence of *Lactobacillus sakei* on the cooling pork half-carcasses has been established. For instance, V. Vovkotrub *et al.* (2023) observed the preservation of transverse and longitudinal striations of muscle fibres during microscopic examination on the fourth day of storage, in contrast to untreated pork half-carcasses.

D.F. Benitez-Chao *et al.* (2021) and D. Bhattacharya *et al.* (2022) noted that bacteriocins are antimicrobial peptides produced by various bacterial strains. They can be added to raw or cooked meat to inhibit pathogenic microflora. The research demonstrated the ability of bacteriocins to suppress pathogenic microorganisms such as *E. coli, Salmonella,* and *Listeria*, highlighting the relevance and potential of these agents. The only commercially available bacteriocin is nisin, which is permitted for use in the food industry, particularly for the processing of meat and meat products. Y.B. DeMartinez *et al.* (2002) applied nisin and a spray of lactic acid (1.5%, at 25°C) to poultry and bovine carcasses to reduce the number of aerobic bacteria, *E. coli*, or coliforms. The use of nisin alone was found to have no effect on most gram-negative bacteria, including pathogenic strains. Combinations of various bacteriocins have improved the inhibition of foodborne pathogens. For example, sakacin G and sakacin P, produced by *L. sakei* CWBI-B1365 and *L. curvatus* CWBI-B28, respectively, showed positive results, with a noted suppression of the growth of *L. monocytogenes* in poultry and beef (Dortu *et al.,* 2008). A. Radaic *et al.* (2020) indicated that the semi-purified bacteriocin BacTN635, produced by *L. Plantarum* sp. TN635 and isolated from meat reduced the proliferation of *Listeria* and other spoilage microorganisms in beef and chicken breast under refrigeration conditions.

Bacteriophages are viruses that specifically infect bacteria and are generally considered harmless to humans, animals, and plants. Due to their strict host specificity, bacteriophages can infect and lyse only certain bacteria, or even only specific strains within a species. Bacteriophages do not have a negative impact on the environment. Numerous studies and examples have shown that bacteriophages can be effectively used to control pathogenic bacteria in food products. A. Ishaq *et al.* (2020) demonstrated that in a study on the effect of the bacteriophage *List-Shield* on reducing the number of *L. monocytogenes* in contaminated beef samples, pathogen growth continued on the surface of raw beef at a refrigerated temperature of 4±1°C. However, over a 15-day storage period of beef samples treated with bacteriophages, a significant reduction in pathogen concentration was observed. Additionally, bacteriophages had no negative impact on the colour or pH of the stored beef samples. This indicated that they can be used as an innovative antibacterial strategy to slow down the growth of pathogenic bacteria on the surface of meat.

Thus, lactic acid bacteria play a crucial role in preserving and extending the shelf life of meat and meat products by producing bacteriocins, organic acids, and other metabolites. The use of these agents necessitates further research, particularly the development

of guidelines and regulations regarding their application as food additives in meat and meat products.

Innovative approaches to meat and meat product processing

Beyond traditional methods, novel and innovative technologies are employed to protect meat products from spoilage and extend their shelf life. As highlighted by W. Khalid *et al.* (2022), these include non-thermal processing technologies such as gamma irradiation, electron beam irradiation, high-pressure processing, and pulsed electric fields.

Irradiation is a process used to control foodborne pathogens, enhance the safety, and extend the shelf life of meat products by maintaining stable nutrient content during storage. This method is also considered an effective way to control microbial growth and eliminate microbiological hazards in meat and meat products. Currently, the irradiation of food products is regulated by national and international standards to ensure product safety. R. Indiarto *et al.* (2023) emphasise the need for labelling such products as "irradiated" and indicating the irradiation dose. In the United States, the Food Safety and Inspection Service (FSIS) of the USDA oversees the safety and labelling of meat, including the inspection and testing of irradiated products. T. Wahyono *et al.* (2024) pointed out the importance of adhering to the irradiation dose as it can have a negative impact on meat quality if not properly controlled.

According to the World Health Organization (WHO), irradiation doses below 10 kGy are generally considered safe and do not adversely affect the quality or safety of food products (Khalid *et al.,* 2022). Ionising radiation, when applied to food, minimises or completely eliminates pathogenic microorganisms that cause spoilage. G.M. Khalafalla *et al.* (2018) demonstrated the effectiveness of irradiation in extending the shelf life of minced meat, particularly beef. A. Amiri *et al.* (2019) indicated that this method is lethal to pathogenic microorganisms such as *Salmonella* and *Escherichia coli*, allowing for a significant extension of food shelf life. Additionally, studies have reported minimal impact of irradiation on the sensory properties and nutritional value of food (Lima *et al.,* 2018). Research also suggests that irradiation can be combined with other chemical substances and technologies to enhance the effectiveness of meat decontamination.

Ultrasonic technology involves the use of sound waves with frequencies between 20 and 100 kHz to damage the cell membranes and DNA of microorganisms (Morild *et al.,* 2011). This technology is particularly suitable for sanitising poultry carcasses, as it typically involves immersing the food product in an ultrasonic bath. Ultrasonic waves are safe and non-toxic to humans. A study combining the effects of ultrasound and lactic acid demonstrated a reduction in the number of gram-negative microorganisms in poultry meat.

Cold plasma is an innovative non-thermal food processing technology that employs energetic chemically reactive gases to inactivate unwanted microorganisms on meat, poultry, fruits, and vegetables. This flexible method, which exerts both bactericidal and bacteriostatic effects on microorganisms, relies on the use of electrical energy and carrier gases such as oxygen, nitrogen, or helium, without the need for antimicrobial chemical agents. The primary mechanism of action is associated with ultraviolet radiation and reactive chemical products generated during the ionisation process in cold plasma. A wide range of cold plasma systems is being developed, operating either at atmospheric pressure or in low-pressure processing chambers. B.A. Niemira (2012) noted that the bactericidal activity of cold plasma can reduce the colony counts of microorganisms by more

than 5 logarithmic units for pathogens such as *Salmonella, Escherichiacoli O157:H7, Listeria monocytogenes* and *Staphylococcus aureus*. The effective processing time for food products using cold plasma can vary from 120 seconds to 3 seconds, depending on the type of food product and processing conditions.

P. Sammanee *et al.* (2022) and M. Gavahian & A.M. Khaneghah (2020) indicated that the method of immersion in cold liquid plasma can be employed to reduce the number of pathogenic microorganisms in pork and poultry meat with skin. This modern innovative technology holds potential for application in the meat industry during the slaughter of pigs and poultry. Liquid plasma contains ions, atoms, and molecules of oxygen, ozone, nitrates, and nitrites, and can generate reactive forms of oxygen and nitrogen that inactivate harmful microorganisms. Recent studies have demonstrated a reduction in the numbers of *S. enteritidis*, *S. typhimurium*, *E. coli* and *C. jejuni* on the surface of pork and poultry meat following 15 minutes of treatment with liquid plasma, evaluated on days 0, 3, 7, and 10. The effectiveness of reducing *S. Aureus* colonies in the meat was observed after a 3-day experiment. However, as noted by P. Sammanee *et al.* (2022), *P. aeruginosa* was not inactivated under the same experimental conditions. The results of the study established the efficacy of liquid plasma in reducing contamination by *Salmonella enteritidis, Salmonella typhimurium, E. coli*, *C. jejuni* and *S. aureus* on the surface of pork and poultry meat over a storage period of three days at temperatures between 4-6°C, with minimal organoleptic changes in the meat. During longer storage periods, a colour change was observed – pork samples took on a lighter hue, while less significant changes were noted in the poultry meat samples. A significant reduction in pH levels was found compared to the control group.

Innovative methods for meat processing include the application of pulsed electric fields (PEF). Despite the considerable number of scientific publications, this technology is still regarded as new in the food industry. The PEF technology is one of the promising non-thermal methods for preserving food products and can effectively manage biological hazards. Pulsed electric fields can be employed for the treatment of food items. K. Kopuk *et al.* (2020) noted that this method utilises short pulses of high voltage. V.R. Krishnamurthi *et al.* (2020) indicated that, under this method, the cell membranes of microorganisms are damaged due to the high voltage, leading to their death. While high voltage also generates heat, it does not adversely affect meat quality because of the extremely short exposure time. Some studies have reported contradictory findings regarding the use of pulsed electric fields for the decontamination of meat and meat products; therefore, further research is necessary to assess the potential of this method before its widespread application in the meat industry can commence.

Combined methods of processing meat and meat products

Currently, in addition to traditional methods such as cooling, pre-slaughter preparation, slaughter hygiene, and primary processing, various combined methods are employed to reduce the contamination of slaughter products by pathogenic microflora. Research findings indicate the individual and combined effects of organic acids, bacteriophages, and ultraviolet light on populations of *Salmonella* in beef mince contaminated with four strains of *Salmonella* bacteria, resulting in a contamination level of 3.5 log CFU/g after grinding. A.S. Danso *et al.* (2018) noted that the individual application of lactic and peracetic acids, or their combinations, did not result in a significant reduction in *Salmonella* numbers in beef mince. The

individual use of bacteriophages and ultraviolet light reduced their numbers by 1 log CFU/g of mince. The combined application of bacteriophages and ultraviolet light resulted in a reduction of *Salmonella* in the mince by 2 log CFU/g.

F. Soyer *et al.* (2020) demonstrated that the application of activated lactoferrin and rosemary extract to pork meat effectively reduced levels of *E. coli, Salmonella* and *Listeria* in *in vitro* studies. However, further research is needed to investigate the synergistic effects of plant extracts and essential oils, as well as to determine optimal dosing and application conditions.

Ultrasonic technology has shown promise for the decontamination of poultry carcasses, as it involves complete immersion in an ultrasonic bath (Sohaib *et al.,* 2016). When combined with lactic acid, ultrasound has been shown to reduce the number of gram-negative bacteria in poultry meat. R.K. Morild *et al.* (2011) proposed a combined thermal (steam) and ultrasonic decontamination method for pork. This method effectively reduces the overall number of microorganisms, including *Salmonella* and *E. coli*. The advantage of this method is its short processing time and reduced water consumption; effective treatment can be achieved within 1-2 seconds without noticeable changes in the appearance of the pork.

S.K. Sadaghiani *et al.* (2019) established that to achieve a synergistic effect, rather than using *LAB* or bacteriocins separately, they should be combined with any natural antimicrobial substance. The scientists conducted a study using *Limosilactobacillus reuteri* and *L. plantarum* separately, as well as with 1% garlic extract in raw beef, which was stored for 12 days at 4°C. When *LAB* strains and garlic extract were used together, they reduced *L. monocytogenes* contamination to 1.5 log CFU/g, indicating a better effect with the synergistic action of the preparations. Thus, the combined use of measures, in particular, two or more, gives a better effect than a single intervention after the primary processing of animals. For carcass decontamination, preparations against pathogenic microorganisms and physical methods are most often used, with low-temperature treatment being the most common.

A significant number of methods have been developed and are currently used for the decontamination of meat and meat products. However, these methods possess several drawbacks, including alterations to organoleptic characteristics, complex application techniques, and insufficient scientific justification, which hinder their widespread adoption in production. Consequently, while numerous antimicrobial agents designed to reduce contamination of animal carcasses after slaughter have demonstrated their effectiveness, they also have drawbacks related to the deterioration of meat's appearance and potential toxicity to humans. This necessitates the search for safe and effective agents that can ensure the long-term storage of meat and meat products.

Conclusions

Both in the European Union and globally, there remains a traditional demand for safe and high-quality meat, which is an essential component of human nutrition. The safety and quality of meat depend significantly on the conditions of animal husbandry, feeding, and care. In addition to these factors, important roles are played by the conditions of animal preparation for slaughter, transportation, pre-slaughter holding, stunning, bleeding, evisceration, splitting carcasses into halves, post-slaughter cleaning, and cooling of the meat. A primary factor contributing to microbial contamination of carcasses during cooling and storage is the unsatisfactory state of the relevant facilities, as well as violations of the technological regimes for refrigeration treatment, which create favourable conditions for

the growth and multiplication of microorganisms. A provisional classification of the main modern methods of meat processing aimed at reducing microbial contamination and extending its shelf life has been presented. These methods include physical techniques, the use of various chemical substances, biological technologies, and combined processing methods. Each technology has its own advantages and disadvantages. When selecting the optimal method to reduce bacterial contamination and prolong shelf life, it is essential to consider the chemical composition of the meat, the species of animal, the technological capabilities of the processing facility, the season, and other factors. The current approach, which takes into account the modern ecological state and the growing trend towards consuming organic products, underscores the importance of focusing on safe methods (without the use of toxic chemicals) and substances of natural origin for meat processing, aimed at improving quality and extending shelf life. It is known that most meat processing enterprises utilise organic acids to treat the surface of carcasses to reduce microbial contamination. However, these acids have several drawbacks, particularly their impact on organoleptic properties, necessitating the development of new methods for prolonging the shelf life of meat in a cooled state. Such methods may include the application of microbiological preparations, specifically special strains of lactic acid bacteria, which exhibit selective fermentation towards mono- and polysaccharides, do not adversely affect the organoleptic properties of meat, and display antagonism towards conditionally pathogenic and pathogenic microorganisms. Therefore, considering the research findings on the use of lactic acid bacteria and bacteriocins, and their detrimental effects on key pathogenic microorganisms for humans, including *E. coli, Salmonella* spp., and *Listeria monocytogenes*, it is essential to combine classical physical processing methods – such as temperature control, pressure application, and mechanical cleaning – with biological technologies to produce safe and high-quality raw meat. Thus, the utilisation of suspensions of lactic acid bacteria starters for the final treatment during the cooling of pork carcasses (or half-carcasses) in refrigeration chambers requires a detailed investigation into their impact on the quality and safety of pork, taking into account sensory properties and the overall appearance of the meat.

In the future, it is planned to investigate the effectiveness of using starter cultures of strains SafePro® B-SF-43 (*Leuconostoc carnosum*) and SafePro® B-2 (*Lactobacillus sakei*) as a final treatment for pork half-carcasses to achieve biopreservation and maintain freshness.

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Conflict of Interest

None.

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Сучасні методи обробки сирого м'яса для зменшення мікробної контамінації

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Анотація. Існують різні методи збереження якості та подовження терміну придатності харчових продуктів. Сире м'ясо різних видів тварин – продукт, що піддається швидкому псуванню. Актуальність дослідження зумовлена необхідністю узагальнення сучасних знань стосовно підходів і методів подовження термінів зберігання безпечного та якісного м'яса та їх наукового обґрунтування. Дана робота була сфокусована на пошуку засобів, які знижують бактеріальне забруднення, оскільки саме мікроорганізми є головною причиною псування м'яса. Метою роботи був пошук найбільш перспективних та оптимальних методів для подовження термінів зберігання свіжості сирого м'яса. Для аналізу використовували інформацію наукових першоджерел, електронні ресурси, вивчали результати експериментальних даних щодо застосування науково обґрунтованих безпечних підходів. Здійснено огляд основних методів та заходів, які спрямовані на подовження термінів зберігання сирого м'яса. Встановлено переваги та недоліки різних методів обробки сирого м'яса, що діють бактерицидно чи бактеріостатично на різні, зокрема, патогенні мікроорганізми, які викликають не лише псування м'яса, але й можуть бути

небезпечними факторами для потенційного споживача. Нині використовують традиційні, різноманітні нові та інноваційні методи. З урахуванням сучасних досягнень науки та технологій найперспективнішим є використання комплексних методів обробки сирого м'яса. Проаналізувавши результати досліджень багатьох науковців зроблено висновок, що найоптимальнішими є біологічні технології з використанням молочнокислих бактерій у поєднанні з традиційними методами фізичної обробки, а саме, охолодженням. Даний підхід зберігає свіжість м'яса та виявляє мінімальний вплив на органолептичні показники за дотримання температурних режимів та технології обробки культурами молочнокислих мікроорганізмів сирого м'яса. Прикладне значення цієї роботи полягає у встановленні найперспективніших напрямків для подальшого дослідження та вдосконалення наявних безпечних протимікробних препаратів, зокрема, використання різних штамів молочнокислих заквасок. Їх практичне застосування дасть можливість зберегти свіжість м'яса та подовжити його термін зберігання

Ключові слова: продукти забою; молочнокислі бактерії; знезараження протимікробними добавками; контроль якості і безпечності; протимікробні хімічні речовини

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Evaluation of the effectiveness of surgical treatment using sonographic diagnostics with pyometra in dogs

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Abstract. The relevance of this study lies in the necessity to evaluate the effectiveness of surgical treatment for pyometra in dogs, employing sonographic diagnosis. Pyometra, a common uterine infection in female dogs, can lead to life-threatening complications such as sepsis and kidney damage if left untreated. Ultrasound imaging, a non-invasive and effective diagnostic tool, plays a crucial role in the early detection of pyometra and can identify potential post-operative complications following ovariohysterectomy. This study aimed to identify the ultrasound characteristics of the abdominal cavity in dogs affected by pyometra and to compare the postoperative ultrasound features of the abdominal cavity following ovariohysterectomy over time in a group of animals with and without complications. The primary research method involved a sequential and targeted ultrasound examination of the abdominal cavity in dogs diagnosed with pyometra. Subsequent ultrasound examinations were performed during ovariohysterectomy and at 1, 5, and 10 days postoperatively. It was established that the pathologically altered uterus on ultrasound often exhibited characteristics of an enlarged, distended tubular structure with anechoic or hypoechoic content. Ultrasound examination of affected dogs also demonstrated diagnostic effectiveness in cases of visualising intrauterine fluid, even when the uterine size was within normal limits. The use of ultrasound diagnostics allowed for the timely detection of pyometra at early stages, significantly improving the effectiveness of subsequent treatment. Ovariohysterectomy proved to be the most effective treatment method for dogs with pyometra, promoting the normalisation of vital functions as early as 2-3 days post-surgery, which reduced the risk of recurrence. Dynamic ultrasound examination of the abdominal cavity after surgery ensured timely adjustment of treatment measures and improved prognosis for patients. The established facts have practical

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significance for veterinarians, which will contribute to improving the quality of pyometra diagnosis in dogs, the effectiveness of their treatment, and reducing mortality

Keywords: uterine pathology; ultrasound diagnosis; surgical intervention; postoperative period; intrauterine fluid; complications

Introduction

Pyometra is a common condition in adult female dogs, caused by an acute or chronic bacterial infection of the uterus. This pathology is characterised by the accumulation of inflammatory exudate within the uterine cavity and presents with various clinical signs. Pyometra most commonly occurs in females during the luteal phase of the cycle due to high levels of progesterone, which plays a key role in its development. The diagnosis is based on medical history, physical examination, laboratory tests, and results from radiography and/or ultrasound, which reveal an enlarged, fluid-filled uterus. Delayed diagnosis of pyometra, when the kidneys are already damaged, can lead to irreversible consequences for their function.

B. Gasser *et al*. (2020) demonstrated the potential for multiple organ dysfunction as a consequence of sepsis and endotoxemia, which can be life-threatening. This research highlighted the importance of early diagnosis of pyometra to prevent serious complications. In this context, ultrasound imaging emerges as an accurate method for diagnosing pyometra in dogs. Pathological changes in the uterus, such as endometrial hyperplasia, can lead to infertility in dogs. These conditions may be collectively referred to as "pyometra" or considered as a symptom complex of endometritis with the accumulation of purulent exudate in the uterine cavity. Pyometra in dogs remains one of the most common and serious reproductive pathologies. In recent years, numerous studies have been dedicated to improving diagnostic and treatment methods for this disease. Scientists have investigated clinical and ultrasound indicators for the early diagnosis of septic acute kidney injury in female dogs with pyometra, underscoring the importance of timely diagnosis.

S. Ahn *et al*. (2021) compared clinical and inflammatory parameters in dogs with pyometra before and after ovariohysterectomy, emphasising the importance of surgical intervention. Their research highlighted that surgery is the key treatment for pyometra, contributing to the normalisation of vital functions in dogs as early as 2-3 days postoperatively. P.R. Batista *et al*. (2022) evaluated the use of Doppler ultrasonography to assess females receiving medical treatment for pyometra, emphasising the effectiveness of this method. Doppler ultrasonography allowed for the detection of changes in uterine blood flow, which is an important indicator for determining the animal's condition and treatment effectiveness.

Ukrainian researchers have also made significant contributions to the study of pyometra. P.M. Sklyarov & V.V. Piatibrat (2021) reviewed the diagnostic aspects of pyometra in female dogs, providing an overview of current information on this topic. Their study includes a detailed analysis of diagnostic methods used in Ukraine and their comparison with international standards. D.V. Zarutska (2022) investigated the application of ultrasound for the diagnosis of pyometra in dogs, confirming its high diagnostic value. Her research highlighted that ultrasound diagnostics allows for the timely detection of pathological changes in the uterus

and the presence of intrauterine fluid, even in cases where uterine size is within normal limits. In recent studies, scientists have focused on various aspects of pyometra development. R. Hagman (2022) updated information on pyometra in small animals, including new approaches to diagnosis and treatment. His research showed that a comprehensive approach to the diagnosis and treatment of pyometra can significantly reduce mortality from this disease.

M.K. Nilsson *et al*. (2023) investigated the sonographic features of uncomplicated post-operative abdominal conditions in dogs treated for pyometra via ovariohysterectomy. Their study confirmed that ultrasound imaging is a valuable tool in monitoring the post-operative condition of patients. A.J.R. Peixoto *et al*. (2023) examined the impact of clinical presentation, the presence of systemic inflammatory response syndrome (SIRS), and organ dysfunction on mortality in female dogs with pyometra, highlighting the importance of a comprehensive approach to the treatment of this disease. Their research confirmed that timely diagnosis and adequate treatment significantly increase the chances of a successful recovery.

E. Porowska *et al.* (2018) argued that in the current context of veterinary medicine, it is important to implement new technologies and diagnostic methods that ensure high accuracy and timeliness in disease detection. The development of ultrasound diagnostics opens up new opportunities for veterinarians in diagnosing reproductive pathologies, such as pyometra, allowing for a significant improvement in the quality of treatment. The application of modern diagnostic and treatment methods not only increases the effectiveness of therapy but also reduces the risk of complications and mortality in animals. Thus, the study of the effectiveness of surgical treatment of pyometra using ultrasound diagnostics is a relevant and important area of research. Therefore, pyometra in female dogs is a serious disease that requires timely diagnosis and adequate treatment. Ultrasound diagnostics emerges as an important tool in the early detection and monitoring of this disease. Surgical treatment, supported by modern diagnostic methods, can significantly increase the chances of a successful recovery.

This study aimed to evaluate the effectiveness of surgical treatment of pyometra in dogs using sonographic diagnosis. The objectives of the study included the analysis of the effectiveness of ultrasound diagnosis in the early detection of pyometra, the assessment of clinical outcomes following surgery, and the comparison of the obtained data with previously described facts in the literature.

Literature Review

Pyometra accounts for over 87% of gynaecological pathologies in dogs. In 80% of cases in female dogs, this condition is caused by false pregnancy, chronic genital inflammation, and functional disorders such as uterine atony and hypotonia (Zarutska, 2022). Pyometra is a potentially life-threatening condition and can have fatal consequences due to toxin poisoning (endotoxemia), the development of peritonitis, and systemic sepsis. P.M. Sklyarov & V.V. Piatibrat (2021) found that in most cases, *Escherichia coli* is often the cause of purulent uterine inflammation.

According to R. Rautela & Katiyar (2019), the disease is not seasonal and can occur due to abnormal sexual cycles, missed matings, pathological births, medication effects, and other factors. Inflammatory processes in the uterus are often accompanied by mastitis, forming the symptom complex “endometritis-mastitis”. V. Sachan *et al.* (2019) established that cats with polycyclic cycles have an increased risk of pyometra. This disease can develop at different ages, even immediately after oestrus during the luteal phase. On average, pyometra is detected

in animals aged 5-6 years, but this pathology is recorded in females from 8 months of age and throughout life (13-16 years). The incidence increases with age, the older the animal, the more likely the pathology. In pyometra, conditions are created in the uterine cavity that adversely affect the survival, movement, and viability of sperm.

The primary cause of endometritis and pyometra is attributed to the influence of infection that enters during oestrus. Depending on the aggressiveness of microorganisms and the resistance of genital tissues, the symptoms of the disease can vary from obvious to hidden. Treating endometritis and pyometra is a complex task. L. Llazani *et al.* (2021) demonstrated that pyometra in dogs is one of the most common reproductive disorders found in female dogs, especially during dioestrus and the dominant progesterone phase of the oestrus cycle. This is an accumulation of exudate in the uterine lumen, which usually occurs during or after a cycle with high progesterone levels. Symptoms of this disorder include weakness, loss of appetite, increased thirst and urination, vomiting, and abnormal vaginal discharge.

Depending on the nature of the exudate in the uterine cavity, inflammatory processes can be classified as serous, catarrhal, purulent, fibrinous, or haemorrhagic. N.P. Ovcharuk & O.O. Kravchuk (2016) established that pyometra can manifest in either acute or chronic forms, with symptoms that may be clinically apparent or subclinical.

