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# Marker changes in the blood lipidogram in the pathogenesis of some internal diseases of horses

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**Abstract.** Disorders in lipid metabolism in the bodies of horses (genus Equus) due to various factors often lead to the development of diseases, including those associated with the onset of metabolic syndrome. Therefore, determining marker changes in the serum lipid profile during metabolic disorders is a relevant issue in investigating the pathogenesis of the most common diseases in this species. The purpose of the study is to identify the features of lipid metabolism indicators in the serum of horses in a physiological state and in the case of the onset of colic, laminitis, and metabolic syndrome symptom complexes. The investigation of lipid metabolism indicators in the serum of these animals was conducted using an enzymatic colorimetric method with the use of a biochemical automatic analyzer COBAS C 311 ("Roche Diagnostics GmbH", Germany). It is established that in the lipid profile of the serum of clinically healthy Ukrainian Hutsul horses, the share of high-density lipoprotein cholesterol was 75.1%, low-density lipoproteins were 15.7%, and

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very low-density lipoproteins were 9.2%. In cases of pathologies with colic symptom complex in the serum of horses, the share of high-density lipoprotein cholesterol significantly decreased, while low-density lipoproteins increased 3.9 times compared to healthy animals. In the lipid profile of the serum of clinically healthy ponies, the share of high-density lipoprotein cholesterol was 26.3%, low-density lipoproteins were 65.1%, and very low-density lipoproteins were 8.61%. In the case of laminitis in ponies, the dynamics of lipid profile indicators corresponded to those in horses. The established regularities regarding changes in the serum lipid profile in different representatives of the horse genus allowed specifying their role in the pathogenesis of certain prevalent pathologies. The results of the study are of practical value for application in clinical veterinary medicine and will contribute to conducting high-quality laboratory diagnostics of the most common horse pathologies (colic symptom complex, laminitis, and metabolic syndrome), characterised by lipid metabolism disorders

**Keywords**: pony; Ukrainian Hutsul breed; cholesterol; lipoproteins; colic symptom complex; laminitis; metabolic syndrome

## Introduction

Horse breeding is considered a substantial area within the agricultural sector and veterinary science. Modern horse breeding provides opportunities for the development of sports, tourism, and recreation and serves as a source of agricultural production. However, there is a negative trend in the development of the horse breeding industry. According to T.A. Yusiuk-Omelnytska (2023), in 2001, the total number of horses in Ukraine was 701.2 thousand, with 209.8 thousand on enterprises and 451.4 thousand in private households. By 2019, there was a significant decrease to 244.0 thousand, mainly due to a reduction in horses in private households. As of 2020, Ukraine has 79 institutions involved in breeding activities in horse breeding, including 20 horse farms, 46 breeding reproducers, 3 breeding centres, and 2 genetic control enterprises, as noted by O. Stetsiuk (2020). State horse farms concentrate 28.0% of the breeding population, while the rest is distributed among farms of various ownership forms.

Nevertheless, proper conditions for the maintenance and feeding of horses are often not observed, especially in recent years, which can have serious negative consequences for the animals. Issues such as insufficient body weight or, conversely, obesity, musculoskeletal problems, digestive system issues, weakened immunity, and nervous disorders can be observed in horses. Therefore, it is crucial to create suitable conditions for the housing, proper nutrition, and regular veterinary care of horses to ensure their health and well-being.

Among the most common pathologies in horses related to metabolic syndrome is laminitis. Researchers like A. Reynolds *et al.* (2019) noted equine metabolic syndrome, classified in 2010 by the American College of Veterinary Internal Medicine as insulin resistance, general or regional obesity, and susceptibility to laminitis. Equine metabolic syndrome is similar to that in humans. It is classified by features such as visceral obesity, hypertriglyceridemia, glucose intolerance, low cholesterol levels, and high-density lipoproteins. A.C. Ericsson *et al.* (2021) compared metabolic syndrome in horses with that in humans, allowing the application of methodologies already developed in medicine for diagnosis and differential diagnosis of metabolic syndrome in horses. The state of hyperlipidemia in horses can develop

in various forms. Therefore, different terms are used to differentiate them based on the severity of these diseases. In this context, four terms are proposed, determined by the concentration of triglycerides in the horse's blood serum: hypertriglyceridemia, hyperlipidemia, severe hypertriglyceridemia, and hyperlipemia. Hyperlipidemia usually results from a negative energy balance, primarily caused by feed restriction, especially during periods of high energy demand, such as pregnancy, lactation, or anorexia induced by illness. However, other findings suggest that obese and stressed mares pose the highest risk of developing hyperlipidemia regardless of pregnancy status.