It has been demonstrated that the pathological changes in the uterine tissues are dependent on the type of pathogen and the immune response of the organism. In cases of serous and catarrhal endometrial inflammation, erythema of the swollen mucosa is observed, accompanied by the discharge of serous exudate. Haemorrhages of various types can be visualised within the uterine tissues. Ulcerations form on the surface of the endometrium following the detachment of necrotic tissues. Changes in the glandular part of the endometrium are characterised by infiltration, dilatation of the glands, thinning between them, filling the cavity with secretion, and the formation of cysts. The walls of the blood vessels undergo thickening, the lumen becomes narrower, and there is evidence of obliteration and calcification (Hagman, 2018).

A.J.R. Peixoto *et al.* (2023) found that pyometra is characterised by the discharge of brown mucus from the vaginal opening with admixtures of exudate. With an open cervical canal, exudate constantly flows out of the body, whereas with a closed canal, the exudate accumulates in the uterine cavity, leading to asymmetry of the uterine horns, their adhesion, and the detection of fluctuation during rectal examination. During oestrus, the exudate discharge increases. During mating, fertilisation does not occur, even with an intact sexual cycle.

With a prolonged course of these diseases, deep morphological changes in the uterine wall occur, leading to infertility in animals. If untreated, pyometra can be fatal. Weakened uterine contractility can be caused by various factors such as lack of exercise, poor nutrition, diseases of other organs, as well as pathological complications of pregnancy, including a large number of foetuses, foetal hydrops, retention of foetal membranes, metritis and endometritis, and the development of ovarian cysts. All of the above factors can eventually lead to pyometra.

In most cases, treatment of any form of pyometra typically involves surgery, as this method is more effective and the risk of recurrence is nearly eliminated (Pailler *et al.*, 2022). Typically, the diagnosis of uterine inflammation includes an analysis of the medical history, clinical manifestations, and the conduct of special examinations such as ultrasound and radiography. M. Woźna-Wysocka *et al.* (2021) determined that timely diagnosis and treatment of

pyometra significantly increase the likelihood of animal survival. The diagnosis of "pyometra" is established taking into account the anamnestic data, clinical symptoms, general examination, results of general and biochemical analysis, and ultrasound of the uterus.

The disease is easily differentiated in typical cases, but in the absence of visible vaginal discharge, diagnosis is difficult, especially if the medical history and clinical symptoms are unclear. As R. Hagman (2022) demonstrated, palpation can detect a distended, segmented uterus, making radiography unnecessary, as it does not always provide clear results.

N. Lansubsakul *et al.* (2022) established that bacteriological analysis of vaginal discharge is not effective for diagnosis, as similar microorganisms can be present in healthy dogs. Palpation of the abdominal wall can reveal an enlarged uterus. Visual diagnosis is quite useful for determining the size of the uterus and ruling out other possible causes of its enlargement. According to research by R.S. Kumar *et al.* (2023), radiography often shows the presence of a large tubular structure in the anteroposterior part of the abdominal cavity. The ultrasound method is better at detecting pathological changes, such as the size of the uterine cavity, especially when exudate is present and the diameter of the uterus remains within normal limits; changes in the tissues of the uterus (thickening) and ovaries (cysts).

L.E. Lavin & L.C. Maki (2023) established that among the possible differential diagnoses, mucometra, hydrometra, and hematometra should be excluded, as they present with similar symptoms and cause similar results on ultrasound examination of the uterus. Vaginal cytology often reveals marked signs of degeneration in leukocytes, neutrophils, macrophages, plasma cells, and lymphocytes, but phagocytosis of bacteria is not always observed. N. Lansubsakul *et al.* (2022) noted that vaginoscopy can be useful for establishing the origin of vaginal discharge and excluding other pathologies, but its use is not typical in new clinical settings.

A preliminary diagnosis of this disease is made based on the medical history, results of clinical and gynaecological examinations, blood tests (haematological and biochemical), and results of ultrasound or radiographic examination. Confirmation of the diagnosis of pyometra occurs through macroscopic and histological analysis of the uterus and ovaries after surgery, as well as through bacteriological analysis of the uterine contents.

The chronic form of pyometra may manifest without clear visible signs (subclinical). The diagnosis of subclinical pyometra can be established using ultrasonography. S. Ahn *et al.* (2021) proved that the traditional method of treating pyometra is the surgical removal of the uterus along with the ovaries, which allows for the rapid and final removal of the pathological contents of the uterus and the cessation of toxins entering the general bloodstream. The disadvantages of surgery include the risk of anaesthesia and the factors of sterility. The optimal and safest way to treat pyometra is surgical removal of both the ovaries and the infected uterus, known as ovariohysterectomy. This procedure is also commonly used to sterilise females to prevent the occurrence of pyometra.

Research by V. Suchan *et al.* (2019) has shown that the simultaneous removal of the uterus and ovaries is the most effective method of treatment, as it eliminates the source of infection, preventing the possibility of the disease recurring. Many experts argue that surgical treatment is the only reliable method for treating the accumulation of purulent exudate in the uterine cavity in dogs with a closed cervix. However, the technique of ovariohysterectomy differs from female sterilisation, as it needs to be performed in such a way that the uterine contents do not enter the abdominal cavity.

If this condition is not met, it can lead to the development of peritonitis and, consequently, the need for additional antibiotic therapy, and in severe cases, additional surgery to clean and drain the abdominal cavity, which can lead to the formation of adhesions in the postoperative period. In any case, whether performed by a retrograde or direct method, the uterus is removed along with the cervix, leaving only the vaginal stump in the abdominal cavity. If the cervix with the stump is left in the abdominal cavity, there is a risk not only of pyometra recurring but also of developing cervical cancer.

C.H. Santana & R.L. Santos (2021) found that pyometra is a common disease in unmated female dogs. Approximately 20-25% of unmated females develop the condition before the age of 10. The recommended treatment is ovariohysterectomy. Complications such as peritonitis, urinary tract infections (UTIs), wound infections, and even death after surgery have been recorded in approximately 25% of cases. If signs of complications arise, an ultrasound examination of the abdominal cavity is usually performed to rule out peritonitis, abscessation of the cervix, bleeding, and/or UTI. However, the echographic characteristics of a normal postoperative abdomen may overlap with potential pathological findings. Common surgical reactions, such as hyperechoic fat, pneumoperitoneum, peritoneal effusion, and postoperative ligature absorption, can be difficult to distinguish from postoperative peritonitis and cervical abscessation (Nilsson *et al.,* 2023).

After a comprehensive review of the available literature, few published studies were found that describe the echographic characteristics of the abdominal cavity following ovariohysterectomy in dogs for the treatment of pyometra. To date, only a few studies have described the typical echographic pattern of the abdomen in dogs after any surgical procedure. Therefore, veterinary clinicians may find it difficult to determine whether the observed reactions during an ultrasound examination are part of the normal postoperative process or indicative of postoperative complications.

Materials and Methods

The study was conducted following ethical standards and principles of humane animal care. All procedures, including diagnostics and surgery, were performed by accepted veterinary medical standards and the recommendations of the IASP Guidelines for the Use of Animals in Research (n.d.). All dog owners provided written informed consent for the inclusion of their animals in the study and the performance of all necessary medical procedures. All animals received adequate pre-operative preparation, anaesthesia, and postoperative care, tailored to their individual needs and promoting rapid recovery from surgery.

All examinations were performed on 23 March 2021, according to a standard protocol and were conducted in B-mode using a GE (Logiq E9) ultrasound machine with a linear transducer (9-11 MHz). The GE LOGIQ E9 ultrasound system is a modernised version of the previous generation flagship LOGIQ 9 equipment from the American company GE (General Electric). During ultrasound scanning, all dogs were positioned in a supine position. The standardised ultrasound examination procedure began with A-FAST (Abdominal Focused Assessment with Sonography for Trauma) to detect free fluid. If an abdominal fluid score (AFS) was detected, this information was recorded in the patient's medical history. Following A-FAST, an assessment of the upper abdomen was performed to detect the presence of pneumoperitoneum.

Several statistical methods were employed to analyse the data, appropriate to the type and nature of the data collected. Descriptive statistics were used to calculate mean, standard deviation, median, minimum, and maximum

values for quantitative variables such as haematological and biochemical blood parameters. To compare mean values between groups, an independent samples t-test or Mann-Whitney U test was used. Correlation analysis was used to assess the relationship between clinical and laboratory parameters, employing Pearson's or Spearman's correlation coefficient. Regression analysis helped to identify dependencies between different factors, and analysis of variance (ANOVA) was used to compare mean values in more than two groups. All statistical analyses were performed using SPSS software (version 25) or R (version 4.0.2), with the significance of differences considered statistically significant at a p-value of <0.05.

A convenience sample of privately owned dogs exhibiting clinical signs typical of pyometra, including vaginal discharge, polyuria, polydipsia, loss of appetite, vomiting, lethargy, fever, and visually confirmed purulent content, were included in the study following informed consent from the owners. The study population consisted of female dogs – patients of the veterinary clinic over the past six months and diagnosed with pyometra based on the results of clinical and laboratory examinations. The progression of pyometra in dogs was assessed based on the conducted studies.

Most of the studies were conducted on animals with clinical signs typical of pyometra, which were hospitalised with obvious symptoms of the disease. The diagnosis of pyometra was based on the results of medical history, clinical and laboratory examinations, and analysis of morphological and biochemical blood parameters using standard methods. For this purpose, an automated haematology analyser Sysmex XS-1000i (Japan) was used to count the number of erythrocytes, leukocytes, and thrombocytes, and a biochemical analyser Roche Cobas c311 (Switzerland) was used to determine the levels of urea, creatinine, total protein, and other parameters. Ultrasound examination of the uterus and ovaries was used for additional diagnostic clarification. An assessment of the animals' living conditions and the nature of their care, including an analysis of their diet, was also conducted. Most of the animals (six individuals) were kept in outdoor environments, while four were housed indoors. The dietary regimens of the animals varied. All animals exhibited satisfactory feeding and housing conditions.

The clinical examination involved a general physical examination of the animals presented to the clinic, taking into account their breed and age. This included inspection, percussion, and palpation, determination of habitus and constitution, assessment of limbs, condition of all body systems, measurement of body temperature, and assessment of pulse and respiratory rate. The general condition of the sick animals was assessed by visual examination. The diagnosis was established using bimanual palpation, revealing an enlarged and thickened uterus, sometimes with the presence of ampullary dilations and septa. Ultrasound diagnosis was used to confirm the diagnosis, showing an enlarged uterus with a hypoechoic structure. When making a diagnosis, the main physiological parameters, the general condition of the animal, the condition of the mucous membranes, the condition of the hair coat, changes in food and water intake, and the nature of the vaginal discharge were taken into account.

Preoperative preparation involved subcutaneous injection of atropine sulphate in a 0.1% solution at a dose of 0.2-0.5 mL depending on the weight of the dog, intramuscular injection of diphenhydramine in a 1% solution (1-2 mL) and acepromazine in a 2% solution (0.5 mL/10 kg weight). For general anaesthesia, a 2% solution of xylazine was used at a dose of 0.1-0.15 mL/kg. Before surgery, the surgical field was disinfected. Access to the surgical

area was gained along the linea alba at the level of the posterior pair of mammary glands, performing a supravaginal extirpation of the uterus together with the ovaries. For premedication 30 minutes before the main anaesthesia, atropine sulphate at a dose of 0.1-0.5 mL/10 kg, diphenhydramine at a dose of 0.2 mg/kg, and xylazine at a dose of 0.15 mL/kg were used. Ketamine was utilised as the primary anaesthetic at a dose of 1 mL/kg. The animal was secured on the surgical table in a supine position.

The surgical procedure involved a laparotomy along the linea alba, during which, after incision of the skin, muscle layer, and peritoneum, the uterus was carefully exteriorised, avoiding the possible rupture of it and its blood vessels. After the uterus was fully exteriorised and placed on a sterile surgical drape, using a haemostatic clamp, the mesovarium was punctured beneath the ovarian-uterine artery. A haemostat was then applied to the ovarian pedicle, and a catgut suture was placed above the clamp. A second clamp was similarly positioned at the uterotubal junction, and a suture was applied beneath this clamp. Using this ligature, the uterine artery was ligated and severed between the two clamps. After that, the broad ligament of the uterus was incised outside the uterine artery up to the body of the uterus, allowing the uterine horn to be freely retracted backwards. The same actions were performed on the other uterine horn.

Before performing the amputation of the uterus, the anterior portion of the abdominal wall wound was closed using several individual sutures to prevent the evisceration of internal organs. A haemostatic clamp wrapped in gauze was placed at the level of the cervix, and the longitudinal arteries of the cervix (the uterine-vaginal arteries) were ligated. Subsequently, a tight ligature was applied as a transverse stitch around the entire body of the uterus, positioned approximately 2-3 cm below the haemostatic clamp. The excision was carried out along the posterior line of the haemostatic clamp. Prior to excision, a gauze swab was placed beneath the incision site. The stump was treated with iodine, creating a groove to fold the uterine wall inward before suturing. Finally, the stump was dusted with an antibiotic and reintroduced into the abdominal cavity.

All dogs included in the study underwent three consecutive standardised ultrasound examinations of the abdominal cavity. These examinations were conducted the day after surgery, on the 5th day post-surgery, and on the 10th day, which was also the day of suture removal. Ultrasound examinations were performed according to a standard protocol using a GE ultrasound machine (Logiq E9) and a linear transducer (9-11 MHz) in B-mode. The dogs were in the supine position during the ultrasound examination.

The protocol for the ultrasound examination began with the performance of A-FAST (Abdominal Focused Assessment with Sonography for Trauma) to detect free fluid. If abdominal fluid was identified, this information was recorded in the patient's medical history. Following A-FAST, an assessment of the upper abdomen was conducted to check for pneumoperitoneum. The video of the cervical hinge was taken in both longitudinal and transverse planes, from left to right and caudally to cranially, respectively. Static images were utilised to capture the maximum longitudinal size of the cross-sectional area of the medial iliac lymph nodes, obtained using a transducer positioned ventrolaterally on the dog's body and angled at approximately 45° to the mid-sagittal plane. The video loop in the sagittal plane over the left and right areas of the mesovarium was recorded from a lateral to a medial direction.

Results and Discussion

In this study, ultrasound examinations were performed on 10 dogs with a confirmed

diagnosis of pyometra. It was found that in 85% of cases, the pathologically altered uterus had the characteristics of an enlarged, distended tubular structure. In 15% of cases, despite the normal size of the uterus, ultrasound examination revealed the presence of intrauterine fluid.

Following ovariohysterectomy, within 2-3 days post-surgery, 90% of dogs showed improvement in their overall condition, normalisation of vital functions, and the absence of clinical signs of disease. According to an ultrasound examination 10 days post-surgery, all animals had a stable condition and no complications. At the same time, the study confirmed that the clinical manifestations of pyometra in female dogs depend significantly on the state of the cervix, i.e., whether it is open or closed. An open cervix allows the drainage of purulent fluid, whereas with a closed form, such discharge is not observed, leading to more severe symptoms due to endotoxemia and an increase in uterine size.

Purulent inflammation of the uterine lining was accompanied by vaginal discharge, which varied in colour from light red to brown. However, these discharges may not be present, depending on the degree of cervical opening. Pyometra is characterised by thick, purulent discharges of a yellowish-red, brown, or dark brown colour with a foul odour, although sometimes odourless, as found in 80% of cases. General nonspecific symptoms included loss of appetite, general weakness, excessive thirst, frequent urination, vomiting, fever, tachycardia, and tachypnoea. In cases of severe intoxication, hyperthermia, lethargy, and diarrhoea may be observed, especially with severe systemic inflammatory response syndrome.

With the open form, external signs can be detected due to vaginal discharge. The closed form often manifests with severe symptoms due to an enlarged uterus and endotoxemia. Sepsis, toxaemia, shock, elevated body temperature, significant abdominal distension, and tension of the hair coat may occur. Ultrasound has been established as the most accurate method for diagnosing pyometra, allowing the detection of even small amounts of intrauterine fluid and abnormal changes in the ovaries and uterine tissues. Ultrasound shows a swollen uterus with anechoic cysts due to pus accumulation. Histopathological analysis revealed cystic endometrial hyperplasia, wall thickening, inflammatory cells, and bacterial colonies.

E. Porowska *et al.* (2018) demonstrated that the severity of clinical signs depends on the degree of cervical opening, which may be sufficient for drainage of the pathological exudate. Purulent endometritis is accompanied by vaginal discharge of red and brown colour. This secretion is characteristic of inflammation of the uterine lining, but its amount directly depends on the degree of cervical opening, and in some cases is completely absent. In cases of pyometra, the discharge has a thick, purulent consistency, a yellowish-red, brown or dark brown colour, and a foul odour, but may sometimes be odourless.

In 80% of cases, such discharges were detected. Animals often exhibited general non-specific symptoms, such as loss of appetite, general weakness, increased thirst, increased urination, vomiting, fever, tachycardia, and tachypnoea. Lethargy and diarrhoea were also observed among these clinical signs, especially with the development of severe systemic inflammatory response syndrome. Hyperthermia in more than 20% indicated the presence of fever. Temperature increased in the early stages of the disease but then often returned to normal or lower values. The animal's condition and the condition of its coat depended on how quickly the pathological process progressed. Occasionally, intoxication, severe anaemia, and shock were observed.

According to R. Hagman (2018), pyometra in female dogs can be classified into two forms based on the condition of the cervix: closed

and open. The clinical manifestations in cases where the cervix is closed are often more severe than in those where it is open, as the animals experience an increase in uterine size and escalating endotoxemia. Such pathological changes frequently lead to uterine rupture and provoke the development of sepsis. In the closed form of purulent inflammation of the endometrium, the clinical symptoms are not pronounced in the initial stages of the disease and typically appear only after general intoxication. In cases of severe intoxication, hyperthermia, significant abdominal distension, and a ruffled coat may be observed. D. Talukdar *et al*. (2022) demonstrated that among the symptoms of pyometra with a closed cervix, depression, lethargy, vomiting, septicaemia, toxaemia, and shock may occur. The researchers emphasised that sepsis and life-threatening toxicosis necessitate the immediate removal of the source of pathogenic microorganisms through the administration of appropriate antibacterial agents.

V. Sachan *et al.* (2019) found that female dogs with pyometra can experience an increase in white blood cell count to levels ranging from 15,000 to 60,000/mm^3. In the "closed" form of pyometra, ultrasound and X-ray examinations revealed a fluid-filled uterus, and histological examination of the uterine horns and body showed cystic endometrial hyperplasia, wall thickening, and inflammatory cells in the glandular and endometrial areas, as well as bacterial colonies. Along with pyometra, a significant increase in the number of macrophages and neutrophils in the endometrium was observed. In most situations where pyometra is suspected, ultrasonography is used for diagnostic imaging. Using this method, accurate information is obtained about the size, wall thickness of the uterus, the presence of cysts or fibrosis in the endometrium, and the presence of uterine fluid. The detection of uterine blood vessels, according to R.G.C. Xavier *et al*. (2024), helps to differentiate pyometra from mucometra in cases where the uterus is filled with fluid.

A.K. Ahuja *et al. (*2019) demonstrated that ultrasonography is the most accurate method for diagnosing pyometra, as it allows for a qualitative and quantitative assessment of the uterine condition. In cases of pyometra, the uterus is usually swollen, and anechoic cysts are visualised due to the accumulation of pus. G. Mantziaras & G. Luvoni (2020) found that the advantage of ultrasonography is the ability to detect even small amounts of intrauterine fluid, as well as abnormal changes in the ovaries and uterine tissues. Depending on the degree of uterine involvement, the ultrasound signs of pyometra may vary. Areas of uterine damage may appear as hypoechoic or anechoic regions. For example, with moderate uterine involvement, the uterus may appear as a hypoechoic, almost round structure, located ventrally and ventrolaterally to the anechoic urinary bladder on transverse scanning.

R.S. Kumar *et al.* (2023) determined that cystic endometrial hyperplasia (CEH), which precedes the development of pyometra, can appear as small, fluid-filled cysts on an ultrasound of the endometrium. Transabdominal ultrasonography is an effective method for diagnosing the closed form of pyometra. Characteristic signs include multiple anechoic cysts with altered uterine wall thickness. Thus, ultrasound can be used as a rapid and non-invasive method for detecting uterine pathologies such as CEH and pyometra.

R.G.C. Xavier *et al.* (2024) noted that in clinically healthy animals, the anatomical and topographical position of the uterus can complicate its visualisation using ultrasonography, as the cervix and body of the uterus are located posteriorly in the pelvic cavity, between the loops of the intestine, while the uterine horns are situated above the bladder in the abdominal area. Meanwhile, a full urinary bladder can

create an acoustic window by displacing the intestinal loops, facilitating the visualisation of a uterus with limited content or a healthy uterus. However, if the uterus is located higher than a full urinary bladder, most often only the cervix and body of the uterus can be seen, which on the ultrasound appear as elongated structures with a hypoechoic structure. Identifying the uterine horns on the sonogram can be challenging, as they are often indistinguishable from the intestinal loops. Nevertheless, when there are secretions, even with a small amount of mucus, the uterine horns can be readily visualised. During ultrasonography, they exhibit smooth walls of moderate echogenicity, and no cavities are observed. In the case of inflammatory processes within the uterus, the cavities of the horns may fill with inflammatory exudate, leading to an increase in horn diameter. The presence of exudate within the cavities of the uterine horns can be clearly visualised from any point on the abdominal wall during ultrasonography.

Typically, female dogs with pyometra exhibited characteristic ultrasonographic findings that included enlarged uterine horns, which appeared as round anechoic structures located beneath the urinary bladder. The uterine body could also be enlarged, with anechoic content in the horn cavities, which could be homogeneous or heterogeneous. The uterine walls were usually of increased echogenicity and heterogeneous, and thickening of the endometrial walls was also observed (Fig. 1).

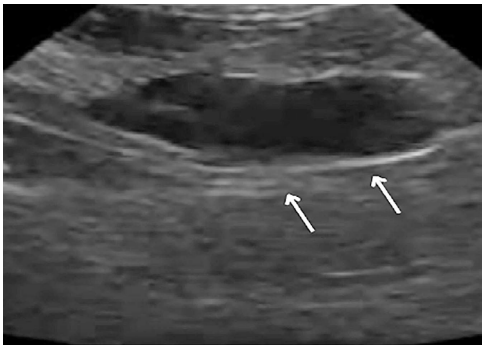

Figure 1. Ultrasound image of pyometra in female dogs
***Note**: arrows in the figure indicate the pathological process within the uterus in cases of pyometra*
***Sourse**: author's material*

Ultrasonography proved to be informative in detecting intrauterine fluid even when the diameter of the uterus remained within normal limits. Additionally, it had the advantage of detecting further pathological changes in tissues and ovaries, such as ovarian cysts or cystic endometrial hyperplasia. During the study, a situation arose where the left uterine horn was enlarged to 4.3 cm, as seen on a transverse section, and it had hypoechoic content, indicating the presence of pyometra.

S. Ahn *et al.* (2021) concluded that endometritis in carnivores is rarely responsive to conservative treatment and recommended surgical treatment – ovariohysterectomy. M.K. Nilsson *et al.* (2023) insisted that the most effective method of treatment and prevention of recurrences is a combination of ovariohysterectomy and antibiotic therapy. This method allowed for rapid removal of purulent content from the uterus and reduced the manifestation of intoxication of the organism. However, it has

its drawbacks. Firstly, surgery is always associated with the risks of anaesthesia and postoperative complications. Secondly, ovariohysterectomy involves the sterilisation of the female, which is not optimal for breeding or purebred animals kept for breeding. Despite the widespread use of ovariohysterectomy, it has its risks and leads to a complete loss of the female's reproductive function.

The blood vessels supplying the uterus are located on both sides: on one side is the ovarian-uterine artery, on the other – the uterine artery. A collateral branch of the posterior aorta is the ovarian-uterine artery, which penetrates the mesovarium and branches into two parts: one supplies the ovary, and the other supplies blood to the anterior part of the uterine horn and broad uterine ligament. This artery connects anteriorly with the ovarian artery, posteriorly with a branch of the vaginal artery. During pregnancy and pyometra, the uterine artery increases significantly in size. During all stages of the operation, special care was taken to interact with the uterus, which contains purulent content. Accidental movements of the instruments can lead to perforation of the uterus and leakage of pus into the abdominal cavity. Adequate anaesthesia during surgery for pyometra is critically important, as it provides continuous monitoring, and prevents complications that may arise due to impaired cardiovascular, respiratory, and haemostatic functions. Since pyometra causes toxic effects on all organs, the choice of anaesthetics should be based on previous clinical studies of the animal.

This study sought to describe the postoperative ultrasonographic features of the cervical stump, mesovarium ligatures, medial iliac lymph nodes, and surrounding abdominal tissues at various time points following surgery in a group of dogs with pyometra and a group of dogs without postoperative complications. The hypotheses predicted that the size and other characteristics of the cervical stump, mesovarium region, and medial iliac lymph nodes would differ at three different time points of examination.

The ultrasound examination revealed the following. Assessment of abdominal fluid volume showed a decrease by the 10th day compared to the 1st and 5th days post-surgery. On the 1st and 5th days, free fluid was found in 60% and 50% of dogs, respectively, while after 10 days, fluid was found in only 20% of dogs. Pneumoperitoneum was observed in 30% of dogs on the 10th day compared to 90% and 70% at the previous control points. The results of the assessment of abdominal fluid volume and the number of dogs with free fluid and pneumoperitoneum at three examination points are presented in Table 1.

Table 1. Assessment of abdominal fluid and the number of dogs with free fluid and pneumoperitoneum at three time points after ovariohysterectomy in 10 female dogs diagnosed with pyometra

Parameter	Day 1, quantity (%)	Day 5, quantity (%)	Day 10, quantity (%)
Free fluid	6 (60%)	5 (50%)	2 (20%)
Pneumoperitoneum	9 (90%)	7 (70%)	3 (30%)
Abdominal fluid indicator			
0	6 (60%)	7 (70%)	9 (90%)
1	4 (40%)	5 (50%)	1 (10%)

Table 1. Continued

Parameter	Day 1, quantity (%)	Day 5, quantity (%)	Day 10, quantity (%)
Abdominal fluid indicator			
2	2 (20%)	0 (0%)	0 (0%)
3	1 (10%)	0 (0%)	0 (0%)
4	0 (0%)	0 (0%)	0 (0%)

Note: *the assessment of the volume of abdominal fluid involved the following values: 0 – no fluid, 1 – the presence of fluid in one quadrant of the abdominal cavity, 2 – in two quadrants of the abdominal cavity, 3 – in three quadrants of the abdominal cavity, 4 – in all four quadrants of the abdominal cavity*
Source*: developed by the author*

In all 10 dogs, the cervical stump was examined at all selected time points. The stump had a mixed echogenicity with a hypoechoic central region and a hyperechoic rim of varying thickness. There was a relationship between body weight and the cross-sectional area of the cervix. The area of the stump varied from 75 to 716 mm^3 on the 1st day, from 156 to 887 mm^3 on the 5th day, and from 108 to 558 mm^3 on the 10th day. Areas of the mesovarium were found in all 10 dogs over the three days of observation. They appeared as hyperechoic heterogeneous areas with shadows at the edges or central shadowing artefacts.

The cervical stump increased in size up to the 5th day post-operatively compared to subsequent days. The size of the mesovarium ligature reaction remained stable at all selected time points of the study. The study suggests that the cervical stump, the reaction to mesovarium ligatures, and the medial iliac lymph nodes can be effectively visualised using ultrasonography after ovariohysterectomy. It is noteworthy that the details and size of the cervical stump varied significantly between individuals.

All images and videos were stored, and video loops were played to assess the margins of the cervical stump and the reaction of the mesovarium ligature. For measurements, frames with the maximum cross-sectional area were used, later defined as size. The maximum length of the cervical stump was measured in the longitudinal plane, placing the callipers at the level of the ligature and the external cranial edge of the hyperechoic area, defined as the cervix. The maximum height of the cervix in the longitudinal view was measured in two places – cranial and caudal to the ligature, placing the callipers at its extreme ventral and dorsal external edges. In the transverse plane, a frame containing the cervical stump at its maximum size was used. At this point, measurements were taken of both the maximum diameter (referred to as width) and the orthogonal diameter (referred to as height). The cross-sectional area of the cervical stump was calculated by indicating the outer hyperechoic edge at eight different points to cover the area of interest. The maximum length of the mesovarium reaction was measured parallel to the long axis of the transducer scanning plane, placing the callipers on its outer hyperechoic edges. The point of its maximum height was identified and measured perpendicular to the length. The maximum height and length of the medial iliac lymph nodes were measured on still images. The characteristics of the cervical stump, mesovarium reaction, and medial iliac lymph nodes were documented.

The present study confirmed the effectiveness of using ultrasound diagnostics for the early detection of pyometra in dogs and for assessing post-operative conditions. Ultrasound

proved useful in detecting intrauterine fluid even in cases where the size of the uterus was within normal limits. Ultrasound examinations conducted on the 1st, 5th, and 10th days post-surgery provided objective data on the animals' condition and revealed even minor complications. This confirms the high diagnostic value of ultrasound.

The results of the study showed that ovariohysterectomy is the most effective method for treating pyometra, contributing to the normalisation of vital functions as early as 2-3 days post-surgery. This is consistent with the findings of a study by S. Ahn *et al.* (2021), who also emphasised the importance of surgical intervention for the treatment of pyometra and the improvement of clinical parameters in dogs.

In the study by B. Gasser *et al.* (2020), the importance of timely diagnosis of pyometra to prevent sepsis and kidney failure was noted, confirming the value of ultrasound as a rapid and non-invasive method. P.R. Batista *et al.* (2022) evaluated the use of Doppler ultrasound to assess the condition of dogs after medical treatment for pyometra, emphasising that this technique also has high diagnostic value, although it requires extensive training and specialised equipment compared to traditional ultrasound.

A.J.R. Peixoto *et al.* (2023) highlighted the importance of a comprehensive approach to the treatment of pyometra, including the assessment of systemic inflammatory response and organ dysfunction, emphasising the need for a multi-faceted diagnostic approach to improve treatment efficacy and reduce mortality. P.M. Sklyarov & V.V. Piatibrat (2021) and D.V. Zarutska (2022) also confirmed the high diagnostic value of ultrasound for detecting pyometra in dogs and its complications. They emphasised the need for early diagnosis and timely surgical intervention to improve treatment outcomes.

Thus, the present study affirmed the effectiveness of ultrasound in the diagnosis of pyometra and postoperative monitoring. Comparison with other studies showed that the results are generally consistent with the existing literature, underlining the importance of ultrasound diagnosis and surgical treatment for reducing mortality and improving the quality of life of dogs with pyometra.

Conclusions

Pyometra in dogs is a dangerous infectious disease that requires immediate diagnosis and treatment to prevent complications such as multiple organ dysfunction, particularly kidney damage due to endotoxins. The study established that ultrasound is a highly effective method for detecting pyometra in dogs, allowing for the timely identification of pathological changes in the uterus and the implementation of appropriate surgical intervention.

Ultrasound examinations performed using a GE (Logiq E9) machine demonstrated high informativeness and accuracy in the diagnosis of pyometra. Diagnostic criteria, including the presence of purulent material in the uterus, increased uterine size, and changes in the uterine wall structure, correlated with the results of clinical and laboratory investigations, confirming their reliability. A comparison of the effectiveness of different treatment methods for pyometra revealed that ovariohysterectomy was the fastest and most effective method. After surgery, the inflammatory process ceased immediately, which had a positive impact on the animal's health. In most cases, the normalisation of vital functions in patients was observed within 2-3 days after surgery, whereas with conservative treatment, this period lasted up to 10 days.

Following surgical treatment, a series of follow-up ultrasound examinations were conducted to assess the state of the abdominal

cavity and the healing process. Ultrasound characteristics were established for each examination, including features of the cervical stump and mesovarium, size and echogenicity of the medial iliac lymph nodes, as well as the presence of free peritoneal fluid and pneumoperitoneum. In all dogs, the cervical stump had a heterogeneous appearance with a hypoechoic centre surrounded by hyperechoic tissue.

It should be noted that ultrasound is a non-invasive and rapid diagnostic method for detecting pyometra, even small fluid collections. Changes in the uterus on ultrasound typically appeared as an enlarged, distended tubular structure with anechoic or hypoechoic content. Therefore, ultrasound is an effective diagnostic tool for both qualitative and quantitative assessment of pyometra in female dogs, significantly contributing to the treatment process both during surgery and in the postoperative recovery period.

Future research directions include analysis of long-term outcomes after ovariohysterectomy and refinement of ultrasound techniques to improve diagnosis and monitoring of animals post-surgery.

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Conflict of Interest

None.

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Оцінка результативності хірургічного лікування із застосуванням сонографічної діагностики за піометри в собак

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Анотація. Актуальність дослідження полягала в необхідності оцінки ефективності хірургічного лікування піометри в собак із використанням сонографічної діагностики. Піометра є однією з найпоширеніших репродуктивних патологій у сук, що у випадку запізнілої діагностики може призвести до розвитку небезпечних ускладнень – сепсису та ураження нирок. Ультразвукове обстеження є неінвазивним та ефективним методом діагностики піометри, яке є важливим інструментом для встановлення навіть незначних ускладнень після проведеної оваріогістеректомії та дозволяє здійснювати моніторинг стану здоров'я тварини. Мета цієї роботи полягала у виявленні ультразвукових характеристик черевної порожнини в собак, хворих на пірометру, та порівняння післяопераційних ультразвукових характеристик черевної порожнини після оваріогістеректомії в динаміці у групі тварин з піометрою та без ускладнень. Провідним методом дослідження було поетапне і цілеспрямоване ультразвукове обстеження черевної порожнини в собак для виявлення піометри, далі під час оваріогістеректомії, а також на 1, 5 та 10 добу після операції. Встановлено, що патологічно змінена матка на ультразвуковому зображенні часто мала характеристики збільшеної, розтягнутої трубчастої структури з анехогенним або гіпоехогенним умістом. Ультразвукове дослідження хворих собак вирізнялось також діагностичною ефективністю у випадках візуалізації внутрішньоматкової рідини, навіть, коли розміри матки відповідали межам норми. Застосування сонографічної діагностики дозволяло своєчасно виявити піометру на ранніх стадіях, що значно поліпшувало ефективність подальшого лікування. Оваріогістеректомія виявилася найефективнішим методом лікування собак з піометрою, сприяючи нормалізації життєвих функцій вже на 2–3 добу після операції, що зменшувало ризик рецидивів. Ультразвукове дослідження черевної порожнини в динаміці після оперативного втручання забезпечувало своєчасну корекцію лікувальних заходів і покращення прогнозу для пацієнтів. Встановлені факти мають практичне значення для лікарів ветеринарної медицини, що сприятиме покращенню якості діагностики піометри у собак, ефективності їх лікування та зменшенню летальності

Ключові слова: патологія матки; ультразвукова діагностика; хірургічне втручання; післяопераційний період; внутрішньоматкова рідина; ускладнення

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Diagnostic studies for enterotoxaemia in rabbits

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Abstract. The relevance of this study is highlighted by the occurrence of enterotoxaemia in rabbits, particularly caused by *Clostridium perfringens*, in the context of industrial rabbit farming. The research aimed to determine the causes of morbidity and increased mortality in young rabbits during the growing period. The study involved a stepwise analysis of feed samples,

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clinical examination of rabbits reared in an industrial rabbit farm, and laboratory examination of the obtained biological material. Additionally, to establish a diagnosis and provide the farm with appropriate recommendations regarding the maintenance and prevention of rabbit diseases, the health status of the rabbits, the causes of digestive disorders with symptoms of diarrhoea and increased mortality under production conditions were analysed. Clinical, haematological, pathological, microbiological, and statistical research methods were used. In diseased rabbits, an increase in rectal body temperature, symptoms of diarrhoea, and in some animals, seizures were established. Several animals succumbed to the disease. During laboratory blood tests of the rabbits, moderate anaemia was detected, with a decrease in the number of erythrocytes and a reduction in haemoglobin content. There was also a disturbance in the qualitative composition of erythrocytes, including the presence of poikilocytosis and altered erythrocyte forms: acanthocytes, echinocytes, schistocytes, dacryocytes, keratocytes, and drepanocytes. A decrease in the immune status of the rabbits was characterised by a reduction in the total number of leukocytes and lymphocytes in the blood and a low neutrophil-to-lymphocyte ratio. Biochemical analysis of rabbit serum revealed a decrease in glucose levels below the physiological range and an increase in the activity of the enzymes alanine aminotransferase, alkaline phosphatase, and gamma-glutamyl transferase. Results of post-mortem examinations of the deceased rabbits indicated the presence of gas-filled small intestines, with a fluid content and no macroscopic signs of inflammation. The feed provided to the rabbits on the farm was found to contain sulphite-reducing clostridia, specifically *Clostridium perfringens*. Identifying the causes of morbidity and mortality in rabbits will enable the selection of appropriate methods for preventing deaths within specific farming conditions

Keywords: enterotoxin; anaemia; digestive disorders; morphological blood studies; biochemical blood studies

Introduction

Clostridia are widespread in nature and found in soil, water bodies, bedding, and feed. They are part of the normal microflora in the gastrointestinal tract of animals. These are highly resistant spores capable of surviving in the environment for extended periods. Not all species of clostridia cause disease, but those that do are usually fatal (Clostridiosis on a dairy…, 2024).

According to A. Camargo *et al*. (2024), *Clostridium perfringens* bacteria, which have an etiological significance in the infectious pathology of both animals and humans, produce a range of toxins and cause various types of diseases. The Clostridia that synthesise toxins of types A, B, C, D, and E can be both an etiological factor in the development of foodborne illnesses in calves, lambs, and piglets and a causative agent of infectious diseases characteristic of each animal species. According to M. Hoonakker *et al*. (2024), a common feature of all *Clostridium perfringens* is their impact on their hosts through the secretion of very potent exotoxins, some of which are spore-forming toxins. Clostridia do not pose a threat to the animal's body until favourable conditions arise. Typically, clostridial infections are very severe and manifest acutely (Clostridiosis on a dairy…, 2024).

Moreover, A. Camargo *et al.* (2024), expressed that the etiological role of clostridia in animal pathology is often controversial, as most species of clostridia can be part of the microbiota of the intestines of healthy animals and

humans, and from there enter and accumulate in the environment, feed, water, and soil. Therefore, infectious diseases can be endogenous in nature, when unfavourable conditions enhance the multiplication and accumulation of *Clostridium perfringens* in the intestine. For example, as a result of poor feeding hygiene (incomplete and sudden changes in diet) or poor animal husbandry conditions, or in the case of using feed contaminated with *Clostridium perfringens*, several different antibiotics and disinfectants are naturally ineffective, especially against their spore forms.

According to research by G.G. Alves *et al*. (2024), the beta-toxin produced by *Clostridium perfringens* types B and C causes necrotic enteritis, characterised by deep segmental necrosis of the mucosa with pronounced haemorrhage in the small intestine, the occurrence of neurological symptoms, and sudden death in animals, and rarely, humans. M. Hoonakker *et al*. (2024) indicated that while *C. perfringens* type C secretes several exotoxins, the beta-toxin is the primary virulence factor for inducing necrohaemorrhagic intestinal lesions. B. Tarek *et al*. (2021) established that beta-toxin plays a pivotal role in the pathogenesis of necrohaemorrhagic enteritis in young animals and humans by targeting endothelial cells in the intestine. M. Hoonakker *et al*. (2024) noted that beta-toxin primarily affects the endothelial cells of blood vessels in the mucosa and may also suppress platelet function. The beta-toxin is produced as a monomeric protein with a molecular weight of 34.86 kDa. *Clostridium perfringens* secrete it in the form of a water-soluble monomer that binds to membrane receptors on target cells, making it one of the most potent clostridial toxins known. Binding to receptors in the plasma membrane of target cells results in local concentration, oligomerisation, and the formation of a multimeric prepore that undergoes conformational changes and inserts into the lipid bilayer, where it creates a functional transmembrane pore. These pores disrupt membrane permeability by allowing ions to pass through, which, according to B. Tarek *et al*. (2021) and G.G. Alves *et al*. (2024), lead to changes in intracellular concentration and the responses of affected cells. Furthermore, A. Camargo *et al*. (2024) discovered that *Clostridium perfringens* produces other clinically significant supplementary toxins, such as *perfringolysin O*, tetanotoxin, and *Clostridium perfringens* beta 2 toxin. Although these toxins are not used for toxinotyping, they can act synergistically with extracellular toxins, influencing the expression of other toxins, their production levels, and virulence factors, thereby contributing to the overall progression of the disease.

I.M. Gohari *et al*. (2021) demonstrated that most of the toxin genes in *C. perfringens* are encoded in conjugative plasmids, including pCW3-like and recently discovered pCP13-like families of plasmids. The production of *C. perfringens* toxins is tightly regulated through processes involving two-component regulatory systems, quorum sensing, and/or alternative sigma factors associated with sporulation. Non-toxin factors, such as degradative enzymes and sialidases, are also involved in the pathogenicity of this bacterium. These factors may contribute to the action of toxins *in vitro* and possibly *in vivo*, and may also enhance intestinal colonisation by *C. perfringens*. For example, sialidase NanI increases the adherence of *C. perfringens* to intestinal tissue and generates nutrients for its growth, at least *in vitro*.

This study aimed to determine the health status of rabbits, taking into account the results of clinical, haematological, pathological, and microbiological examinations, and to elucidate the causes of digestive disorders with diarrhoeal syndrome and increased mortality in these animals under production conditions.

Literature Review

Modern industrial rabbit farming typically involves slaughtering rabbits at the age of three months. By this time, rabbits should have gained approximately 2.5-3 kg of body weight. Although rabbits are considered fast-growing animals, such results can only be achieved with a complete and balanced diet (Abdelazeem *et al*., 2019a; 2019b) and a healthy gastrointestinal tract, which allows the animal's body to be provided with everything necessary for its growth.

Digestive disorders are one of the primary causes of mortality in young rabbits. C. Romero *et al.* (2011) noted that *Clostridium perfringens* is a widespread pathogenic bacterium associated with intestinal diseases in domestic animals, and its pathogenicity is linked to the production of potent exotoxins. A. Shrestha *et al*. (2022) indicated that *Clostridium perfringens* enterotoxin (CPE) induces gastrointestinal symptoms of enterotoxaemia in animals and is one of the most common bacterial foodborne diseases.

Enterotoxaemia is characterised by symptoms of diarrhoea, primarily in rabbits aged between 4 and 8 weeks, although according to M.E. Ensminger *et al*. (1990), it can affect rabbits of any age. The main symptoms of this disease in rabbits are severe diarrhoea, loss of appetite, and the presence of a rough coat. Death of the animal occurs within the first forty-eight hours of the onset of symptoms. This disease is caused by several microorganisms: *Clostridium spiroforme*, *Clostridium perfringens*, and *Escherichia coli*.

According to C. Romero *et al*. (2011), a rabbit exhibiting mild diarrhoea, distension of the foregut with gaseous fluid content, and lesions of the caecum without macroscopic signs of inflammation is considered to have epizootic rabbit enteropathy (ERE). I.M. Gohari *et al*. (2021) demonstrated that during gastrointestinal tract lesions, *C. perfringens* produces enterotoxin in the intestine. *Clostridium perfringens* enterotoxin (CPE) is a primary cause of food poisoning and antibiotic-associated diarrhoea. D.C. Briggs *et al*. (2011) noted that this enterotoxin structurally belongs to the family of aerolysin pore-forming toxins. CPE binds to receptors belonging to the claudin family of proteins (Jorge *et al*., 2014; Shrestha *et al.,* 2022). Claudins are a family of integral membrane proteins that mediate interactions between cells of the mammalian intestinal epithelium by localising and stimulating the formation of intercellular tight junctions (Anderson & Van Itallie, 2009). *Clostridium perfringens* causes widespread gastrointestinal disorders in mammals due to the action of enterotoxin, which affects claudins. CPE binds to claudins at or near tight junctions in the intestine and disrupts their barrier function (Ogbu *et al.*, 2022). CPE widens β-hairpins in the cell membranes of enterocytes, forming active pores (Jorge *et al*., 2014; Shrestha *et al*., 2022). The formation of this pore causes an influx of free calcium into intestinal enterocytes, killing these cells in a dose-dependent manner. Specifically, according to M.I. Gohari *et al*. (2021), even low doses of CPE induce classical apoptosis. In contrast, according to A. Shrestha *et al*. (2022), low doses of CPE form only a small number of CPE pores, leading to a limited influx of free calcium and moderate activation of calpain. This, in turn, leads to cell death via caspase-3-mediated classical apoptosis. Higher concentrations of CPE induce the formation of many CPE pores, greater influx of free calcium into enterocytes, and stronger activation of calpain, which induces the death of these cells via necroptosis. Furthermore, according to C. Ogbu *et al*. (2022), CPE ultimately destroys cells expressing claudin through the formation of a cytotoxic membrane penetrating β-barrel.

A. Shrestha *et al*. (2022) noted that in the loops of the rabbit small intestine, the cytotoxicity of CPE leads to fluid accumulation in

the intestinal lumen and histological damage to the epithelium, including villus shortening and epithelial desquamation. Considering the data of previous authors and the information from M.I. Gohari *et al*. (2021), it was found that CPE is a significant pathogenicity factor of *C. perfringens* type F strains. Type F strains cause 5-10% of cases of antibiotic-associated diarrhoea in animals.

Therefore, based on the analysis of the literature, the impact of enterotoxin on the mechanisms underlying the development of the corresponding symptoms in diseased rabbits has been determined.

Materials and Methods

The study was conducted in 2023 within an industrial farm in the central region of Ukraine, involving rabbits aged between 35 and 68 days. The rabbits were housed in wire mesh cages with wire mesh floors, with 3-5 animals per cage. Each cage was supplied with water through a system equipped with nipple drinkers, allowing free access to water for each animal. The rabbits were fed a granulated feed produced on the farm.

Blood samples were collected from the jugular vein of diseased rabbits into vacuum tubes containing ethylenediaminetetraacetic acid (EDTA). Blood smears were immediately prepared during venipuncture to prevent changes in blood cell morphology. The blood samples were transported in a thermally insulated bag with ice, separated by a layer of cotton, to inhibit the processes of lactic acid formation from glucose in the erythrocytes. For post-mortem examination, carcasses of animals that had just died were collected. Post-mortem examinations of the rabbits were carried out immediately on the rabbit farm in a separate, equipped room. During the experiments on the rabbit farm, all bioethical requirements for animals were adhered to, by the Law of Ukraine No. 249 (2012) and the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (1986)

The clinical condition of the animals was assessed using standard methods. During the study, attention was paid to the animal's general appearance, visible mucous membranes, condition of the coat and skin, lymph nodes, organ systems, and individual organs. Body temperature was measured rectally using a mercury thermometer manufactured by Tetafarm LLC (Ukraine). Quantitative analysis of the morphological composition of the blood was performed using a haematology analyser "Mindray animal care" BC – 5000 Vet (China). The haematocrit value was determined using a haematology analyser "VetAutoread", manufactured by "IDEXX Laboratories" (USA). The qualitative composition of erythrocytes was examined in blood smears stained with haematological dyes "Leucodif 200", manufactured by "Erba lachema" (Czech Republic).

Biochemical parameters of rabbit serum, namely: total protein, albumin, glucose, urea, creatinine, inorganic calcium, total phosphorus, total bilirubin, and the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), and alkaline phosphatase (ALP), were determined using standard methods with a photometric biochemical analyser "Lab Line 010" (Austria). Reagents from the company SpinaLab (USA) were used for the biochemical studies.

Microbiological analysis of the feed was conducted following the following testing methods: determination of the number of yeasts and mould fungi – DSTU ISO 7954:2006 (2006) Microbiology of food and animal feed. General guidelines for counting yeasts and microscopic fungi. Colony counting technique cultivated at a temperature of 25°C (DSTU ISO 7954:1997,

IDT); determination of *Bacillus cereus* – DSTU ISO 7932:2007 (2009); Microbiology of food products and animal feeds. Horizontal method for the enumeration of presumptive *Bacillus cereus*. Counting technique at a temperature of 30°C (ISO 7932:2004, IDT); determination of the number of sulphite-reducing clostridia *Clostridium perfringens* – (6) DSTU ISO 7937:2006 (State standard..., 2006); Microbiology of food products and animal feeds. Horizontal method for the enumeration of *Clostridium perfringens*. Colony counting technique.

Samples of pathological material from rabbits (heart blood, liver, kidneys, stomach, and intestine) were examined for the presence of pathogens of pasteurellosis (Pavlenko *et al.,* 1995), streptococcosis (Methodological guidelines..., 1983), staphylococcosis (Methodological recommendations..., 1999), salmonellosis (DSTU 4769:2007, 2009), yersiniosis (Melnychuk *et al.,* 2013), listeriosis (Bondar *et al.,* 2007), and clostridial diseases (DSTU 8492:2015, 2017). Statistical analysis of the research results was performed using the computer program Microsoft Excel 2016.

Results and Discussion

The results of the clinical examination of the animals indicated that a portion of the rabbit population experienced periodic digestive disorders during the period from 35 days of age (the weaning period from the female rabbit) until slaughter at 68 days. These disorders included loose stools and soiling of the fur around the anal region with faeces. At the onset of diarrhoea, the rectal temperature increased to 40-41°C. Subsequently, the body temperature decreased to within physiological ranges (38.5-39.5°C), but diarrhoea persisted. The rabbits exhibited stunted growth and poor weight gain, and some individuals displayed seizure-like symptoms with backward head tilting A portion of the rabbits succumbed to the condition. Palpation of the abdomen revealed signs of intestinal gas, but no signs of pain were observed.

According to the results of the morphological study of rabbit blood, moderate anaemia was recorded, as indicated by a haemoglobin level lower than the physiological range, a reduced erythrocyte count, and a decreased haematocrit value (Table 1).

Table 1. Morphological blood parameters of rabbits, M ± m, n = 5

	Parameter	Research results	Physiological range*
%	Ht, Haematocrit	33.0 ± 0.15	36.6-47.4
g/L	HB, Haemoglobin,	104.0 ± 2.0	115-151
10^{12}/L	RBC, Erythrocytes	5.05 ± 0.18	5.2-6.8
pg	MCH, Haemoglobin content in an erythrocyte	20.5 ± 0.44	21.1-24.5
%	MCHC, Haemoglobin concentration in an erythrocyte	31.7 ± 0.5	29.5-33.9
fl	MCV, Erythrocyte volume	65.34 ± 1.16	64.6-76.2
10^9/L	WBC, Leukocytes	4.2 ± 0.6	6.30-10.06
10^9/L	Basophils	0	0.06-0.36
10^9/L	Eosinophils	0.05 ± 0.13	0.01-0.15
10^9/L	Band Neutrophils	0	0
10^9/L	Segmented Neutrophils	1.47 ± 0.06	1.49-3.21
10^9/L	Lymphocytes	2.48 ± 0.34	3.36-7.00
10^9/L	Monocytes	0.21 ± 0.05	0.05-0.45

Note: **Physiological ranges according to M.A. Thral et al. (2012)*
Source*: authors' development*

M.I. Tsvilikhovskyi *et al*. (2023) demonstrated that a decrease in haematocrit can occur in anaemia of various origins or due to hyperhydration. With a normal concentration of total plasma protein, a low haematocrit can be a result of increased erythrocyte destruction, decreased erythrocyte production, or chronic blood loss. These authors note that the causes of decreased haemoglobin in the blood can include iron deficiency anaemia, anaemia due to acute blood loss, hypoplastic anaemia, haemolytic anaemia after a haemolytic crisis, B_{12}-deficiency anaemia, anaemia associated with neoplasms or leukaemia, and hyperhydration. The development of anaemia in an animal, according to A.H. Rebar *et al*. (1999), is associated with the development of hypoxia, which, in turn, causes damage to the cell membranes of parenchymal organs (such as the liver) and an increase in the activity of cytoplasmic enzymes.

After centrifugation, the blood plasma had a red colour (Fig. 1), which may indicate intravascular haemolysis and the development of hemoglobinemia and, consequently, haemoglobinuria in the experimental rabbits.

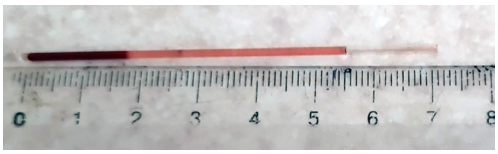

Figure 1. Haematocrit content determination
***Sourse**: authors' material*

According to L.M. Perry-Clark & L.D. Meunier (1991), this could also be due to the fact that rabbit blood readily haemolyzes and clots quickly. However, colour indices did not indicate a deficiency of iron and copper in the experimental rabbits. The size of the erythrocytes was not reduced. The authors of this article believe that the decrease in the number of erythrocytes occurred as a result of haemolytic processes, i.e., the development of haemolytic anaemia. This is confirmed by polychromasia (an increase in the number of polychromatophils greater than 4/100 erythrocytes) (Figs. 2-5). According to M. Suckow *et al*. (2002), in rabbits, 1-4% of circulating erythrocytes can be polychromatophils. The presence of a few nucleated erythrocytes (1-2×100 leukocytes) should be considered within the normal reference range for rabbits, rather than an indicator of cell regeneration (Melillo, 2007). However, according to A. Bodnariu (2022), in rabbits up to two months of age, the percentage of reticulocytes can be as high as 12% of the number of erythrocytes. Polychromasia and the presence of polychromatophils in rabbit blood smears are explained by the short lifespan of the animals and the intensity of erythropoiesis (Suckow *et al*., 2002). The increased number of polychromatophils in the blood of the examined rabbits indicates that the red bone marrow of the animals is undamaged and actively restores erythrocytes lost due to haemolysis. A. Bodnariu (2022) found that erythrocyte regeneration is characterised by increased anisocytosis and polychromasia. The authors of this article believe that a likely cause of haemolysis in rabbits may be the influence of toxins with a haemolytic effect (feed toxins, bacterial toxins, in particular alpha-toxin of *Clostridium perfringens*, etc.).

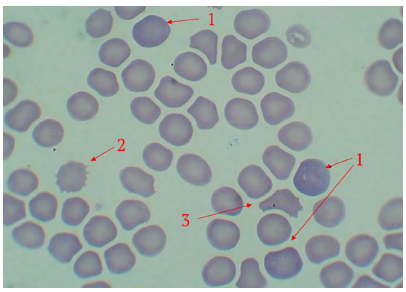

Figure 2. Blood smear from an experimental rabbit. Marked polychromasia
***Note**: Polychromatophils – 1, echinocyte – 2, and acanthocyte – 3 are visible under the microscope. Leucodif 200 staining. Magnification ×1000*
***Sourse**: authors' material*

During the examination of blood smears from the rabbits, no nucleated erythrocytes or Howell-Jolly bodies were detected. Erythrocytes in the blood smear exhibited marked poikilocytosis. The presence of the latter indicates the development of processes in the rabbits' bodies that lead to a disruption of the erythrocyte membrane structure due to changes in lipid and protein metabolism. Poikilocytosis can be caused by both a disruption of the erythrocyte membrane structure and a disruption of redox processes in the erythrocyte and at the level of the whole organism. Almost all erythrocytes had an altered shape: many acanthocytes (Figs. 2-6), echinocytes (Figs. 2-6), schistocytes (Fig. 3), dacryocytes (Fig. 4), keratocytes (Fig. 5), and drepanocytes (Fig. 6). In the literature presented above, the appearance of acanthocytes in the blood of experimental rabbits is associated with an increase in the concentration of cholesterol relative to phospholipids in erythrocyte membranes, which occurs with an increase in the level of cholesterol in the blood plasma or the presence of abnormal lipoprotein in it. In blood smears of humans, acanthocytes are found with impaired lipid metabolism, which manifests itself in liver pathology. However, in the blood of dogs, acanthocytes are found quite rarely with hepatopathology. As a rule, acanthocytes are recorded in blood smears of domestic cats with hepatosteatosis, and in the blood of dogs with hemangiosarcoma. Echinocytes, like acanthocytes, appear in the blood of animals with uraemia, vitamin E deficiency, and hyperlipidaemia caused by liver dysfunction. J.S. Owen *et al*. (1985) proved that plasma membrane receptors of erythrocytes bind to high-density lipoproteins and this causes a change in their shape. According to M.I. Tsvilikhovskyi *et al*. (2023), the presence of schistocytes in rabbit blood can be attributed to intravascular damage, particularly in cases of disseminated intravascular coagulation (DIC). During DIC, fibrin strands trap and fragment erythrocytes, leading to the formation of schistocytes. Animals experiencing DIC may also exhibit thrombocytopenia. In calves, the appearance of schistocytes is widely recognised as a characteristic feature of DIC. In the blood of horses and cats, schistocytes are rarely observed, and their presence is associated with hepatopathy (Fig. 4).

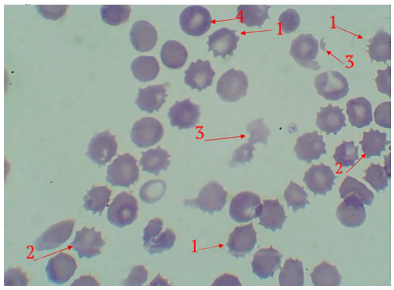

Figure 3. Blood smear from an experimental rabbit. Marked poikilocytosis of erythrocytes
***Note**: Echinocytes – 1, acanthocytes – 2, schistocytes – 3, and polychromatophils – 4 are visible under the microscope. Leucodif 200 staining. Magnification ×1000*
***Sourse**: authors' material*

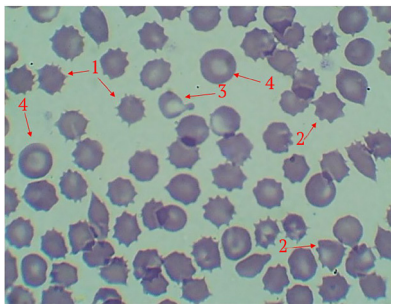

Figure 4. Blood smear from an experimental rabbit. Marked poikilocytosis
***Note**: Echinocytes – 1, acanthocytes – 2, dacryocytes – 3, and polychromatophils – 4 are visible under the microscope. Leucodif 200 staining. Magnification ×1000*
***Sourse**: authors' material*

The presence of dacryocytes in rabbit blood may indicate haemolytic anaemia, a condition where erythrocytes are destroyed by the body's own immune system. As a result, the bone marrow produces a larger number of blood cells,

including those with structural abnormalities, which are then released into the bloodstream. J.W. Harvey (2012) noted that dacryocytosis is commonly found in the blood of dogs with myelofibrosis, glomerulonephritis, and hypersplenism, as well as in dogs and cats with myeloid neoplasms. In ruminants, dacryocytes are often seen in cases of iron deficiency anaemia.

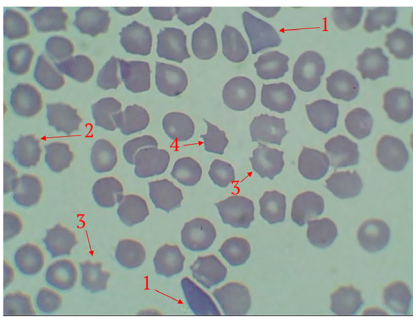

Figure 5. Blood smear of the experimental rabbit
***Note**: Polychromatophils – 1, echinocytes – 2, acanthocytes – 3, and keratocyte – 4 are visible under the microscope. Leucodif 200 staining. Magnification ×1000*
***Sourse**: authors' material*

The presence of keratocytes in rabbit blood can be indicative of various pathological conditions, including iron deficiency anaemia and liver diseases such as hepatosteatosis. In these cases, damage to the erythrocyte membrane occurs as a result of oxidative stress. For instance, in iron deficiency, a rounded vacuole initially forms within the erythrocyte. This vacuole then moves to the cell's exterior due to oxidative damage to the inner surface of the membrane. According to the literature previously cited, one reason for the formation of this clear zone (vacuole) could be a deficiency of haemoglobin. The same literature mentioned that drepanocytes appear in the blood of animals due to haemoglobin polymerisation. This phenomenon occurs *in vitro* when the partial pressure of oxygen increases and the pH level exceeds 7.4.

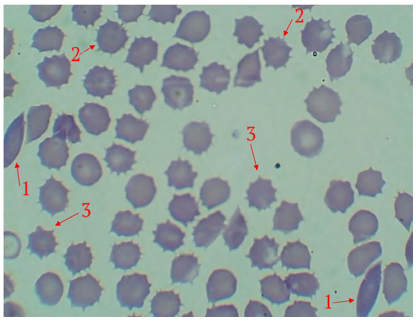

Figure 6. Blood smear of the experimental rabbit
***Note**: Drepanocytes – 1, echinocytes – 2, and acanthocytes – 3 are visible under the microscope. Leucodif 200 staining. Magnification ×1000*
***Sourse**: authors' material*

A reduction in the white blood cell (WBC) count in the experimental rabbits indicated a suppression of their immune system. Absolute lymphopenia was diagnosed in the blood of these animals, further supporting the conclusion of immune suppression. Lymphopenia is more commonly associated with acute or severe inflammatory processes and can be linked to chronic viral, bacterial, and fungal infections. The release of endogenous glucocorticoids in response to severe systemic disturbances may play a major role in the development of lymphopenia, which often accompanies these disturbances. According to M.I. Tsvilikhovskyi *et al*. (2023), glucocorticoids can potentiate apoptosis in susceptible lymphocytes. In rabbits, lymphocytes are primarily found in the blood, spleen, bone marrow, lymph nodes, and gastrointestinal lymphatic tissues. The number of circulating lymphocytes represents a balance between cells entering and leaving the bloodstream, and this does not necessarily reflect changes in lymphopoiesis. A. Melillo (2007) demonstrated that an increase in adrenaline levels in rabbit blood (acute stress) induces lymphocytosis, while an increase in cortisol levels (chronic stress) leads to lymphopenia.

The data from this author and D.R. Reavill & R.E. Schmidt (2000) suggest that viral diseases may not affect the lymphocyte count in these animals or may even lead to lymphocytosis. The extremely low neutrophil to lymphocyte ratio (0.59), combined with a low absolute lymphocyte count ($2.48 \pm 0.34 \times 10^9$/L) in the blood of the experimental animals (Table 1), indicates immunosuppression rather than stress. The neutrophil-to-lymphocyte ratio in healthy adult rabbits is approximately 1:1. Changes in this ratio can be associated with stress (heterophilia and lymphopenia). A. Bodnariu (2022) noted that rabbits do not develop significant leukocytosis in response to bacterial infection. However, a change in the neutrophil-to-lymphocyte ratio is typically observed. During this study, band neutrophils were not detected in the rabbit blood. According to A. Melillo (2007), these neutrophils are a rare finding in the blood of rabbits with clinical infection, but the absence of a left shift in the leukocyte profile does not rule out an infectious process.

The researcher demonstrated that four days of fasting did not decrease blood glucose levels in healthy rabbits. Glucose metabolism in rabbits differs from that in dogs or cats. Rabbits not only eat continuously throughout the day but also utilise volatile fatty acids produced by the caecal flora as a primary energy source. Obtaining a fasting blood sample is impossible as rabbits practice coprophagy. A rabbit deprived of food may continue to consume cecotropes.

Biochemical analysis of the blood plasma (Table 2) of the experimental rabbits revealed a decrease in glucose levels below the minimum physiological range. This may indicate a reduced level of energy and fibre in the rabbits' diet, as well as impaired synthesis of this metabolite in the animals' bodies, particularly in the liver.

Table 2. Biochemical analysis of rabbit plasma, M ± m, n = 5

Parameter	Result	Physiological range *
Total protein, g/L	60.9 ± 2.38	54-75
Albumin, g/L	38.72 ± 2.34	27-46
Glucose, mmol/L	5.22 ± 0.21	6.1-15.9
Calcium, mmol/L	3.96 ± 0.14	2.4-4.2
Phosphorus, mmol/L	1.34 ± 0.18	0.6-2.7
Urea, mmol/L	5.47 ± 0.41	2.3-6.6
Creatinine, µmol/L	68.04 ± 5.46	44.2-141.4
Alkaline phosphatase, units/L	174.14 ± 4.28	19-173
ALT, U/L	77.48 ± 4.09	25-60
AST, U/L	27.89 ± 1.87	5-31
GGT, U/L	7.54 ± 0.29	0-7
Total bilirubin, µmol/L	6.34 ± 0.64	3.4-8.5

Note: **Physiological ranges according to M.A. Thral et al. (2012)*
Source*: authors' development*

Hypoglycaemia can be induced by anorexia, starvation, or impaired liver function. Additionally, according to A. Bodnariu (2022), it leads to the mobilisation of free fatty acids from adipose tissue, causing ketoacidosis and hepatic lipidosis. A negative consequence of hypoglycaemia can also be impaired detoxification function of the liver, synthesis of heparin, hyaluronic acid, and chondroitin. Insufficient synthesis of the latter can lead to impaired vascular permeability, resulting in haemorrhages in such animals. R.A. Saunders & R.R. Davies (2005) noted that

hypoglycaemia is a rare occurrence in rabbits. In anorexic animals, it suggests that the rabbit's body is utilising fat stores and is at risk of developing fatty liver disease. Hypoglycaemia can occur in cases of terminal mucoid enteropathy, hepatic insufficiency, or other chronic diseases. Rabbits with acute sepsis may also experience hypoglycaemia.

Elevated levels of alanine aminotransferase (ALT), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) in the blood plasma of the experimental rabbits indicated destructive changes in cell membranes, primarily in the liver, and the development of cholestasis. It is known that ALT activity is lower and less organ-specific in rabbits compared to other mammals. A. Bodnariu (2022) noted that increased ALT activity in rabbits suggests possible hepatocyte damage, however, the degree of elevation does not correlate with the severity of hepatopathy and is not a prognostic indicator. Slightly elevated ALT activity in blood plasma is a common occurrence in healthy rabbits. A slight increase in ALT activity in the blood of healthy rabbits can be explained by the influence of low concentrations of toxic substances, such as resins in wooden bedding or aflatoxins in feed. A. Melillo (2007) demonstrated that increased ALT activity in blood plasma in combination with elevated ALP and GGT can be associated with hepatic lipidosis, and was also observed in rabbits with hepatic coccidiosis (*Eimeria steidae*). Thus, increased ALT activity in rabbit blood can accompany both pathological changes in the liver parenchyma and biliary tract of an inflammatory nature, as well as in dystrophy caused by intoxication of various origins.

Alkaline phosphatase (ALP) is a non-specific enzyme. The highest concentrations of ALP are found in the liver and bones, but it is also abundant in the intestinal epithelium, kidney tubules, and placenta. Similar to other species, according to the two aforementioned authors, higher concentrations of ALP are found in the plasma of young rabbits compared to adults due to increased osteoblastic activity. As a liver enzyme, ALP activity does not increase due to hepatocellular damage but indicates bile stasis (e.g., hepatic coccidiosis, liver abscesses, neoplasia, lipidosis). The authors found that increased GGT activity in rabbit plasma is most often associated with obstructive lesions of the bile ducts (cholestasis) but with less sensitivity than in other species.

During necropsy of the rabbit carcasses, the presence of gas-filled loops of small intestine with fluid contents and no macroscopic signs of inflammation was diagnosed (Fig. 7).

Figure 7. Meteorism of the intestine in an experimental rabbit
***Note**: The arrow indicates the location of the pathological process*
***Sourse**: authors' material*

Samples of pathological material from rabbits (heart blood, liver, kidneys, stomach, intestine) were examined for the presence of the causative agents of pasteurellosis, streptococcosis, staphylococcosis, salmonellosis, yersiniosis, listeriosis, and clostridial diseases. No pathogens were detected. Given the clinical symptoms, blood test results, and post-mortem findings, it was necessary to determine the cause of these changes. To this end, feed samples were collected and subjected to microbiological analysis.

Samples of plant-based rabbit feed (grist, compound feed, bran, premix, sunflower seed hulls) were examined for microbiological indicators (number of yeasts and mould fungi; quantity of *Bacillus cereus* and sulphite-reducing clostridia *Clostridium perfringens*; detection of *Enterobacteriaceae*, pathogenic *Yersinia*, *Salmonella* spp., coagulase-positive *Staphylococcus aureus*, and *Listeria monocytogenes*). The results of the studies are presented in Table 3.

Table 3. Results of the microbiological analysis of rabbit feed samples

Indicator assessed	Rabbit feed samples								[(2)] Norms according to regulatory documentation
	10/1	10/2	10/3	10/4	10/5	10/6	10/7	10/8	
Yeast count, [(1)] CFU/g	<10 (not detected)	<10 (not detected)	<10 (not detected)	<10 (not detected)	<10 (not detected)	<10 (not detected)	<10 (not detected)	<10 (not detected)	Not more than 5×10^5
Mould fungi count, [(1)] CFU/g	4.5×10^1	<10 (not detected)	3.5×10^4	1.4×10^1	<10 (not detected)	3.2×10^2	9.0×10^2	<10 (not detected)	
Bacillus cereus count, [(1)] CFU/g	<10 (not detected)	<10 (not detected)	<10 (not detected)	<10 (not detected)	<10 (not detected)	<10 (not detected)	<10 (not detected)	<10 (not detected)	Not established
Sulphite-reducing clostridia *Clostridium perfringens* count, [(1)] CFU/g	<10 (not detected)	3.6×10^1	1.4×10^1	<10 (not detected)	1.8×10^1	<10 (not detected)	<10 (not detected)	<10 (not detected)	Not permitted in 1 g
Coagulase-positive staphylococci *Staphylococcus aureus* count, [(1)] CFU/g	not detected								Not permitted in 1 g
Presence of bacteria from the family *Enterobacteriaceae*, [(3)] MPN in 1.0 g	No *Enterobacteriaceae bacteria* detected (The actual number of microorganisms in 1 g is within 0.0-9.4 at a 95% confidence level)								Not more than 300 CFU in 1 g
Pathogenic *Yersinia*, in 25 g	not detected								Not more than 300 CFU in 1 g
Pathogenic microorganisms, including *Salmonella* spp., in 25 g	not detected								
Presence of Listeria monocytogenes, in 25 g	not detected								Not established

Note: *[(1)]CFU – Colony-forming units. [(2)]List of maximum permissible levels of undesirable substances in animal feeds and feed raw materials. [(3)]MPN – Most probable number*
Source*: authors' development*

No yeasts, *Enterobacteriaceae*, pathogenic *Yersinia*, *Salmonella* spp., coagulase-positive *Staphylococcus aureus*, or *Listeria monocytogenes* were detected in any of the feed samples examined. According to the test results, samples 10/1 – Grist (No. 2); 10/4 – Bran; 10/6 – Grist (No. 1); 10/7 – Bran; and 10/8 – Compound feed PK-90 Kremiks complied with the requirements of the normative documentation (ND) (On the approval..., 2012).

Samples that did not meet the requirements of the ND were: 10/2 – Compound feed (due to "Quantity of sulphite-reducing clostridia *Clostridium perfringens*"); 10/3 – Sunflower seed hulls (due to "Mould fungi count" and "Quantity of sulphite-reducing clostridia *Clostridium perfringens*"); and 10/5 – Premix "Kremiks" (due to "Quantity of sulphite-reducing clostridia *Clostridium perfringens*").

Based on the research conducted, the most probable diagnosis is enterotoxaemia in rabbits caused by *Clostridium perfringens*. Due to the epidemiological data, clinical symptoms, and post-mortem findings, it is quite difficult to establish a definitive diagnosis for this disease. Therefore, the results of bacteriological and toxicological studies are crucial for confirming the diagnosis. The absence of positive results from culturing the pathological material from rabbit tissues and the inability to detect the presence of toxins cannot confirm but also cannot refute this diagnosis. R.M.C. Guedes (2016) suggested that certain strains of *Clostridium perfringens* are pathogenic to piglets and can cause diarrhoea and reduced growth rates in these animals, but how to identify them is still unknown. This, of course, poses a significant challenge in combating this disease. Some of the lesions observed in the experimental rabbits may partially reflect the effects of α-toxin on the organism, specifically causing gas production and tissue damage.

In fact, J.G. Songer (1996) and C. Romero *et al.* (2011) noted that this toxin hydrolyses lecithin and sphingomyelin, causing membrane destruction. According to these authors, some rabbits in which α-toxin was not detected had lesions similar to those caused by high concentrations of α-toxin. D. Marlier *et al.* (2006) were also unable to detect the presence of α-toxin in the intestinal contents of rabbits that died from anaerobic enterotoxaemia or other digestive disorders. *Clostridium* type C produces a potent toxin – leukocidin – which almost completely blocks the immune system of the diseased animal. This makes it vulnerable to other diseases and leads to the development of new, non-specific clinical symptoms for enterotoxaemia (Moraru, 2020). Additionally, according to M.E. Fernandez-Miyakawa *et al.* (2007), isolates of *C. perfringens* type B also produce epsilon toxin, which exhibits potent neurotoxic activity. In their opinion, there is currently limited information on the pathogenesis of diseases associated with type B, although these same authors indicated that both CPE and epsilon toxin can contribute to lethality.

As a result of enterotoxaemia, the death of a rabbit occurs quite rapidly; therefore, treatment is rarely employed. The choice of feed has a significant impact on the development of the disease – the higher the fibre content in the diet, the lower the incidence of enterotoxaemia. M.E. Ensminger *et al.* (1990) have demonstrated that a diet consisting of hay, straw, and oats significantly reduces the risk of developing this disease. Therefore, preventive measures to prevent the development and spread of enterotoxaemia in rabbit populations must be considered when formulating diets and preparing feed for feeding.

Conclusions

A comprehensive study of the health status of the experimental rabbits, including clinical, morphological, and biochemical analyses of blood samples, as well as microscopic examination of feed components and tissues from deceased animals, revealed changes characteristic of enterotoxaemia. Affected rabbits exhibited digestive disturbances, watery faeces, and intermittent fever of 40-41°C. These rabbits demonstrated stunted growth, poor weight gain, and, in some cases, experienced convulsions; several of them succumbed to the condition. Blood analysis revealed a decrease in the number of erythrocytes, leukocytes, neutrophils, and lymphocytes, as well as low haemoglobin levels and

reduced haematocrit. The blood plasma had a red colour, indicating intravascular haemolysis and the development of hemoglobinemia and, consequently, haemoglobinuria. The obtained colour indices refuted the deficiency of iron and copper in the experimental rabbits. Erythrocyte size was not reduced. An increase in the number of polychromatophils to more than 4/100 erythrocytes confirmed the presence of haemolytic processes in the sick rabbits. Rabbit erythrocytes exhibited altered morphology, which also indicated the development of anaemia and a decreased immune status in the diseased animals. The sick rabbits developed hypoglycaemia, suggesting an energy imbalance in the body. Simultaneously, an increase in the activity of liver-specific enzymes – alkaline phosphatase, alanine aminotransferase, and gamma-glutamyl transferase – was detected in the blood serum of these animals, indicating destructive changes in hepatocyte membranes and the development of cholestasis. During the post-mortem examination, the anterior loops of the small intestine were found to be distended with gas and fluid content, but macroscopic signs of inflammation were absent. Samples of the feed components did not meet the requirements of the normative documentation regarding the quantity of sulphite-reducing clostridia – *Clostridium perfringens*, suggesting the possible influence of their toxins on the rabbit organism.

A promising avenue for future research involves identifying the specific types and quantities of *Clostridium perfringens* toxins that cause mortality in rabbits, as well as developing effective preventive measures.

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None.

Conflict of Interest

None.

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Діагностичні дослідження за ентеротоксемії кролів

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Анотація. Актуальність дослідження зумовлена проблемами ентеротоксемії у кролів, що викликається *Clostridium perfringens* в умовах промислового кролівництва. Мета дослідження – визначення причин захворюваності та підвищеної летальності в молодняку кролів у період дорощування. У роботі поетапно проведено аналіз зразків корму, клінічне обстеження кролів, вирощених в умовах промислової кролеферми, та лабораторне дослідження отриманого від них біологічного матеріалу. Крім того, для встановлення діагнозу та надання господарству відповідних рекомендацій щодо утримання та профілактики захворювань кролів аналізували стан їх здоров'я, причини розладів травлення з симптомами діареї та підвищеної смертності в умовах виробництва. Використовували клінічні, гематологічні, патологоанатомічні, мікробіологічні та статистичні методи дослідження. У хворих кролів встановлено підвищення ректальної температури тіла, симптоми діареї, в окремих тварин – напади судом. Частина тварин загинула. Під час лабораторного дослідження крові кролів виявили помірну анемію зі зменшенням кількості еритроцитів і зниженням вмісту гемоглобіну та порушення якісного складу еритроцитів, зокрема наявність пойкілоцитозу, змінених форм еритроцитів: акантоцитів, ехіноцитів, шистоцитів, дакріоцитів, кератоцитів і дрепаноцитів. Зниження імунного статусу

організму кролів характеризувалося зменшенням у крові загальної кількості лейкоцитів, лімфоцитів, низьким співвідношенням нейтрофілів до лімфоцитів. Під час біохімічного аналізу сироватки крові кролів встановлено зниження рівня глюкози нижче межі фізіологічних коливань та підвищення активності ферментів – аланінамінотрансферази, лужної фосфатази і гамма-глутамілтранспептидази. Результати патологічного розтину кролів, що загинули, свідчили про наявність переповнених газами передніх петель тонкого кишечнику, з рідким умістом та без макроскопічних ознак запалення. У кормі, яким годували кролів у господарстві, виявлено сульфітредукуючі клостридії – *Clostridium perfringens*. Визначення причин захворюваності та летальності кролів надасть можливість підібрати належні методи профілактики падежу в умовах конкретного господарства

Ключові слова: ентеротоксин; анемія; розлади травлення; морфологічні дослідження крові; біохімічні дослідження крові

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Clinical and morphological analysis of splenic neoplasms

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Abstract. The relevance of this study arises from the pressing need for a deeper investigation into the morphological aspects of identifying focal and localised lesions in the spleen. The spleen performs several vital functions, including immune, haematological, storage, metabolic,

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haemostatic, hormonal, and antiblastic roles, among others. Therefore, this study aimed to determine the clinical characteristics of splenic tumour lesions and their structural changes, as well as to identify the macroscopic appearance of the organ post-splenectomy. In diagnosing splenic diseases, a key instrumental method was employed – ultrasound examination with Doppler imaging – which enabled comprehensive investigation, analysis, and characterisation of parenchymal pathologies. Animals underwent clinical examinations during routine health checks or upon presentation of symptoms. When a splenic neoplasm was detected, surgical treatment was carried out, specifically through an open laparotomy and splenectomy. The surgery was performed under combined general anaesthesia (using both non-inhalational and inhalational anaesthetics). The intraoperatively collected material from the affected organ was subjected to histological examination. It was found that the pathomorphosis of splenic lesions varies, and the author's pathological and histological studies highlight the key morphological characteristics of the neoplasms. Accordingly, any splenic neoplasm can pose a life-threatening condition for several reasons: organ rupture and haemorrhage, organ torsion, and malignancy, among others. The findings are crucial for verifying splenic pathology, enabling the establishment of a definitive clinical diagnosis, facilitating treatment of the animal, and extending its lifespan. The practical significance of the results lies in identifying the need for comprehensive clinical and morphological investigations in diagnosing splenic diseases, as well as examining histological preparations for cellular and tissue changes. In veterinary medicine, the method of surgical treatment for splenic lesions in animals may be a subject of debate. In contrast, in human medicine, established protocols exist for performing organ-destructive operations such as splenectomy (by open or laparoscopic approach), organ-preserving procedures like splenic resection or splenectomy with autotransplantation of splenic tissue into the greater omentum (by open or laparoscopic approach), and percutaneous punctures for splenic cysts

Keywords: ultrasound examination; lymphogranulomatosis; myelolipoma; structural changes; laparotomy; endothelial cells; non-Hodgkin lymphoma

Introduction

According to the classification provided by A.Y Mazurkevich *et al.* (2023), mesenchymal-origin neoplasms are categorised into tumours of fibrous, nervous, adipose, muscular, and synovial tissues, as well as of vascular origin and myxomas. In dogs, fibromas, fibrosarcomas, leiomyomas, haemangiomas, and malignant fibrous histiocytomas are more common. In cats, fibrosarcoma predominates, often associated with the feline fibrosarcoma virus. Vascular-origin tumours are relatively frequently diagnosed in dogs, ranking 1st to 3rd among soft tissue neoplasms. The average age of affected animals is 8-9 years, with an almost equal gender ratio. These tumours typically localise in the neck, torso, and limbs (with the pelvic limbs being affected 2.6 times more often than the thoracic limbs). The spleen, like soft tissues, is also frequently affected. This is a heterogeneous group of tumours. According to the classification from the 1990s, the following are distinguished: angioma, angiosarcoma, epithelioid haemangiosarcoma, spindle-cell haemangiosarcoma, malignant endovascular papillary angioendothelioma (Dabska tumour), haemangiopericytoma, and retiform haemangiosarcoma. A distinctive feature of the latter is the presence of long, branching vessels

lined by monomorphic endothelial cells and pronounced lymphoid infiltrates. Cytological differentiation of these tumours is not always possible. The vascular origin of the tumour is indicated by the presence of blood vessels in the material for examination.

J.M.M. Rodriguez *et al.* (2020) conducted histological and immunohistochemical investigations of a haemangioma found in the spleen of a grey wolf (*Canis lupus lupus*) and compared the results with those obtained in dogs. The authors note that there are relatively few published reports on the pathology of the spleen in carnivores. The results show similar characteristics to dogs, in which neoplastic cells express vimentin, von Willebrand factor, antibodies to smooth muscle actin and endothelial growth factor. Special staining with toluidine blue demonstrated a moderate increase in the number of mast cells infiltrating the tumour. The study focuses on this particular feature of the neoplasms.

In the studies of M.V. Utechenko (2022) and M. Quinci *et al.* (2020), it was highlighted that one of the reliable methods for differentiating focal splenic lesions is the use of ultrasonography results, microscopy, and imaging of neoplastic processes, which assist in determining treatment strategies and prognosis. A review of the specialised literature reveals differing views on the functional role of the spleen in mammals. Some researchers do not classify this organ as essential for survival, although they do not deny its significance for the proper functioning of the body. When pathological processes affect the spleen, splenomegaly is often observed, which may result from the acute or chronic impact of a pathological factor.

S. Griffin (2021) highlights the importance of ultrasound examination of the abdominal organs, especially when splenic neoplasms are discovered incidentally. The author emphasised that diagnostic accuracy is quite high when performed by a skilled operator. However, all detected splenic neoplasms, whether localised or diffuse, are indications for surgical intervention.

G. Corvera *et al.* (2024) demonstrated the critical role of the spleen in the body, performing at least three essential functions: eliminating foreign particles and bacteria from the blood; participating in immune responses; and producing blood cells when bone marrow haematopoiesis is suppressed, as seen in myeloid metaplasia of the spleen, which occurs as a restoration of the haematopoietic function originally active during embryogenesis.

The spleen also plays a role in stress adaptation. Through the activity of beta-adrenoreceptors, its capsule contracts, releasing stored erythrocytes into the bloodstream, thereby increasing the blood's oxygen-carrying capacity. In some animal species, the spleen functions solely as an organ of lymphopoiesis. During embryogenesis, it acts as a mixed lymphomyeloid structure and regulates the cytological composition of the blood.

N. Kim *et al.* (2022) found that following a splenectomy, the body's resistance to bacterial and viral agents decreases, leading to complex changes in both the humoral and cellular branches of the immune system. This includes a reduction in the clearance of particulate antigens, inhibition of phagocytic activity, and suppression of antibody production in response to non-specific antigens. Alterations in the spleen's filtration function result in hypercoagulopathy and thrombocytosis, with an increased risk of excessive thrombus formation post-surgery, which is explained by elevated levels of TNF-α and interleukin-6 in the blood, as well as overproduction of platelet antibodies. The immune response is also weakened, and IgG catabolism increases. Post-splenectomy immune disorders involve reduced levels of IgG1 and IgG4 fractions, as well as lower concentrations of fibronectin and IgM, accompanied by impaired functional ability of neutrophil granulocytes, monocyte cytotoxicity, and shifts in lymphocyte population ratios.

Consequently, the authors emphasise the importance of differential diagnosis in the study of splenic neoplasms.

Based on previous research reflected in specialised publications, this study aimed to conduct post-operative histological studies of splenic neoplasms detected by ultrasound.

Literature Review

In their study, T. Fossum *et al.* (2019) demonstrated that the spleen plays a crucial role in animals by producing blood cells, which, under conditions of myeloid metaplasia, support the restoration of haematopoiesis. A. Biriş *et al.* (2019) noted that splenic tumours are common in dogs and are often diagnosed during routine screening using ultrasound examination of the abdominal organs, as well as in patients showing clinical symptoms of the disease. Statistical data were collected from April 2007 to June 2018, indicating that splenic tumours accounted for 6% (97 cases) of the total (1,629 cases) neoplasms examined. Histologically, malignant tumours were found in 89% of cases, benign tumours in 11%, with haemangiosarcoma diagnosed in 75% and splenic lymphoma in 22%. Of the 97 splenic tumours included in this research, 64 were diagnosed as primary haemangiosarcoma, with 56% exhibiting metastases in various organs of the body.

According to the research of J.M. Connors *et al.* (2020), Hodgkin's lymphoma is classified as a B-cell lymphoma. The clonal proliferation of so-called Hodgkin and Reed-Sternberg cells, which belong to the B-cell population and are surrounded by reactive cells, occurs primarily in the lymph nodes before spreading to the spleen and other organs. The aetiology of the disease remains unknown. In human medicine, the condition is predominantly observed in adolescents and young adults, although it can also affect the elderly. Diagnosis is based on histological and immunohistochemical examination of tissue following a biopsy. Morphological changes in the tissue and the antigen expression profile allow for the classification of the lymphoma subtype (nodular sclerosis, mixed cellularity, lymphocyte-depleted, or lymphocyte-rich lymphoma). A thorough understanding of the pathology is essential for determining the approach to multimodal therapy, including radiation and multi-agent chemotherapy.

G. Corvera *et al.* (2024) determined that in dogs, the spleen functions as a secondary lymphoid organ where both neoplastic and non-tumorous nodular formations of varying sizes can develop. The research aimed to characterise several focal lesions and identify risk factors specifically associated with tumour development. A total of 507 histological reports were examined. Hemangiosarcoma and hyperplasia were most commonly observed in Cocker Spaniels, German Shepherds, and Labrador Retrievers. The collected data were analysed using descriptive statistics and multiple logistic regression. Similarly, E.J. Herman *et al.* (2019) reported that hemangiosarcoma is a prevalent tumour in geriatric dogs. To confirm the pathohistological changes, it is necessary to examine the entire organ. M.A. Griffin *et al.* (2021) suggested that hemangiosarcoma in dogs and cats originates from pluripotent bone marrow with a multifactorial pathogenesis. In dogs, the disease is recorded more frequently than in cats. According to the authors' studies, affected animals have an unfavourable prognosis with rapid metastasis. The primary treatment method is complete splenectomy combined with chemotherapy based on anthracyclines. J.M. Rozolen *et al.* (2021) reported that splenic hemangiosarcoma is a malignant tumour arising from endothelial cells and predominantly affects middle-aged and older dogs. The tumour leads to extensive necrosis and haemorrhages within the organ's parenchyma. Their study utilised 53 samples of splenic tumour

tissue from dogs that had been previously diagnosed through histopathological examination, which were fixed in formalin and embedded in paraffin. The expression levels of Claudin-5, PSMA, and Ki67 proteins were evaluated using immunohistochemistry, while gene expression was assessed through quantitative polymerase chain reaction. Overexpression of Claudin-5 was observed in patients with metastases and those with stage III tumours compared to patients with stages I and II. The findings indicated that the expression of Claudin-5 protein is associated with the metastatic process, whereas the expression of the PSMA gene and Ki67 protein correlates with survival time.

F.J. Leyva *et al.* (2018) histopathologically investigated the characteristics of concurrent neoplasms of the spleen and liver in dogs that underwent complete splenectomy and biopsy. The study included 125 patients (dogs), of which 27% (34/125) showed no signs of malignant neoplasms, while 48% (60/125) had malignant neoplasms of both the liver and spleen. Notably, 56 of these 60 dogs presented with identical malignant neoplasms in both organs.

The relevance of splenectomy in cases of diffuse splenic lesions was discussed by A. Ampese *et al.* (2022). The authors noted that myelolipoma of the spleen is a benign neoplasm composed of adipocytes and haematopoietic tissues. In comparison to the incidence of tumours such as haemangiosarcoma, fibrosarcoma, haemangioma, and lymphoma, myelolipoma is relatively rare. Typically, the disease remains asymptomatic in animals. In some cases, symptoms such as vomiting, abdominal distension, and signs of pain were observed. The presence of a neoplasm was confirmed through ultrasound diagnostics. In cases of significant neoplasms and splenomegaly, adjacent internal organs, including the liver, pancreas, and kidneys, may become compressed. The surgical treatment for myelolipoma involves total splenectomy L. Cassaro *et al.* (2021) reported that myelolipoma can occur in both dogs and cats and may develop in various organs and tissues. A clinical case of splenic myelolipoma in a dog was described, detailing its clinical-pathological status and ultrasound examination findings. Ultrasound imaging revealed splenomegaly with formations along the entire length of the parenchyma, exhibiting mixed echogenicity and heterogeneous echostructure, likely due to the mixing of tumour components with adipose tissue. Macroscopically, the spleen appeared enlarged, with the presence of irregularly shaped nodules on the surface of the organ, which were red with small yellowish areas. Microscopically, a well-defined encapsulated formation was observed, consisting of differentiated neoplastic adipose tissue with islands of haematopoietic elements in varying proportions. J.A. Nitz *et al.* (2020) described a clinical case of splenic myelolipoma in a human patient. Ultrasound imaging, along with computed tomography, identified a splenic neoplasm with characteristics consistent with adipose tissue. Following a total splenectomy, a pathological examination of the organ was conducted, confirming the diagnosis. The authors emphasised that the differential diagnosis of splenic neoplasms containing adipose tissue, as seen on computed tomography, ranges from benign tumours to malignant mesenchymal origins.

J.L. Kutok & C.D.M. Fletcher (2005) established the existence of both benign and malignant tumours of the spleen. Benign tumour types include lymphangiomas, haemangiomas, and endothelial tumours, with osteomas, chondromas, and fibromas being exceedingly rare. Malignant tumours encompass specific types of sarcomas, such as lymphosarcoma, fibrosarcoma, angiosarcoma, and reticulosarcoma, among others. The authors focused on the differential diagnosis of vascular tumours of the spleen in their contemporary context.

The splenic angiomata were investigated by P.P. Arcuri *et al.* (2022). The authors noted that primary angiomata of the spleen are generally benign; however, instances of malignancy have been reported. They cited a case study involving a patient who underwent diagnostic investigations using both computed tomography with contrast agents and magnetic resonance imaging.

P.A. Cole (2012) and K. O`Byrne & G. Hosgood (2019) demonstrated that various diseases can lead to neoplasms of the spleen. Their research was based on ultrasonography and computed tomography. The comparisons were conducted across different breed sizes: small, medium, and large. They found that the prevalence of malignant and benign tumours did not significantly differ; however, small-breed dogs were more likely to have benign neoplasms. This information is important to consider during initial consultations with pet owners.

In summary, the literature indicates that splenic neoplasms are relatively common in animals. Pre-operative clinical examinations and post-operative histological studies are crucial for accurate diagnosis. Splenic lesions can be both benign and malignant.

Materials and Methods

The study was conducted between 2020 and 2023 at the "Universal Veterinary Care" clinic and the pathology department of the "Universal Clinic Oberih" medical centre in Kyiv. Thirteen illustrative clinical cases of splenic neoplasia were documented. All experimental procedures involving animals were conducted following the fundamental principles of bioethics, following the European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (1986) and the Procedure for Conducting Research and Experiments on Animals by Scientific Institutions (2012).

In particular, the results of surgical treatment for various species of animals are presented: an 11-year-old female Dachshund; an 11-year-old female Yorkshire Terrier; an 11-year-old female Jack Russell Terrier; a 10-year-old female French Bulldog; a 10-year-old male Yorkshire Terrier; a 10-year-old male Jack Russell Terrier; a 10-year-old male mixed-breed dog; a 10-year-old female Yorkshire Terrier; a 9-year-old female Pug; an 8-year-old male Yorkshire Terrier; a 3-year-old male cheetah (*Acinonyx jubatus*); a 6-year-old male cheetah (*Acinonyx jubatus*); and a 6-year-old female cheetah (*Acinonyx jubatus*).

All affected animals underwent clinical and instrumental examinations, including an ultrasound of the abdominal organs using the MyLabOneVet veterinary scanner (Esaote). The detected neoplasm was measured and characterised in terms of the echogenicity of the affected area, its relation to surrounding organs, and the blood circulation in the affected region was assessed using Doppler ultrasound.

The protocol for general anaesthesia used was as follows: an anaesthetic mixture was administered intramuscularly, consisting of Domitor Vet (medetomidine hydrochloride 1 mg/mL) at a dose of 0.01 mg/kg and Butomidor (butorphanol) at 0.1 mg/kg of the animal's body weight (Orion Pharma, Finland). Surgical intervention was conducted under isoflurane endotracheal anaesthesia (Iso-Vet, isoflurane 1000 mg/g, United Kingdom). For this purpose, the Komesaroff small animal closed circuit anaesthetic machine MINI-KOMV (Australia) was used, along with endotracheal tubes with a cuff.

The examination of animals under anaesthesia was conducted using clinical methods and a device that monitored pulse rate, respiratory rate, blood oxygen saturation, rectal body temperature, and arterial blood pressure (via an automatic cuff placed on the brachial area of the limb) known as InnoCare-Vet (Hungary).

The surgical procedure involved an open laparotomy of the upper midline abdominal wall along the linea alba. Small bleeding vessels were coagulated during the operation using bipolar forceps connected to the device EXBA-350M/120Б "Nadiya-2" (Ukraine). Ligation was applied to the larger vessels supplying the spleen. The surgical wound was closed in layers, primarily using simple interrupted sutures. Absorbable surgical suture material, Syntil No. 3 and 4, was employed for ligatures, soft tissue of the abdominal wall, and skin.

Subsequently, in pursuance of the aim, tissue samples were taken from the pathologically altered areas of the spleen and fixed in 10% neutral formalin for further investigation.

Subsequently, histotechnical processing of the material was performed using the STP120 Microm / Thermo Scientific (Germany) tissue processing machine of carousel type, which involved dehydration in a series of ascending concentrations of alcohol, followed by embedding in paraffin. Paraffin blocks were prepared using the EC350 Microm/Thermo Scientific (Germany) tissue embedding centre. From these blocks, serial sections were produced with a thickness of 3-5 μm using the HM340E Microm/Thermo Scientific (Germany) rotary microtome, which was additionally equipped with the STS Microm/Thermo Scientific (Germany) transfer system for sections. The samples were placed on standard glass slides, followed by deparaffinisation and histological staining in the HMS 740 Microm (Germany) staining machine. The staining protocol involved haematoxylin (Harris Haematoxylin Accified series S, Epredia, Spain)/ eosin (Eosin Y Alcoholic series R/ Epredia, Spain). Upon completion of the staining process, the tissue samples were mounted under glass using the mounting medium Micromount/DiaPath (Italy). Subsequently, the histological preparations were examined using light microscopy with an Olympus BX 46 microscope (Japan) equipped with an Olympus SC 50 camera (Japan), with sequential observations at magnifications of ×4, ×10, ×20, and ×40 (with a ×10 ocular).

Results and Discussion

According to patient medical histories, it was established that the symptoms of splenic neoplasms may not manifest clinically, or the disease could follow a subclinical course. In cases of malignant tumour development, the symptoms may be more pronounced, particularly with rapid metastasis. Clinical cases of 13 patients diagnosed with splenic neoplasms through ultrasonographic examination, followed by splenectomy and morphological studies, are presented.

Non-Hodgkin lymphoma. Dog, Dachshund, 11-year-old, female**.**

Medical history: Clinical symptoms included abdominal wall pain, with noted tension in the area. During clinical examination using ultrasound of the abdominal organs, the following findings were recorded: the spleen was not enlarged, crescent-shaped, with smooth contours and clear boundaries. The echogenicity was increased, and the structure was homogeneous. A mass, measuring 1.2×1.1 cm, was visualised in the middle third of the spleen. It had a homogeneous hypoechoic internal structure with irregular and unclear borders, and blood flow to the mass was not enhanced (Fig. 1). A laparotomic splenectomy was performed. Macroscopic changes: The spleen was not enlarged, palpably not compacted, with a 1.2×1.1 cm nodular growth of light grey tissue with unclear margins, penetrating into the normal splenic parenchyma (Fig. 2). Morphological changes: The splenic parenchyma was histologically normal, with typical white and red pulp organoid structure. Tumour growth focus: a focus of proliferating lymphoid tissue forming atypical follicular structures,

composed of neoplastic cells of the lymphoid series. These cells were of medium size, with well-defined nucleoli, and sporadic figures of mitotic divisions were observed (Fig. 3). This histological pattern is indicative of non-Hodgkin lymphoma.

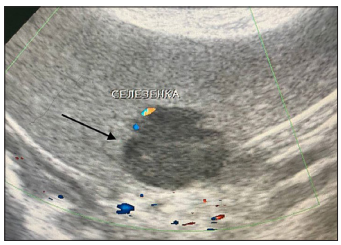

Figure 1. Ultrasound image of the neoplasm
***Note**: hypoechoic neoplasm measuring 1.2×1.1 cm*
***Sourse**: authors' material*

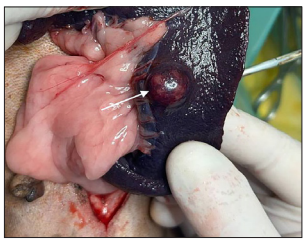

Figure 2. Intraoperative photo of the splenic neoplasm
***Note**: neoplasm localisation*
***Sourse**: authors' material*

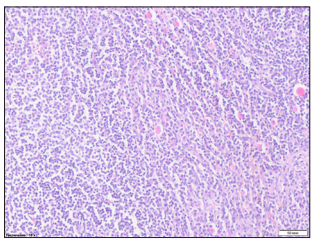

Figure 3. Atypical lymphoid proliferation in splenic tissue
***Note**: Haematoxylin-eosin staining, magnification ×100*
***Sourse**: authors' material*

Cavernous haemangioma. Dog, Yorkshire Terrier, 11-years-old, female.

Medical history: The splenic tumour was discovered incidentally during a dispensary examination. No clinical symptoms of the disease were observed. Ultrasound examination: The spleen was not enlarged, with a typical crescent shape, smooth borders, and well-defined edges. The echogenicity was increased, and the parenchymal structure was homogeneous. In the lower pole of the middle third of the spleen, a lesion measuring 1.8×1.6 cm with a heterogeneous internal structure and poorly defined borders was visualised, with no increase in blood flow to the lesion (Fig. 4). A laparotomic splenectomy was performed. Macroscopic changes: Palpation of the spleen was not compressed, blood-filled, with a soft-consistency neoplasm located superficially (Fig. 5). Morphological changes: Histologically, the spleen showed age-related involutive changes, such as widespread hyalinisation of the capsule and sclerosis of the organ's parenchyma. There were areas of proliferating thick-walled fibrous structures branching and anastomosing to each other. These septa were lined with endothelial cells without signs of cellular atypia. The formations corresponded to cavernous structures, with the lumens partially filled with blood (Figs. 6, 7). The histological findings are consistent with a cavernous haemangioma.

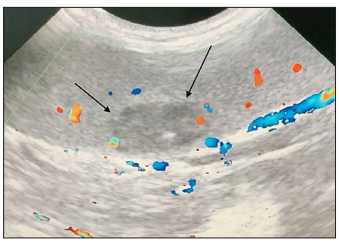

Figure 4. Ultrasound image of the splenic neoplasm
***Note**: neoplasm 1.8×1.6 cm*
***Sourse**: authors' material*

Figure 5. Intraoperative photo of the same splenic neoplasm
***Note**: neoplasm visualisation*
***Sourse**: authors' material*

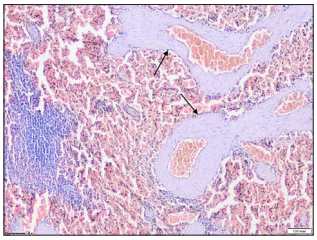

Figure 6. Proliferation of thick-walled septa lined with endothelial cells, forming cavernous structures
***Note**: Haematoxylin-eosin staining, magnification ×40*
***Sourse**: authors' material*

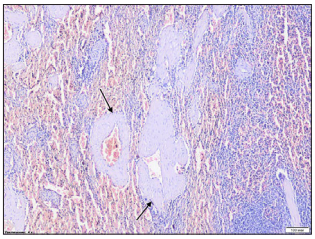

Figure 7. Proliferation of thick-walled septa lined with endothelial cells, forming cavernous structures
***Note**: Haematoxylin-eosin staining, magnification ×40*
***Sourse**: authors' material*

Neoplasm complicated by haemorrhage. Dog, Jack Russell Terrier, 11-years-old, female.

Medical history: The splenic neoplasm was detected during a dispensary examination of the animal via ultrasound examination. No clinical symptoms of the disease were recorded. Ultrasound examination: The spleen was neither enlarged nor displaced, maintaining its typical shape, with smooth contours and well-defined borders. The parenchymal structure was homogeneous, and coarse-grained, with unchanged echogenicity. In the mid-third of the spleen, a formation was found in the central pole, with clear contours measuring 1.5×1.4 cm, with a hypoechoic internal structure (Fig. 8). A laparotomic splenectomy was performed. Macroscopically, the spleen was not enlarged, palpably compacted, blood-filled, and a focal point with structural disorders was found in the parenchyma. On incision, this area revealed a disruption in tissue integrity due to the accumulation of unstructured, dark red masses (Fig. 9). Morphological changes: Histologically, fragments of splenic parenchyma showed areas of haemorrhage replaced by erythrocytes, with a focus of haemorrhage in the white pulp, and areas of capsular hyalinisation (Fig. 10). The histological presentation is consistent with haemorrhage.

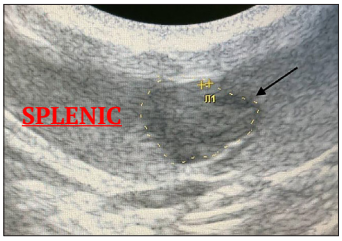

Figure 8. Ultrasound image of the splenic neoplasm
***Note**: neoplasm with mixed echogenicity 1.50×1.4 cm*
***Sourse**: authors' material*

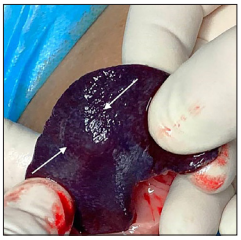

Figure 9. Intraoperative photo of the same splenic neoplasm
***Note**: visualisation of haemorrhage within the parenchyma*
***Sourse**: authors' material*

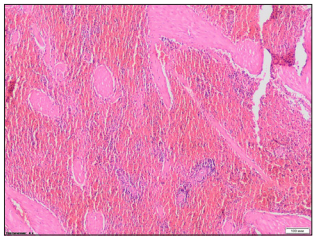

Figure 10. Spleen showing disrupted typical histostructure due to haemorrhage
***Note**: Haematoxylin-eosin staining, magnification ×40*
***Sourse**: authors' material*

Lymphangiohaemangioma. Dog, French Bulldog, 11-years-old, female.

Medical history: The animal underwent surgery for purulent endometritis three months

prior. The splenic neoplasm was detected during a dispensary examination. Ultrasound examination: The spleen was not enlarged, its shape was typical, with smooth contours and well-defined borders. The parenchyma appeared homogenous, and echogenicity was unchanged. In the lower pole, an oval-shaped neoplasm measuring 2.2×1.5 cm was detected, with indistinct borders and a hypoechoic internal structure. A laparotomic splenectomy was performed. Macroscopic changes: The spleen was not enlarged, with a doughy consistency on palpation and filled with blood. In the lower pole, a mass measuring 2.2×1.5 cm with a firm consistency was observed (Fig. 11). Morphological changes: Histologically, a tumour was found in the splenic parenchyma, consisting of proliferating thick- and thin-walled vessels with branching, narrow lumens. Some vascular spaces showed occlusion, while the majority were filled with lymph and blood. The endothelium exhibited signs of hyperchromasia and oedema (Figs. 12, 13). The histological findings are consistent with lymphangiohaemangioma.

Figure 11. Intraoperative photo of the splenic neoplasm
***Note**: neoplasm localisation*
***Sourse**: authors' material*

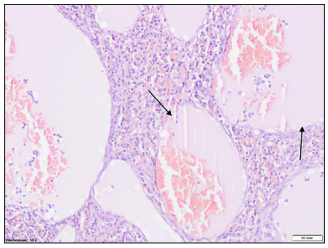

Figure 12. Splenic tissue with the proliferation of lymphangiohaemangioma, filled with lymph and blood
***Note**: Haematoxylin-eosin staining, magnification ×100*
***Sourse**: authors' material*

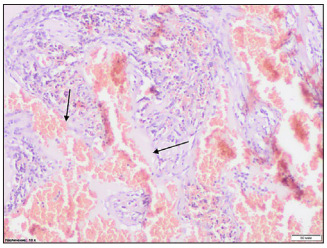

Figure 13. Lymphangiohaemangioma proliferation
***Note**: Haematoxylin-eosin staining, magnification ×100*
***Sourse**: authors' material*

Vascular malformation and haemorrhage. Dog, Yorkshire Terrier, 10-years-old, male.

Medical history: Clinical symptoms included abdominal discomfort, abdominal wall tremors, and a reaction to bimanual palpation. Ultrasound examination: The spleen was not enlarged, with a typical shape, smooth contours, and well-defined boundaries. Echogenicity was mixed, and the parenchymal structure appeared heterogeneous (Fig. 14). A laparotomic splenectomy was performed. Macroscopic changes: The spleen was palpably compacted with a focal structural disorder in the form of grey-brown foci with relatively clear contours (Fig. 15). Morphological changes: Histological examination of the splenic tissue revealed vascular malformation characterised by thick-walled vessels, branching and anastomosing with each other, alongside fields of haemorrhage with varying degrees of organisation. The white pulp was almost entirely displaced, with no signs of cellular atypia (Fig. 16). The histological findings indicate vascular malformation and haemorrhage.

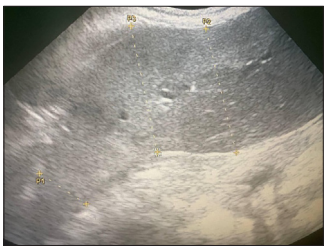

Figure 14. Ultrasound image of the splenic
***Note**: mixed echogenicity*
***Sourse**: authors' material*

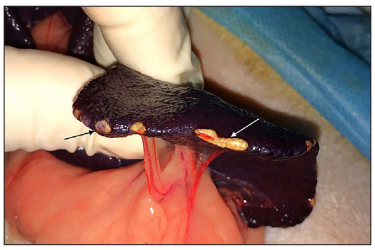

Figure 15. Intraoperative photo | of the same splenic
***Note**: isualisation of spleen tissue*
***Sourse**: authors' material*

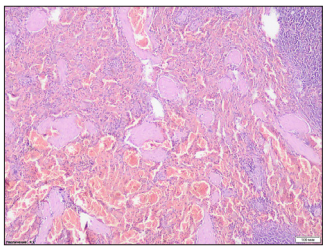

Figure 16. Spleen specimen demonstrating a focus of vascular malformation
***Note**: Haematoxylin-eosin staining, magnification ×40*
***Sourse**: authors' material*

Old haemorrhage. Dog, Jack Russell Terrier, 10-years-old, male.

Medical history: A splenic neoplasm was discovered incidentally during the animal's routine check-up. No clinical symptoms of the disease were recorded. An ultrasound examination of the abdominal organs revealed that the spleen was not enlarged or displaced, but a localised area with clear contours and altered echostructure was found within the parenchyma (Fig. 17). A laparotomy was performed for splenectomy. A laparotomic splenectomy was performed. Macroscopic changes: The spleen was not enlarged, had a doughy consistency upon palpation, and was filled with blood. An area measuring 2.5×2.3 cm was present in the middle third of the organ (Fig. 18). Morphological changes: Histologically, the typical architecture was disrupted due to the formation of a cavity, the walls of which comprised the parenchyma of the spleen, with areas of mildly expressed fibrosis. The cavity was filled with erythrocytes exhibiting signs of decay (Figs. 19, 20). The histological findings are characteristic of old haemorrhage.

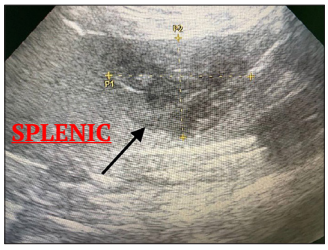

Figure 17. Ultrasound image of the splenic neoplasm
***Note**: neoplasm with mixed echogenicity 2.50×2.3 cm*
***Sourse**: authors' material*

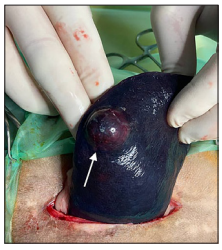

Figure 18. Intraoperative photo of the same splenic neoplasm
***Note**: visualisation of haemorrhage*
***Sourse**: authors' material*

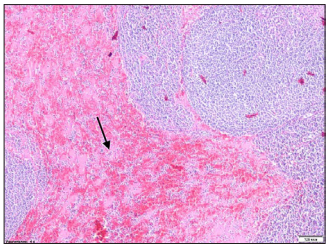

Figure 19. Spleen showing disrupted typical histostructure due to haemorrhage
***Note**: Haematoxylin-eosin staining, magnification ×100*
***Sourse**: authors' material*

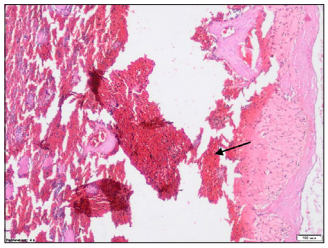

Figure 20. Subcapsular area of the spleen with disrupted typical histostructure due to haemorrhage
***Note**: Haematoxylin-eosin staining, magnification ×100*
***Sourse**: authors' material*

Splenic lymphoma. Dog, mixed breed, 10-years-old, male.

Medical history: The animal exhibited weight loss and intermittent vomiting, particularly in the morning on an empty stomach. During a clinical examination using ultrasound of the abdominal organs, echostructural signs of a neoplasm were observed in the parenchyma of the spleen. The spleen was not enlarged, retained its typical shape, had smooth contours, and clear margins. Echogenicity was increased, and the parenchymal structure was homogeneous. A formation with increased echogenicity measuring 1.8×1.6 cm was visualised in the upper pole of the parenchyma (Fig. 21). A laparotomic splenectomy was performed. Macroscopic changes: The spleen was palpably compressed and blood-filled, with a dense formation in the upper pole of the parenchyma measuring 1.8×1.6 cm (Fig. 22).

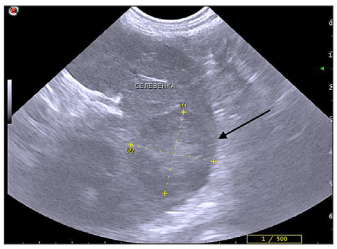

Figure 21. Ultrasound image of a splenic neoplasm (local changes in echostructure)
***Note**: neoplasm of the spleen with increased echogenicity*
***Sourse**: authors' material*

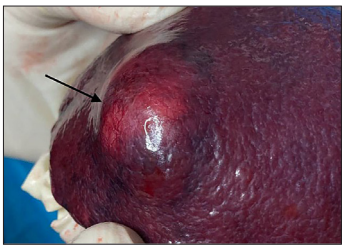

Figure 22. Intraoperative photo of the same neoplasm in the spleen
***Note**: localisation of lymphoma*
***Sourse**: authors' material*

Morphological changes: The histological structure of the spleen's parenchyma was completely disrupted due to the proliferation of neoplastic lymphoid-type cells, resulting in a diffuse growth pattern. The tumour cells were medium-sized, possessing a single enlarged nucleus with convoluted and diffuse chromatin, and exhibited figures of mitotic division. The cytoplasm of the cells appeared limited, amphiphilic, or weakly basophilic (Figs. 23, 24). The histological findings are consistent with lymphoma.

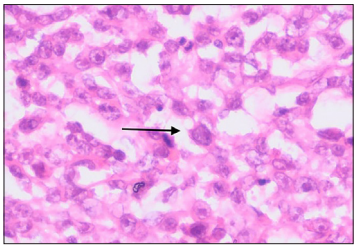

Figure 23. Spleen exhibiting disrupted typical histostructure due to the proliferation of pleomorphic neoplastic cells of lymphoid origin
***Note**: Haematoxylin-eosin staining, magnification ×200*
***Sourse**: authors' material*

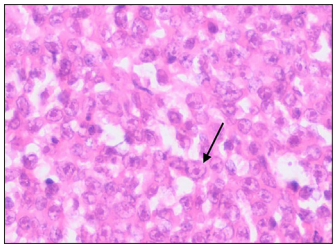

Figure 24. Spleen exhibiting disrupted typical histostructure due to the proliferation of pleomorphic neoplastic cells of lymphoid origin
***Note**: Haematoxylin-eosin staining, magnification ×100*
***Sourse**: authors' material*

Haemangiolymphangioma. Dog, Yorkshire Terrier, 10-years-old, female.

Medical history: No clinical symptoms of the disease were recorded. Ultrasound examination: The spleen was not enlarged, with a typical shape, smooth contours, and clear margins. Echogenicity was elevated, and the parenchyma exhibited a homogeneous structure. In the middle third of the organ, a formation measuring 1.1×1.0 cm was visualised, characterised by indistinct contours and a hypoechoic internal structure (Fig. 25).

A laparotomic splenectomy was performed. Macroscopic changes: The spleen was not enlarged, and the parenchyma was full of blood, with a moderately dense neoplasm palpable beneath the capsule (Fig. 26). Morphological changes: Histologically, the neoplasm was represented by a system of vascular structures separated by thin connective tissue septa. The lumens of the vessels varied in size, and the walls were lined with endothelial-type epithelial cells. The lumens of the vascular structures were partially filled with homogeneous eosinophilic material corresponding to lymph, and partially with erythrocytes (Figs. 27, 28). The histological picture is characteristic of haemangiolymphangioma.

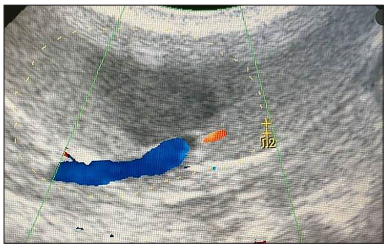

Figure 25. Ultrasound and Doppler examination of the spleen
***Note**: area with a hypoechoic internal structure*
***Sourse**: authors' material*

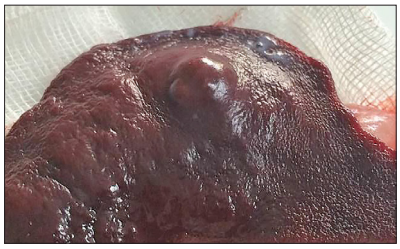

Figure 26. Intraoperative photo of the neoplasm in the spleen
***Note**: haemangiolymphangioma*
***Sourse**: authors' material*

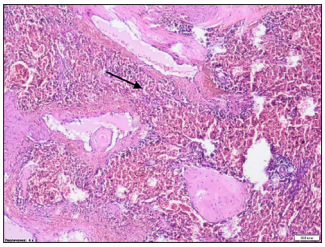

Figure 27. Tumour composed of lymphatic and blood vessel types
***Note**: Haematoxylin-eosin staining, magnification ×40*
***Sourse**: authors' material*

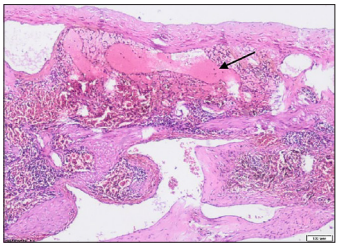

Figure 28. Structures of the haemangiolymphangioma that branch and anastomose with one another
***Note**: Haematoxylin-eosin staining, magnification ×40*
***Sourse**: authors' material*

Cavernous lymphangioma of the spleen. Dog, Pug, 9-year-old, female.

Medical history: Clinical symptoms of the disease were not observed. During a screening examination using ultrasound of the abdominal organs, a neoplasm was identified in the parenchyma of the spleen. A laparotomic splenectomy was performed. Macroscopic changes: The spleen was enlarged and engorged with blood, a compacted area with a hard consistency was palpated in the parenchyma (Fig. 29). Morphological changes: Histologically, the lumens of the vascular structures were filled with a homogeneous light eosinophilic substance corresponding to lymph, within which a few lymphoid cells were diffusely distributed, showing no signs of cellular atypia (Figs. 30, 31). The histological picture is consistent with cavernous lymphangioma.

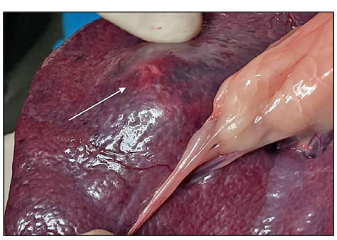

Figure 29. Intraoperative photo of the cavernous lymphangioma
***Note**: area with parenchymal damage*
***Sourse**: authors' material*

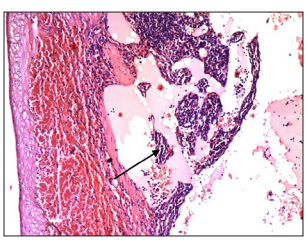

Figure 30. Cavernous lymphangioma of the spleen
***Note**: Haematoxylin-eosin staining, magnification ×100*
***Sourse**: authors' material*

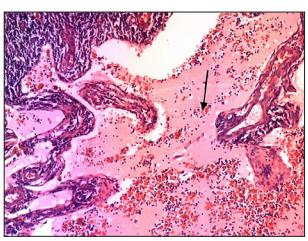

Figure 31. Cavernous lymphangioma of the spleen
***Note**: Haematoxylin-eosin staining, magnification ×100*
***Sourse**: authors' material*

Cavernous haemangioma. Dog, Yorkshire Terrier, 8-years-old, male.

Medical history: The animal exhibited no clinical signs of illness. A neoplasm in the parenchyma of the spleen was discovered incidentally during an ultrasound examination of the abdominal organs. Ultrasound examination: The spleen was not enlarged, and had a typical shape, with smooth contours and clear boundaries. Echogenicity was elevated, and

the structure of the parenchyma was coarse-grained and homogeneous. A formation measuring 1.02×1.3 cm was identified, with indistinct contours and an internal structure of mixed echogenicity (Fig. 32). A laparotomic splenectomy was performed. Macroscopic changes: The spleen was not enlarged, engorged with blood, and palpably not compacted. Under the capsule, a neoplasm was visualised that differed in colour from the parenchyma, exhibiting a soft consistency (Fig. 33). Morphological changes: Histologically, a tumorous formation was identified, comprising thick-walled vessels with cavernous structures. The inner walls of the cavities were lined with endothelial cells, the vessel is surrounded by serocytes, without histological features. The cavities of the formation were filled with erythrocytes exhibiting signs of decay, along with the formation of mural thrombi (Figs. 34, 35). The histological picture supports a diagnosis of cavernous haemangioma.

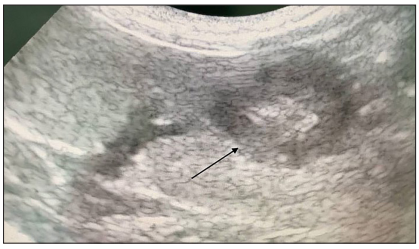

Figure 32. Echostructural changes in the parenchyma with a neoplasm of mixed echogenicity
***Note**: neoplasm 1.02×1.30 cm*
***Sourse**: authors' material*

Figure 33. Intraoperative material. Visual changes in the spleen
***Note**: localised cavernous haemangioma*
***Sourse**: authors' material*

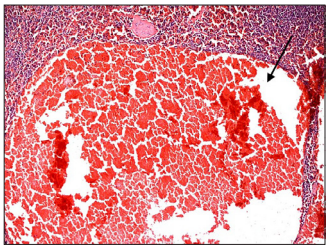

Figure 34. Cavernous haemangioma composed of dilated vessels forming cavities filled with blood
***Note**: Haematoxylin-eosin staining, magnification ×100*
***Sourse**: authors' material*

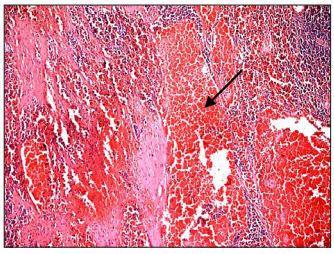

Figure 35. Cavernous haemangioma composed of dilated vessels forming cavities filled with blood
***Note**: Haematoxylin-eosin staining, magnification ×100*
***Sourse**: authors' material*

Lymphogranulomatosis (Hodgkin's lymphoma). Cheetah (*Acinonyx jubatus*), name: Casper, 3-years-old, male. Medical history: The animal exhibited a decrease in appetite, gradual weight loss, and digestive disturbances. During an abdominal ultrasound examination, diffuse neoplasms of varying sizes and mixed echogenicity were identified within the splenic parenchyma (Fig. 36). A laparotomic splenectomy was performed. Macroscopic changes: The spleen was not enlarged, exhibited insufficient blood supply, and had a doughy consistency.

Diffuse subcapsular neoplasms were present, which felt soft upon palpation (Fig. 37). Morphological changes: Histologically, there was the proliferation of lymphoid elements of varying degrees of maturity within the splenic tissue. Reed-Sternberg multinucleated cells and Hodgkin's single-nucleus cells were identified. The background was comprised of an infiltrate of lymphocytes and eosinophils. Tumour structures were partially separated by connective tissue strands (Figs. 38, 39). The histological appearance is characteristic of lymphogranulomatosis.

Figure 36. Ultrasound image of the diffuse lesion in the splenic parenchyma
***Note**: mixed echogenicity of the parenchyma*
***Sourse**: authors' material*

Figure 37. Intraoperative photo. Macroscopic changes
***Note**: diffuse lesions in the splenic parenchyma*
***Sourse**: authors' material*

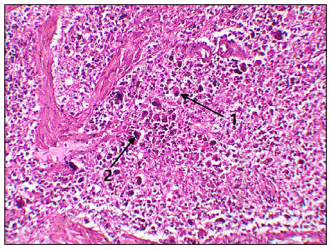

Figure 38. Lymphogranulomatosis (Hodgkin's lymphoma)
***Note**: a significant number of diagnostic cells: 1 – Hodgkin's cells and 2 – Reed-Sternberg cells. Haematoxylin-eosin staining, magnification ×200*
***Sourse**: authors' material*

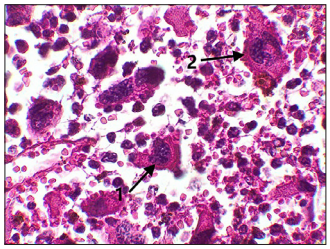

Figure 39. Lymphogranulomatosis (Hodgkin's lymphoma)
***Note**: a significant number of diagnostic cells: 1 – Hodgkin's cells and 2 – Reed-Sternberg cells. Haematoxylin-eosin staining, magnification ×1000*
***Sourse**: authors' material*

Myelolipoma. Cheetah (*Acinonyx jubatus*), name: Orion, 6-years-old, male.

Medical history: The animal exhibited limited physical activity, an increase in the contour of the abdominal wall, and dyspeptic disorders. Ultrasound examination revealed diffuse changes in the echostructure of the spleen, characterised by mixed echogenicity. A laparotomic splenectomy was performed. Macroscopic changes: The spleen was enlarged and firm, with diffuse neoplastic formations of solid consistency noted within the parenchyma (Fig. 40). Morphological changes: Histologically, the tumour tissue was composed of mature lipocytes with large, optically clear cytoplasm and a single eccentrically located nucleus, showing no signs of nuclear pleomorphism. Deposits of myeloid-derived cells were observed among the proliferates of mature lipocytes, with no evidence of cellular atypia (Figs. 41, 42). The histological appearance is characteristic of myelolipoma.

Figure 40. Intraoperative photo of the spleen
Note: *diffuse splenic lesions*
Sourse: *authors' material*

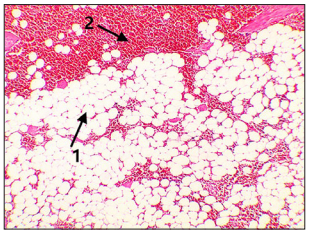

Figure 41. Myelolipoma. Proliferation of lipocytes and myeloid cells
Note: *1 – lipocytes; 2 – myeloid cells. Haematoxylin-eosin staining, magnification ×100*
Sourse: *authors' material*

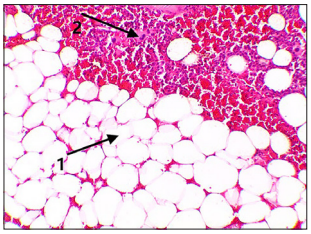

Figure 42. Myelolipoma. Proliferation of lipocytes and myeloid cells
Note: *1 – lipocytes; 2 – myeloid cells. Haematoxylin-eosin staining, magnification ×200*
Sourse: *authors' material*

Myelolipoma. Cheetah (*Acinonyx jubatus*), name: Ariel, 6-years-old, female. Medical history: No clinical symptoms of the disease were reported. The examination was carried out in a dispensary using ultrasound. Splenomegaly and solitary hyperechoic inclusions were detected in the splenic parenchyma (Fig. 43). A laparotomic splenectomy was performed. Macroscopic changes in the organ: The spleen was engorged with blood, enlarged, and had a doughy consistency upon palpation. Various-sized compacted inclusions were noted beneath the capsule (Fig. 44). Morphological changes: Histologically, the tumour tissue consisted of mature lipocytes with voluminous optically clear cytoplasm and a single eccentrically located nucleus, with no signs of nuclear pleomorphism. Between the proliferation of mature lipocytes, deposits of myeloid cells were observed, with no evidence of cellular atypia (Figs. 45, 46). The histological picture is consistent with myelolipoma.

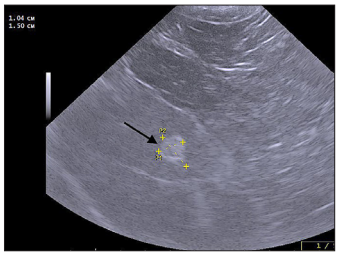

Figure 43. Ultrasound image of the neoplasm
Note: *area with a hyperechoic lesion measuring 1.04×1.5 cm*
Sourse: *authors' material*

Figure 44. Intraoperative photo of the spleen
Note: *hyperaemia, splenomegaly, and solitary neoplasms in the spleen*
Sourse: *authors' material*

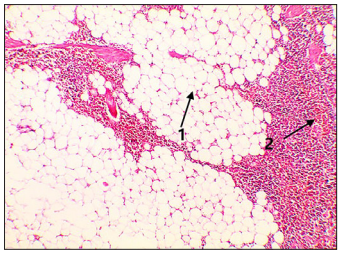

Figure 45. Lipoma (myelolipoma). Proliferation of lipocytes and myeloid cells
***Note**: 1 – lipocytes; 2 – myeloid cells. Haematoxylin-eosin staining, magnification ×100*
***Sourse**: authors' material*

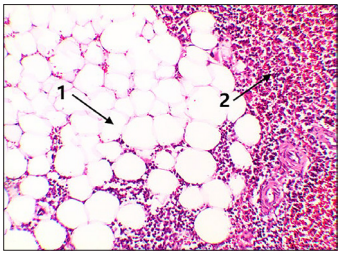

Figure 46. Lipoma (myelolipoma). Proliferation of lipocytes and myeloid cells
***Note**: 1 – lipocytes; 2 – myeloid cells. Haematoxylin-eosin staining, magnification ×100*
***Sourse**: authors' material*

Thus, the described results of ultrasound diagnostics, surgical interventions (splenectomy), and histopathological studies of the spleen are consistent with the findings of C.H. Spröhnle-Barrera *et al.* (2022), which state that the spleen can be affected by various tumours, such as tumour-like formations, vascular tumours, lymphoid tumours, and non-lymphoid tumours. Establishing a clinical diagnosis during the animal's lifetime remains challenging when dealing with pathological changes directly within the organ. Ultrasound diagnostics clearly revealed pathological structural changes, their size, quantity, and whether the organ was enlarged or reduced (hypersplenism or hyposplenism); however, the diagnosis is confirmed through morphological examination.

A. Auerbach *et al.* (2023) highlighted the challenges of differential diagnosis of splenic neoplasms, particularly regarding surgical biopsy methods and the complications that may arise following the procedure. The authors emphasised the importance of developing molecular-genetic markers for tumour identification. In their studies, patients in the postoperative period were provided with adequate antibacterial therapy and postoperative pain management. Special attention was given to wild animals, particularly cheetahs (*Acinonyx jubatus*), as postoperative access to these animals was limited (making it difficult to maintain surgical sutures). In the operated animals, the surgical wound healed by primary intention (*prima intentio*) on the 10^{th}-12^{th} day, without signs of complications.

A.M. Pîrvu *et al.* (2021) demonstrated the diversity of morphological changes using 194 cases of surgically removed spleens. The authors characterised the macroscopic appearance of the spleen in cases of splenomegaly, cystic, and tumourous pathological lesions. The study focused on postoperative cytological and histopathological modifications. In cases of splenomegaly, the absence of follicles and dendritic cells, as well as poorly developed fibrous trabeculae, were noteworthy. Additionally, foci of extramedullary haematopoiesis may be observed. In cavernous haemangiomas, the tumour was composed of vascular cavities resembling sinusoids of varying sizes and shapes, interconnected with one another. These cavities were lined by a single layer of compressed endothelial cells. Similar findings were obtained in this study. The authors also emphasised that such haemangiomas can lead to spontaneous splenic rupture and may give rise to the following vascular tumours: cavernous haemangioma, littoral cell angioma, multinodular haemangioma, capillary haemangioma, benign (infantile) haemangioendothelioma, diffuse

sinusoidal haemangiomatosis, angiosarcoma, and haemangiopericytoma.

According to Y.U. Ko *et al.* (2023), histopathological studies are crucial for predicting the course of angiosarcoma. Angiosarcoma was found to be the most common malignant vascular tumour of the spleen. Its microstructure is polymorphic, characterised by spindle-shaped, polygonal, or round cells forming vascular channels and slits. In these studies, angiosarcoma appeared macroscopically as a distinct haemorrhagic nodule, with a risk of organ rupture. Microscopically, large vascular cavities filled with blood were observed, lined by a thin layer of immature endothelial cells (tumoural angioblasts). Focal areas of loose connective tissue fibres were found between tumour cells. The neoplastic endothelium was loose, leading to frequent signs of wall integrity disruption within the tumoural vessels, which resulted in haemorrhages and necrosis. The nuclei of tumoural angioblasts were enlarged, polymorphic, and hyperchromatic.

One type of malignant transformation of lymphoid tissue is lymphogranulomatosis, or Hodgkin's lymphoma, characterised by the presence of Reed-Sternberg giant cells, which are identified during microscopic examination of affected lymph nodes. In this study, numerous Reed-Sternberg cell proliferations were found not only in the lymph nodes but also in the spleen, indicating an aggressive malignant progression and extensive spread of the disease.

Y.U. Ko *et al.* (2023) demonstrated that myelolipoma belongs to the category of non-lymphoid tumours and is significantly less common compared to other spleen neoplasms. This polyetiological disease is based on various toxic injuries to the spleen, such as infections, lymphatic system tumours, autoimmune and systemic diseases, haemolytic anaemias, and others. It leads to splenomegaly and replacement of the parenchyma with adipose and myeloid tissue. This condition poses a threat as it alters the organ's topography and structure, potentially leading to torsion or even rupture. In the research described in this study, myelolipoma was recorded in two cheetahs (*Acinonyx jubatus*) and confirmed morphologically. In one case, clinical symptoms characteristic of spleen neoplasms were observed, while the second case was subclinical. After surgical intervention (splenectomy), the symptoms in the affected animal disappeared.

Clinical and morphological studies of splenic lesions confirm that neoplasms can be of various types. Early detection, surgical intervention, and histological examination are crucial for the detailed characterisation of the pathological condition and saving the animal's life.

Conclusions

When neoplasms develop in the spleen of animals, it becomes necessary to verify the structural changes to refine the clinical diagnosis and, consequently, the prognosis. Currently, the primary diagnostic method for splenic neoplasms (such as cysts and tumours) in veterinary medicine is abdominal ultrasound, as it is the most accessible for patients. However, based on experience and the capabilities of ultrasound equipment, the nature of the changes can be inferred from the echographic image of the organ's structure. In human medicine, diagnostic computed tomography and magnetic resonance imaging are also widely used.

All methods of instrumental visual diagnostics were aimed at a more detailed study of the pathomorphological changes in the spleen and the development of individualised therapeutic strategies or surgical interventions. The conducted studies show that neoplastic processes in the spleen parenchyma appeared in most cases in adult animals, predominantly in the second half of life. In the case of diagnosed benign neoplasms, the possibility of

threatening conditions cannot be excluded, namely: rupture of the spleen capsule and leakage of the contained neoplasm into the abdominal cavity, which may lead to the development of haemoperitoneum or peritonitis; or the abscessing of the neoplasm if a bacterial infection occurs.

In cases where a malignant tumour or even signs of its presence are established, splenectomy is warranted. All detected neoplasms in the splenic parenchyma should undergo further morphological examination following the splenectomy. Post-splenectomy morphological studies provide the opportunity to establish a definitive clinical diagnosis and to predict the course of the disease, enabling the development of an individualised therapeutic strategy for patient management.

According to the conducted research, splenic neoplasms are often interpreted as "incidental findings" in dispensary ultrasound screening. In such cases, disease symptoms are absent. Timely surgical intervention and subsequent morphological examination can objectively contribute to extending the animal's life when benign neoplasms are present (such as haemangiolymphangioma, lymphangiohaemangioma, cavernous haemangioma, cavernous lymphangioma, haemorrhage, and myelolipoma). In cases of malignant neoplasms (lymphoma, lymphogranulomatosis, non-Hodgkin lymphoma), it has also been possible to manage the disease effectively and alleviate the recovery period.

In the future, it is planned to implement the developed experimental immunohistochemical techniques and oncological markers.

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None.

Conflict of Interest

The authors declare no conflict of interest.

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Клініко-морфологічний аналіз новоутворень селезінки

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Анотація. Актуальність дослідження на сьогодні зумовлена гострою необхідністю у поглибленому вивченні морфологічних аспектів ідентифікації вогнищевих і осередкових уражень в селезінці. Остання здійснює низку життєво необхідних функцій, а саме: імунну, гематологічну, депонуючу, обмінну, гемостатичну, гормональну, антибластичну тощо. Тому,

мета цього дослідження стосувалася визначення особливостей клінічного прояву пухлинного ураження селезінки та її структурних змін , а також визначення макроскопічної картини органу після спленектомії. У діагностиці захворювань селезінки використовували провідний інструментальний метод, такий як ультразвукове дослідження з доплерографією, що дозволило комплексно дослідити, проаналізувати та охарактеризувати патології в паренхімі органу. Тваринам, під час диспансеризації або за ознак захворювання проводили клінічне обстеження. Після виявлення новоутворення селезінки здійснювали хірургічне лікування, а саме, оперативне втручання методом відкритої лапаротомії та спленектомію. Операція проводилася під комбінованим загальним знеболюванням (неінгаляційні та інгаляційні анестетики). Відібраний інтраопераційно матеріал із ураженого органу підлягав гістологічному дослідженню. З'ясували, що патоморфоз уражень селезінки різний, а власні патологогістологічні дослідження висвітлюють основні морфологічні особливості новоутворень. Відповідно, будь-яке новоутворення селезінки може спричинити загрозливий стан з багатьох причин: розрив органу та кровотечу, скручування органу, малігнізацію тощо. Отримані результати мають вирішальне значення у верифікації патології селезінки, що дозволяє поставити заключний клінічний діагноз, провести лікування тварини та подовжити тривалість її життя. Практичне значення отриманих результатів полягає у виявленні необхідності комплексного проведення клінічних і морфологічних досліджень у діагностиці захворювань селезінки, а також дослідженні гістопрепаратів на предмет клітинних та тканинних змін. Дискусійним у ветеринарній медицині може бути метод хірургічного лікування в тварин уражень селезінки. На противагу, в гуманній медицині вже визначені алгоритми проведення органоруйнуючої операції, – спленектомії (відкритим або лапароскопічним доступом), органозберігаючої – резекція селезінки або спленектомія з аутотрансплантацією селезінкової тканини у великий сальник (відкритим або лапароскопічним доступом), через шкірні пункції при кістозних утвореннях селезінки

Ключові слова: ультразвукове дослідження; лімфогранулематоз; міелоліпома; структурні зміни; лапаротомія; ендотеліальні клітини; неходжкінська лімфома

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Clinical and morphological manifestation of the visceral form of candidiasis in the domestic dog (*Canis familiaris*)

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Abstract. The relevance of this research lies in the current lack of information regarding the pathological manifestations of fungal infections in the parenchymal organs of animals, the manifestations and the host's response to the fungus. Diagnosis of visceral mycoses is quite complex and insufficiently substantiated. A significant factor is the absence of clear criteria for pathological changes in visceral mycoses and differential diagnosis from similar diseases. This study aimed to reveal and establish the features of the clinical and

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morphological manifestation of the visceral form of candidiasis in the domestic dog (*Canis familiaris*). The study employed the following main methods: morphologic and biochemical blood analysis, post-mortem examination, and cytological and histological studies. When conducting biochemical analyses of blood serum in dogs, it was found that the glucose concentration was 2.17 mmol/L, which is almost three times lower than the lower limit of the norm, while the creatinine content exceeded the norm by four times and corresponded to a value of 560.4 mmol/L. As a result of serum analysis, an increase in the content of total and direct bilirubin was also recorded. In particular, the content of total bilirubin exceeded the physiological limit by 20 times and amounted to 222.68 μmol/L, while the level of direct bilirubin increased almost 10-fold and corresponded to a value of 59.4 μmol/L. The activity of aminotransferases, gamma-glutamyl transpeptidase, amylase, and alkaline phosphatase in the blood serum of sick animals increased significantly. Key pathological features of visceral mycoses in domestic dogs include haemolytic jaundice with extensive haemorrhages in organs and tissues. Hepatitis and nephritis, diagnosed in sick dogs, are the result of a generalised infectious process, the etiological factor of which is visceral candidiasis. The obtained data are of practical value for practising veterinarians in the issue of differential diagnosis of candidiasis, revealing the features of clinical and morphologic changes in the visceral form of mycoses in dogs

Keywords: mycoses; hepatitis; nephritis; haemolysis; haemolytic jaundice; pathological changes; *Candida albicans*

Introduction

Candidiasis is an opportunistic infection caused by yeasts of the genus *Candida*. According to studies by S. Allert *et al*. (2023), these fungi account for over 80% of yeast infections, and their prevalence has increased dramatically in recent years. K.G. Suprun (2020) and H. Hizlisoy *et al.* (2024) argued that due to the resurgence of diseases that weaken the immune system and the widespread use of immunosuppressive chemotherapy, there is a great deal of interest in *Candida* infections. The clinical spectrum of candidiasis ranges from superficial infections, such as candidiasis of the skin, gastrointestinal tract, and genital organs, to systemic diseases, including candidemia.

J. Lopes & M. Lionakis (2021) noted that yeasts of the genus *Candida* are the primary cause of these infections. Over 200 species of *Candida* have been identified, but only around 20 are responsible for infections in both animals and humans. Typically, these are commensal microbes that reside on the skin, inside the body, in the oral cavity, throat, intestines, and vagina without causing problems. They only exhibit their pathogenic potential when there are factors that promote the transition of an endogenous commensal into a disease-causing parasite. B. Beckwith-Cohen & S.M. Petersen-Jones (2024) found that these factors can be either intrinsic or extrinsic to the host.

Animals with obesity, those undergoing prolonged broad-spectrum antibiotic therapy and corticosteroid treatment, are more susceptible. Immunosuppression remains one of the most common risk factors. The resurgence of diseases that weaken the immune system, such as immunosuppressive treatments and intensive chemotherapy, has led to a dramatic increase in *Candida* infections, which have become a major cause of morbidity and mortality.

S. Headley *et al.* (2023) noted that yeast is a common component of the surface microorganisms of mammals. They inhabit external and internal moist surfaces, such as the skin, ear canal, conjunctival sac, mouth, digestive tract, and perineal area. Studies by J. Lopes & M. Lionakis (2021) have shown that among the various species of *Candida*, *C. albicans* is the most frequently encountered, being a commensal yeast found in the oral cavity, gastrointestinal tract, mucous membranes of the genitals, and skin. Antibiotic-induced dysbiosis, iatrogenic immunosuppression, or medical interventions that compromise the integrity of the skin-mucosal barrier and disrupt host defence mechanisms allow *C. albicans* to become an opportunistic pathogen, causing debilitating mucocutaneous diseases and life-threatening systemic diseases and infections. Depending on the site of infection, candidiasis can be superficial or systemic.

A. Rodrigues Hoffmann *et al.* (2023) established that lesions in mammals are often invasive and ulcerative. Systemic Candida infection is typically rare in dogs and cats. However, surgical procedures and trauma, such as foreign body ingestion, can lead to the penetration of the pathogen into deeper tissues or the abdominal cavity, resulting in granuloma formation or peritonitis in cats and dogs. T. Disha & F. Haque (2022) noted that in *non-invasive* forms of the disease, *Candida* species begin to actively proliferate in the intestinal lumen without penetrating the mucosal layer. At the same time, these microscopic fungi begin to release specific fungal toxins and poisonous fermentation products, leading to mucosal irritation. The effects of these toxic agents result in mycotic allergy and the development of secondary immunodeficiency.

In the *invasive form* of candidiasis, both local and systemic immunity are weakened. *Candida* species invade the intestinal epithelium (tropic to stratified squamous epithelium), penetrate deeper into the tissue, and transform into a filamentous form. Against a background of cell-mediated immune suppression, *Candida* fungi enter the bloodstream and spread throughout the body. In the context of significantly weakened immunity, visceral candidiasis is essentially an infectious disease of the gastrointestinal tract caused by the host's own fungal flora. The clinical presentation of candidiasis depends on the form of the disease and manifests as pain, intestinal meteorism, liquefaction of stools, ulcerative colitis, and fungal sepsis.

Difficulties in diagnosing the disease can be caused by nonspecific symptoms that mimic those of myocarditis, endocarditis, or endophthalmitis.

Therefore, considering the significant prevalence of fungal infections in animals and the severity of the disease, the study aimed to thoroughly investigate the pathological changes in the affected internal organs of dogs with intestinal candidiasis.

Literature Review

Over the past decade, there has been a surge in the incidence of mycoses (fungal diseases) in many countries worldwide, including Ukraine, while viral and bacterial diseases have become less prevalent (Alves *et al.*, 2023). T. Shimamura *et al.*, (2012) and S.P. Bouopda Tamo (2020) have demonstrated that neoplastic diseases, autoimmune disorders, primary bacterial or viral

diseases, metabolic disturbances, hypovitaminosis and avitaminosis, infectious and non-infectious diseases of the digestive and respiratory systems, the use of antibacterial agents, hormone therapy, immunosuppressive and cytostatics drugs, dysbiosis, hormonal imbalances, and primary and secondary immunodeficiencies are all endogenous factors that compromise the body's defences against mycoses.

The term "candidiasis" refers to a pathological process primarily characterized by the overgrowth of Candida in the gastrointestinal tract, as initially suggested by H.R. Conti *et al.* (2012), and secondarily in other organs, most commonly the bronchial mucosa and parenchymal organs, according to F.A. Uzal *et al.* (2016). E. Segal & M. Frenkel (2018) established that visceral mycoses (*Aspergillus, Candida, Mucor* and *Nocardia*) are frequently encountered in veterinary practice across various species and age groups, posing diagnostic challenges. The structure of individual visceral mycoses is as follows: *Aspergillus* accounts for 45% of cases, *Candida* for 35%, Mucor for 15%, and *Nocardia* for 5%. The development of candidiasis in dogs and cats, similar to humans, is associated with multiple factors, primarily a weakened immune system.

According to R. Arenas *et al.* (2012), data from the World Health Organization has shown that *Candida* spp. are the most common and prevalent causative agents of invasive mycoses in patients from intensive care units. The incidence of invasive candidiasis in patients varies from 1 to 10% depending on the unit's profile. The attributable mortality rate for invasive candidiasis is high, ranging from 10 to 47%. Additionally, researchers have noted high resistance to antifungal drugs used in modern therapy, as well as the emergence of new fungal pathogens.

Fungi of the genus *Candida* are widely distributed in the environment. They can be found in the air, soil, water, household items, and various animal and plant-based food products. *Candida* is a saprophytic fungus and a component of the natural microflora of the mucous membranes in animals and humans. T.J. Curiel (2008) indicated that fungal pathogens possess certain factors that can disrupt the host's immune defence mechanisms. The antigenic components of *Candida* have similar antigenic components to those of the host. This fact disrupts the formation of a protective immune response and can provoke autoimmune reactions. As stated by R. Tunca *et al.* (2006), in mycoses (*Candida albicans*, *Aspergillus fumigatus*), the main mechanisms of impaired host defences are their ability to suppress the protective activity of phagocytes and disrupt the processes of specific immune response formation. G.J. Galiza *et al.* (2012) found that suppression of the host's immune system occurs through direct inhibition of lymphocyte proliferation and mediator secretion, as well as through the activation of suppressor immune cells. Due to their powerful adaptive mechanisms, which are absent in most viruses and bacteria, fungi have proven to be well-adapted to survival in extreme conditions. E.P.F. Souto, E.P.F *et al.* (2018) distinguished three forms of mycosis:

1. The superficial form, which affects the mucous membranes of the oral and nasal cavities, the anus, external genitalia, the stratum corneum of the skin, and its derivatives;

2. The subcutaneous form, characterised by the involvement of deeper layers of the skin, the dermis, and/or subcutaneous fat;

3. The systemic form, which involves the spread of the infection to internal organs, manifests as systemic pathological changes.

Mucous membranes possess specific mechanisms that provide antifungal resistance. In addition to the normal microbiota, several other substances can inhibit the adhesion of fungi to epithelial cells, including lysozyme and secretory immunoglobulins such as sIgA.

Fungi can penetrate these protective barriers and enter the internal environment either through mechanical disruption or after colonising the barriers. It is important to note that humoral immune factors exert only a fungistatic, rather than a fungicidal, effect on fungi. Among the nonspecific cellular immune factors, phagocytes are of the greatest importance in protecting the body from fungal infections. Phagocytes interact with fungi through direct contact. Smaller fungal cells are engulfed by phagocytes and undergo intracellular killing. It has been shown that even massive colonisation by *Candida* can lead to the development of visceral or systemic candidiasis only in the presence of neutropenia.

A. Bufalari *et al*. (2016) noted that differentiating internal organ candidiasis from other diseases can be quite challenging, as the symptoms of candidiasis are non-specific. Therefore, comprehensive laboratory and instrumental studies are necessary. According to the research of A.R. Khosravi *et al*. (2009) and E.P. Souto *et al*. (2018), the visceral form of candidiasis is accompanied by severe gastrointestinal disorders. One of the internal factors contributing to reduced resistance and the development of fungal diseases is a decrease in the activity of serum fungistasis, which should inhibit the vital activity of yeast flora. It has been established that approximately half of all domestic animals have the conditionally pathogenic microorganism *Candida* on their mucous membranes. In the samples studied, *C. albicans* was the predominant species.

Consequently, the detection of fungal pathogens in the oral and vaginal cavities is quite common in veterinary medicine, often with asymptomatic courses. The number of carriers among dogs and cats is approximately the same, but the average number of *Candida* per infected animal is an order of magnitude higher in dogs. No sex-related predisposition to candidiasis has been found, but an age-related predisposition has been observed: the older the animal, the more frequent oral and vaginal candidiasis.

Materials and Methods

The study was carried out in the histopathological laboratory of the Department of Normal and Pathological Morphology and Forensic Veterinary Medicine at the Odesa National Agrarian University, and the "Animal Health" veterinary clinic (Hlevakha, Kyiv Region) during 2023. The subject of the study was the internal organs and tissues (blood, lungs, liver, kidneys, spleen, pancreas, heart, intestine) of a dog (8 years old) with spontaneous *Candida* infection. Morphological and biochemical blood analysis was performed using automated analysers. The results of the clinical blood analysis were obtained using a URIT-2900 Vet Plus haematology analyser (China), and biochemical parameters were determined using a VetScan VS2 analyser (USA). The complete blood count included the determination of the number of leukocytes, lymphocytes, intermediate cells, granulocytes, percentage of lymphocytes, percentage of intermediate cells, percentage of granulocytes, number of erythrocytes, mean erythrocyte volume, mean haemoglobin content in erythrocyte, mean haemoglobin concentration in erythrocytes, erythrocyte distribution width, haematocrit, platelet count, mean platelet volume, platelet distribution width, thrombocrit. The biochemical parameters of serum included the determination of glucose, total protein, albumin, urea, creatinine, triacylglycerols, cholesterol, total bilirubin, conjugated bilirubin, calcium, phosphorus, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), amylase, and alkaline phosphatase. Coagulation tests on native blood samples were also performed. All experimental procedures adhered to the main principles of bioethics outlined in the European

Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986) and the Procedure for Conducting Research and Experiments on Animals by Scientific Institutions (2012).

Post-mortem examination of the dog was conducted by full evisceration, following the generally accepted sequence. Before the necropsy, a visual examination of the deceased animal was carried out, assessing the general condition of the body, nutritional status, presence of injuries, and paying attention to the condition of the skin and mucous membranes of the respiratory tract, eyes, and oral cavity. After the necropsy, a visual examination of the internal organs and tissues was conducted with a detailed description and recording of the obtained data in a protocol. After a visual assessment of the condition of the internal organs, affected pieces of organs and tissues measuring 1x1 cm were taken for histological examination. Pieces of lung, liver, kidney, stomach, intestine, spleen, heart, and adipose tissue served as material for histological examination. Several pieces were cut at the border of healthy and affected tissue, taking into account the macro- and microscopic structure of the organs, and volumetric granulomas were completely excised. Subsequently, the taken material was fixed in a 10% solution of neutral formalin (pH 72-74). Some samples of the affected organs were prepared on a freezing microtome, and the main part of the pieces was embedded in paraffin and serial sections were prepared on a sliding microtome. In order to avoid errors and successfully carry out differential diagnosis between different types of fungal pathogens, as well as to assess the features of the macroorganism's reaction in response to the pathogen, sections were stained in several ways. Histological sections were stained with haematoxylin and eosin, according to Van Gieson, and according to Mallory. The general histological structure and microstructural changes in histological preparations were studied under a light microscope MC 100LED (Micros Austria).

To assess the cytological composition of the samples, biological material was collected, namely bronchoalveolar lavage, exudate from the mucous membranes of the mouth, and urogenital organs, obtained by deep scraping and impression smears. The obtained material was applied to a glass slide, stained with the special dye "Diff-Quik" (Croatia), according to the standard method, then the preparation was covered with a coverslip and examined under an MC 100LED microscope (Micros Austria).

Results and Discussion

During a clinical examination of an 8-year-old domestic dog, to conduct a planned general anaesthesia, liver and kidney dysfunction was detected. Following this, the animal underwent outpatient treatment. Less than a week passed from the clinically significant deterioration of the animal's general condition to its death. The preliminary diagnosis was liver cirrhosis (hepatitis).

The results of the general clinical blood analysis of the diseased animal are presented in Table 1.

Table 1. General clinical blood analysis of the dog

Indicator	Normal range	The first day of the study	The second day of the study
Leukocytes, x 10^9/L	6-17	27.4	44
Lymphocytes, x 10^9/L	0.8-5.1	25.3	22.3
Intermediate cells, x 10^9/L	0-1.8	1.2	6.0
Granulocytes, x 10^9/L	4-12.6	0.9	15.7

Table 1. Continued

Indicator	Normal range	The first day of the study	The second day of the study
Percentage of lymphocytes, %	12-30	92.3	50.8
Percentage of intermediate cells, %	2-9	4.2	13.7
Percentage of granulocytes, %	60-83	3.5	35.5
Erythrocytes, x 10^{12}/L	5.5-8.5	6.67	6.5
Haemoglobin, g/L	110-190	164.0	160.0
Mean erythrocyte volume, fl	62-72	67.8	66.4
Mean haemoglobin content in an erythrocyte, pg	20-25	24.6	24.7
Mean haemoglobin concentration in an erythrocyte, g/L	300-380	363	371
Distribution width of erythrocytes, CV, %	11-15.5	13.3	13.7
Distribution width of erythrocytes, MCV, fL	35-56	35.7	35.2
Haematocrit, %	39-56	45.2	43.2
Platelet, x 10^9/L	117-460	337	327
Mean platelet volume, fl	7-12.9	7.2	7.2
Platelet distribution width, %	10-18	17.4	17
Thrombocrit, %	0.1-0.5	0.243	0.234
Large platelet coefficient, %	13-43	4.5 ↓	5.1 ↓

***Source**: compiled by the authors*

On the first day of the blood examination, an increase in the number of leukocytes to 27.4×10^9/L was observed, compared to the normal range of 6-17×10^9/L. On the second day, the number of leukocytes in the blood increased by 1.6 times. The number of lymphocytes was three times higher than the upper limit of the normal range (normal range 12-30%). On the second day, the number of lymphocytes in the blood decreased by 12%. However, the number of lymphocytes decreased by 45% compared to the first day, which is almost twice the upper limit of the norm. At the same time, the number of granulocytes in the dog's blood increased 17-fold (normal range 4-12.6×10^9/L). The number of granulocytes on the first day of the examination was 3.5%, and on the second day – 35.5%, which is significantly below the lower limit of the normal range of 60-83%, at 17 and 1.7 times, respectively.

The number of intermediate blood cells (monocytes, eosinophils, basophils, and their precursors) on the first day of the examination was within the physiological range (normal range 0-1.8×10^9/L). On the second day of the examination, the number of intermediate blood cells increased 5-fold. The number of erythrocytes (Table 1), haemoglobin concentration, mean erythrocyte volume, mean haemoglobin content in erythrocytes, hematocrit, platelet count, thrombocrit, and other parameters of the clinical blood analysis on the first and second days of the examination were within the physiological range. At the same time, a decrease in the large platelet coefficient was observed: on the first day of the examination, it was 4.5%, and on the second day – 5.1%, which is 3 times lower than the lower limit of the norm, which was 13-43%.

The increasing incidence of fungal diseases in animals is primarily linked to the immunosuppressive effects of modern technological civilisation on animal organisms, which weakens the body's defences against infectious diseases. In recent years, there has been a noticeable trend towards increased carriage of

Candida due to the frequent use of antibiotics. The role of irrational antibacterial therapy in the development of this mycosis is substantiated by the suppression of the normal microflora of the animal host, which competes with *Candida* for mucosal receptors and glucose as a nutrient source. Broad-spectrum antibiotics (such as penicillin, streptomycin, amoxicillin, baytril, tetravet, etc.) and, especially, their combinations, play the most significant role in the development of dysbiosis. The use of antibiotics disrupts the vitamin balance in the animal's body (suppressing the vital activity of *Escherichia coli*, which actively participates in the synthesis and replenishment of various vitamins), leading to vitamin deficiency and promoting the development of candidiasis. The results of studies by S. Allert *et al.* (2018) also indicated that the likelihood of developing invasive fungal infections increases significantly in case of impaired immune system function, including immunodeficiency states, neutropenia, disorders of cellular immunity, age, and severe conditions against the background of the underlying disease. One may agree with the data of N. Willems *et al.* (2017), who proved that disruption of the endogenous microflora by antibiotics or immunosuppressive therapy, or disruption of the normal skin or mucosal barriers as a result of surgery, the installation of permanent catheters, or trauma, contributes to the penetration of *Candida* into the body. H.R. Conti *et al.* (2014) noted that primary susceptibility to yeast infections can be associated with diseases such as endocrinopathies, hyperadrenocorticism, diabetes mellitus, cirrhosis of the liver, neoplasms, and hypothyroidism.

On the second day of the examination, the animal underwent a biochemical analysis of its blood (Table 2).

Table 2. Results of the biochemical analysis of the blood of the affected animal

Indicator	Unit of measurement	Normal range	Result
Glucose	mmol/L	3.33-6.38	2.17
Total protein	g/L	55.1-75.2	58
Albumin	g/L	22-39	30.04
Total bilirubin	μmol/L	0.9-10.6	222.68
Conjugated bilirubin	μmol/L	0-5.5	59.4
ALT	U/L	8.2-57.3	938.96
AST	U/L	8.9-48.5	1376.84
GGT	U/L	1-10	19.92
Amylase	U/L	269.5-1462.4	4530.11
Alkaline phosphatase	U/L	10.6-100.7	286.59
Urea	mmol/L	3.1-8.5	8.33
Creatinine	μmol/L	44.3-138.4	560.4
Ca	mmol/L	2.2-3.0	2.68
P	mmol/L	0.7-1.8	4.4
Triglycerides	mmol/L	0.11-5.56	2.46
Cholesterol	mmol/L	2.9-6.5	1.27

***Source**: compiled by the authors*

Specifically, the glucose concentration in the dog's serum was found to be 35% below the lower limit of the normal range (3.33-6.38 mmol/L). Levels of total protein, albumin, and urea were within normal physiological limits. However, creatinine levels were four times higher than the upper limit of the normal range for a physiological serum concen-

tration of 44.3-138.4 μmol/L. Serum analysis revealed a significant increase in both total and conjugated bilirubin, 20 and 10 times higher than the physiological limits, respectively (total bilirubin 0.9-10.6 μmol/L, conjugated bilirubin 0-5.5 μmol/L). The cholesterol level in the serum was 2.2 times lower than the physiological parameters, which range from 2.9 to 6.6 mmol/L. Triacylglycerol levels remained within the normal physiological range of 0.11-5.56 mmol/L.

Regarding the mineral metabolism parameters of the diseased animal, the calcium level in the blood serum was within the physiological range (normal range 2.2-3.0 mmol/L). However, phosphorus levels were 2.5 times higher than the maximum normal value (0.7-1.8 mmol/L). The activity of all enzymes tested was significantly elevated. ALT activity increased 16-fold (normal range 8.2-57.3 U/L). AST activity increased 28-fold (normal range 8.9-48.5 U/L). GGTP activity increased 2-fold (normal range 1-10 U/L). Amylase and alkaline phosphatase activity increased nearly threefold, compared to the normal ranges of 269.5-1462.4 U/L and 10.6-100.7 U/L, respectively. As shown in Table 3, the coagulation profile of the dog's blood indicated that no clot formation occurred.

Table 3. Coagulation profile of the affected animal's blood

Indicator	Result	Unit of measurement	Normal range
International normalised ratio (INR)	No clot formed	-	Total: 0.8-1.0 Anticoagulant treatment: 2.0-3.5
Quick prothrombin time	No clot formed	%	Total: 70-130
Prothrombin time	No clot formed	sec	Total: 9-16
Activated partial thromboplastin time (APTT)	No clot formed	sec	9-18
Fibrinogen	No clot formed	g/L	1.0-3.7

Note: *no clot was formed, comparing the obtained results with the indicators of the normal range*
Source*: compiled by the authors*

The causative agents of systemic mycoses exhibit several common biological characteristics. Depending on temperature and growth conditions, they are capable of forming either mycelial or yeast thalli. They have a high degree of adaptation to parasitism: in natural conditions, at a temperature of 20-30°C, these organisms form mycelium, which feeds on dead organic matter. At a temperature of 37°C, corresponding to the conditions within the body, the pathogens shift to yeast growth, which is more efficient in semi-liquid, organic-rich environments. This characteristic is widely used in their diagnosis.

The findings of U. Binder *et al.* (2014) indicated that *Candida* species can remain localised on the skin and mucous membranes, causing superficial mycoses in immunocompetent hosts, or can disseminate via the bloodstream throughout the body, leading to systemic mycoses. J.C. Heseltine *et al.* (2003) noted that systemic candidiasis is reported in a limited number of cases in dogs. According to S. Seyedmousavi *et al.* (2018), most cases of systemic candidiasis in dogs, for which the species has been identified, are caused by *Candida albicans*. The authors also reported a case of disseminated candidiasis caused by *C. glabrata*

in two dogs with concurrent other systemic fungal infections.

According to a study by A. Khosravi *et al.* (2009), infected dogs exhibited clinical signs including fever, dyspnoea, and neurological symptoms, along with agitation. Congestion and petechial haemorrhages were observed in all visceral organs of the dogs. Small, multiple necrotic foci and greyish-white, nodular abscesses were detected in the lungs, liver, heart, brain, meninges, spleen, and kidneys. Microscopic examination for *Candida* revealed masses of branched, septate hyphae, pseudohyphae, and yeast cells (blastospores) ranging from round to oval, budding, and measuring 3-5 µm in diameter, within various tissues. Yeast forms predominated in most tissues, while filamentous forms, especially true *Candida* hyphae, were predominant in the brain and eye. Additionally, significant true hyphae were detected in the lungs, kidneys, and liver. Tissue cultures from various organs showed that in the study, the lungs contained the highest load of *C. albicans*. Furthermore, fungal load was observed in the tissues of the kidneys, heart, liver, brain, spleen, adrenal glands, lymph nodes, skeletal muscles, pancreas, eyes, and thymus.

S.A. Headley *et al.* (2023) were the first to report a case of mammary candidiasis in a dog. Cytological examination of purulent mastitis revealed *Candida* fungal hyphae within the lesions. A general examination of the dog revealed multiple lesions in the kidneys, liver, myocardium, pancreas, and brain. Histopathological and histochemical methods diagnosed fungal nephritis, hepatitis, myocarditis, pancreatitis, and encephalitis, associated with fungal hyphae within the lesions, often with fungal emboli and vasculitis. Pure cultures of *C. albicans* were obtained from fragments of lesions observed in the myocardium and kidneys. The study results suggested that the dog experienced an initial skin infection, which likely served as the entry point to the mammary gland, leading to mammary gland candidiasis and subsequent embolic dissemination to numerous organs. The results of this study confirm and expand upon the findings of the aforementioned authors.

A post-mortem examination revealed marked jaundice of the skin, serous membranes, and mucous membranes. The oral mucosa of the dog was yellow with haemorrhages and areas of thickening (mainly swelling of the gums). A distinct diffuse yellow colouring with a lemon tint was observed, along with small haemorrhages in the subcutaneous tissue and patchy bleeding in both superficial and deep muscles (Fig. 1).

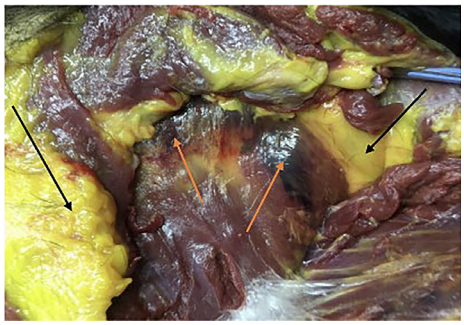

Figure 1. Icterus of the subcutaneous tissue and haemorrhages in the muscles
Note: *black arrows indicate the icterus (jaundice) of the subcutaneous tissue, while orange arrows show the location of haemorrhages in the muscles*
Source*: authors' material*

Up to 500 mL of dark red fluid with a brownish tint was found in the thoracic cavity. The serous membrane of the thoracic wall had a yellow tint and multiple haemorrhages located along the ribs and in the muscles of the ventral part of the thoracic spine (Fig. 2).

Figure 2. Icterus and haemorrhages on the serous membrane of the chest cavity
Note: *black arrows indicate icterus (jaundice) of the serous membrane of the chest, while green arrows show the location of haemorrhages on the serous membrane in the chest area*
Source*: authors' material*

Pericardial adipose tissue was abundant, yellow in colour, and contained numerous haemorrhages, ranging from pinpoint to blotchy. The heart membranes displayed a yellowish tint, with the epicardium and endocardium showing predominantly pinpoint haemorrhages. The endothelium of blood vessels also exhibited icteric discolouration. The ratio of the right to left ventricular wall was 1:4. The lungs had an uneven colour, containing haemorrhages, with flat, diffuse greyish-white foci observed under the pleura (similar signs were found under the liver capsule) (Fig. 3 A, B).

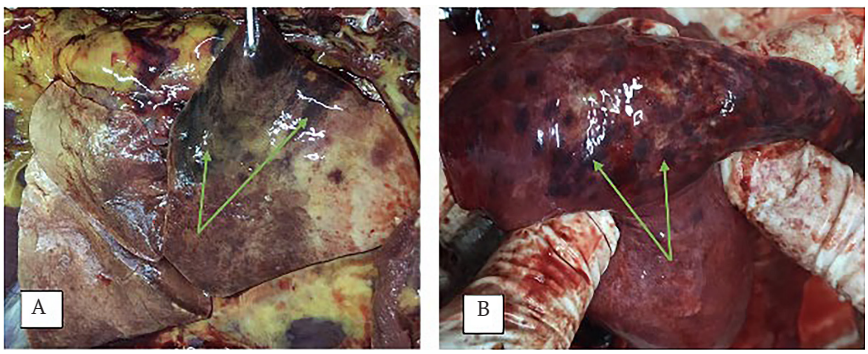

Figure 3. Overall appearance of the lungs from the pleura (A).
Multiple haemorrhages and fungal mycelium beneath the lung pleura (B)
Note: *arrows indicate the location of haemorrhages under the lung pleura*
Source*: authors' material*

In cross-sections, foamy, reddish-brown fluid was observed seeping from the lumens of certain alveolar areas. The tracheal and bronchial mucosa contained small clots of dark

red, semi-fluid material. Subpleural haemorrhagic lesions were also found in the lungs, extending deep into the parenchyma, along with accumulation of serofibrinous exudate in the alveolar spaces and marked infiltration of neutrophils, mononuclear cells, and macrophages in the alveolar walls. The abdominal cavity contained a fluid similar to that found in the thoracic cavity, with a volume of up to 300 mL. The serous membranes and visceral fat had a jaundiced appearance and contained haemorrhages. Multiple formations, flat and round with scalloped edges and a jaundiced colour, ranging in size from a grain of rice to larger, were noted on the surface of the mesentery (Fig. 4 A, B).

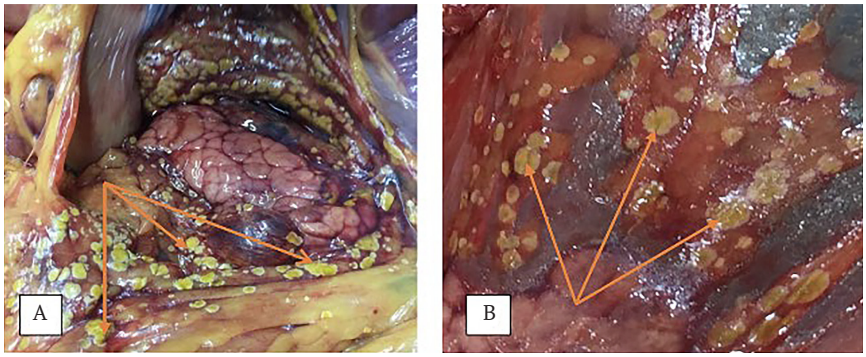

Figure 4. Generalised form of visceral mycosis (serous membranes of the abdominal cavity)
Note: *arrows indicate the location of the formations on the serous membranes of the abdominal cavity*
Source*: authors' material*

These formations consisted of granulomas with a central necrotic core surrounded by peripheral neutrophils, followed by epithelioid macrophages and multinucleated giant cells, enclosed by a layer of fibroblasts mixed with a moderate number of lymphocytes and plasma cells. Within the granulomas, myriads of intracellular and extracellular yeasts and extracellular hyphae were found. The liver was not enlarged. It appeared flabby in consistency with an uneven dark red colour, and under the capsule, there were flat, diffuse grey-white foci (similar findings were noted beneath the pleura of the lungs and the splenic capsule). The gallbladder was above average in fullness, with a dark brown, fluid bile. The pancreas was enlarged, gelatinous, and light with a jaundiced hue, with occasional patchy haemorrhages. The mesenteric lymph nodes were enlarged, displaying an uneven red colour, and the blood vessels were notably engorged.

The spleen was slightly enlarged, with a wrinkled capsule and a diffuse dark red colour, and produced a moderate scraping of the parenchyma. Up to 4 small, barely visible neoplasms were detected, becoming noticeable upon palpation. One of the neoplasms was round, up to 3 cm in diameter, with a firm consistency and a jaundiced colour, containing a fine network of blood vessels and had a structured appearance on the cut surface. Small formations in the form of grey cords were visible beneath the capsule (similar in colour and consistency to the lesions found in the lungs and liver) (Fig. 5A). The serous membrane of the mid and posterior sections of the digestive tract exhibited uneven red discolouration with a jaundiced tint and contained haemorrhages. The contents were below average, dark red, and semi-fluid. The mucosa

was red, with haemorrhages but remained intact. The adipose capsule of the kidneys showed diffuse jaundiced discolouration and contained haemorrhages. The renal cortex was clay-yellow in colour, with blood vessels in the medulla displaying prominent blood engorgement, and haemorrhages were observed. The bladder was empty, with a thickened wall, and both the serous and mucous membranes had a jaundiced tint and contained haemorrhages (Fig. 4B). The kidneys exhibited congestion, haemorrhages, tubular degeneration, and neutrophil infiltration. Numerous necrotic foci and an inflammatory reaction were found in the cortex, and to a lesser extent in the medulla, initially dominated by neutrophils and later by macrophages.

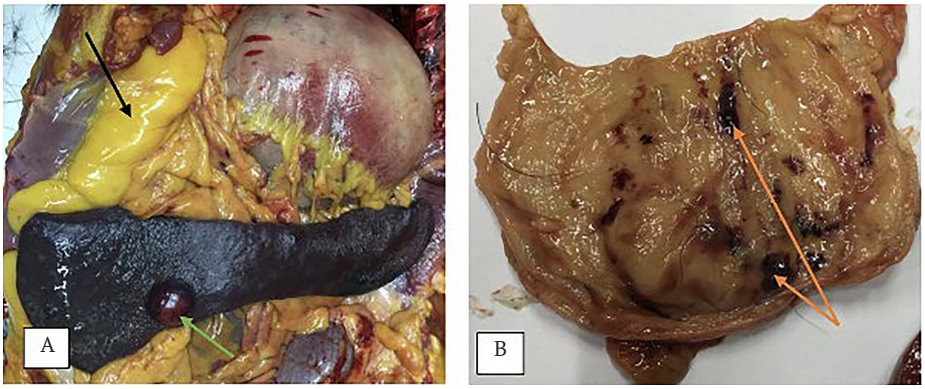

Figure 5. Neoplasms of the spleen (A). Bladder wall from the mucosal side (B)
Note: *in Figure 4A, the black arrow indicates visceral fat with a jaundiced tint, while the green arrow points to the location of the neoplasm in the spleen. In Figure 4B, the orange arrows indicate haemorrhages on the thickened bladder wall from the mucosal side*
Source*: authors' material*

Thus, the post-mortem examination revealed haemolysis of erythrocytes, leading to haemolytic jaundice with corresponding icteric and haemorrhagic manifestations in organs and tissues. The fungal mycelium (*Candida* species) was localised in the mesentery (diffuse form), lungs, liver, and spleen, contributing to the development of pneumonia, degenerative hepatitis, splenitis, and pancreatitis. The formation of dental calculus, candidiasis and neoplasms (tumours) in the spleen and liver may have contributed to the development of degenerative stomatitis. Nephrosis, impaired haemodynamics of the renal medulla, and haemorrhagic gastroenterocolitis were also present. Histological examination identified the neoplasms as predominantly haemangiomatosis of the spleen and liver (benign vascular tumours of the abdominal cavity).

Conclusions

In the case of a dog suffering from visceral candidiasis, serum analysis revealed a significant elevation in total bilirubin levels to 222.68 µmol/L, which was 20 times higher than the physiological range, and conjugated bilirubin reached 59.4 µmol/L, exceeding normal levels by 10 times. The serum creatinine concentration in this animal was 4 times increased, measuring 560.4 µmol/L, which is four times the normal range of 44.3-138.4 µmol/L. These changes are characteristic of liver and kidney dysfunction associated with visceral candidiasis.

A notable aspect of the infectious process in the domestic dog was the nearly 5-fold increase in lymphocyte count. A marker for fungal infection was found to be a decrease in granulocytes compared to the norm, down to 0.9 10^9/L, compared to the physiological range of 4-12.6 10^9/L. In this context, the relative proportion of granulocytes in the blood of the affected animal decreased 17 times below the lower limit of normal, which is 60-83%, resulting in a value of only 3.5%.

Lesions caused by visceral mycoses can be purulent, granulomatous, or mixed, influencing the variability of histopathological manifestations. More often, the body's response may be mixed. Candidiasis is rarely recorded as a standalone disease. It most commonly occurs against a background of other acute or debilitating chronic diseases and therefore presents as a mixed infection. In the examined dog, a severe manifestation of haemolytic jaundice with massive haemorrhages in organs and tissues, hepatitis, and nephritis were diagnosed, which are consequences of a generalised infectious process, the etiological factor of which is visceral candidiasis (mycosis of the serous membranes). Considering the rapid development of clinical manifestations and the characteristics (and severity of the lesions) of pathological changes in organs and tissues, the possibility of the disease co-occurring with viral diseases of carnivores cannot be ruled out. The established patterns regarding the clinical and morphological manifestations of visceral candidiasis in domestic dogs hold significant practical importance for veterinarians in terms of facilitating timely and effective diagnosis of this condition in this species.

Future research should focus on delineating the clinical and morphological characteristics of visceral candidiasis in cats.

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Conflict of Interest

None.

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Клініко-морфологічний прояв вісцеральної форми кандидозу в собаки свійського (*Canis familiaris*)

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Анотація. Актуальність дослідження обумовлена відсутністю на сьогодні інформації щодо особливостей патоморфологічного прояву грибкових інфекцій у паренхіматозних органах тварин, особливостей прояву і реакції макроорганізму у відповідь на дію гриба. Діагностика вісцеральних мікозів досить складна і недостатньо обґрунтована. Важливим фактором є відсутність чітких критеріїв патоморфологічних змін при вісцеральних мікозах та диференціальна діагностика від схожих хвороб. Метою цієї роботи було розкриття та встановлення особливостей клініко-морфологічного прояву вісцеральної форми кандидозу в собаки свійського (*Canis familiaris*). У роботі використовували такі основні методи: морфологічне і біохімічне дослідження крові, патологоанатомічний розтин та цитологічне і гістологічне дослідження. При проведенні біохімічних досліджень показників сироватки крові в собак виявлено, що концентрація глюкози становила 2,17 ммоль/л, що майже втричі менше нижньої межі норми, а вміст креатиніну в чотири рази перевищував норму і відповідав значенню 560,4 ммоль/л. У результаті дослідження сироватки крові також реєстрували зростання вмісту загального та прямого білірубіну. Зокрема, вміст загального білірубіну в 20 разів перевищував показник фізіологічної межі та становив 222,68 мкмоль/л, а рівень прямого білірубіну зростав майже у 10 разів

та відповідав значенню 59,4 мкмоль/л. Активність в сироватці крові хворих тварин амінотрансфераз, гамма-глутамілтранспептидази, амілази та лужної фосфатази зазнавала істотного підвищення. До особливостей патоморфологічного прояву вісцеральних мікозів у собаки свійського слід віднести гемолітичну жовтяницю з масивними крововиливами в органи та тканини. Гепатит та нефрит, які діагностували у хворих собак, є наслідком генералізованого інфекційного процесу, етіологічним чинником якого є вісцеральний кандідоз. Отримані дані становлять практичну цінність для практикуючих ветеринарних лікарів в питанні диференційної діагностики кандидозів, розкриваючи особливості клініко-морфологічних змін за вісцеральної форми мікозів у собак

Ключові слова: мікози; гепатит; нефрит; гемоліз; гемолітична жовтяниця; патологоанатомічні зміни; *Candida albicans*

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