There are several factors that can cause disruptions in energy metabolism in horses, but the most common cause is insulin resistance, as described in most publications, including Z. Daradics *et al.* (2021) and J. Delarocque *et al.* (2021). Various studies have been conducted to demonstrate that horses with insulin resistance are prone to hyperlipidemia; however, the etiology of this condition remains unknown. Hyperlipidemia most often arises as a primary sign. However, through clinical trials, it has been proven that its occurrence is secondary, resulting from the development of pathologies in various systemic diseases leading to a negative energy balance. Among the most common pathologies in miniature horse breeds are enterocolitis, dental diseases, bacterial and parasitic infections, pneumonia, colic, and laminitis.

Therefore, the purpose of this study is to investigate lipid metabolism indicators in the blood serum of horses and ponies under normal conditions and in the case of colic, laminitis, and metabolic syndrome.

## Literature Review

In the last decade, lipid metabolism in horses and other animals has become the subject of in-depth research in global veterinary medicine. J.J. Kaneko *et al.* (2008) and C.M.M. Loos *et al.* (2019) indicate that disorders in lipid metabolism are associated with diseases of the liver and biliary tract, kidneys, pancreas, and lungs. J. Delarocque *et al.* (2021) note that changes in lipid metabolism play a crucial role in the onset of dystrophic processes, endocrine pathology, and obesity, negatively affecting the animals' bodies and leading to the development of other pathologies. Equine metabolic syndrome has significant prevalence and is a current issue in horse breeding, as evidenced by a considerable number of publications. It is a complex disorder, with more questions than answers, as A.C. Ericsson *et al.* (2021) note that the main components of metabolic syndrome in horses are intense obesity, insulin resistance, and laminitis. This is complemented by the results of studies by N. Heliczer *et al.* (2017) and R. Lu *et al.* (2018), indicating that this syndrome encompasses a much broader range of disorders affecting energy metabolism, adipocyte function, promoting thrombosis, causing inflammation and oxidative stress, and altering the function of vascular endothelial cells in affected horses.

The role of adipose tissue in the development of metabolic syndrome is not limited to the excessive accumulation of nutrients. K. Marycz *et al.* (2018a; 2018b) show that adipokines released from adipocytes and other cells in adipose tissues, including leptin, resistin, adiponectin, visfatin, and apelin, as well as inflammatory cytokines, play a crucial role. A. Reynolds *et al.* (2019) state that disruptions in lipid metabolism, particularly enhanced lipid peroxidation, are one of the primary links in the stress response associated with metabolic syndrome. According to the research conducted in Ukraine, specifically by A. Andriichuk *et al.* (2014) and M.A. de Laat & D.M. Fitzgerald (2023), the causes of lipid metabolism disorders in farm animals were identified.

C.M.M. Loos *et al.* (2019) and Z. Daradics *et al.* (2021) developed and implemented sufficient diagnostic methods for this syndrome,

including biochemical ones. These methods include determining glucose, insulin, interleukins, and lipid exchange indicators in the blood, including lipoproteins. Researchers note that blood lipid profile indicators undergo specific changes and depend on the region, breed, and age characteristics of the animals.

Therewith, information regarding this problem in horses is lacking, although these animals often experience pathological conditions and diseases accompanied by disruptions in lipid metabolism, including lipoproteins – obesity, hepatic lipidosis, nephropathy, cachexia, tumors, lung diseases, and others. This is mentioned in individual studies by domestic researchers such as I.A. Zhukova (2014) and L.I. Posternak (2017).

R.B. Olley *et al.* (2019), N.P. Karikoski *et al.* (2022) and C. Cantarelli *et al.* (2018) emphasise that there is currently a lack of in-depth research on the role of lipid metabolism disorders in the development of pathologies in the internal organs of horses. The causative factors of these disorders may include the pathogenic influence of environmental factors, especially inappropriate conditions of maintenance and feeding, neurogenic stress, various physical, chemical, and other factors. Therefore, exploring the features of lipid metabolism disorders, determining the informativeness of their indicators for the diagnosis and assessment of the effectiveness of horse treatment for various internal diseases, is a relevant task for veterinary science and practice.

## Materials and Methods

During the study, Ukrainian riding breed horses and ponies housed at the State Biotechnological University Equestrian Sports Complex (Kharkiv) and at the Feldman Eco Park Public Organisation (Kharkiv region) were examined. Specifically, 10 Ukrainian riding breed horses, males and females, of sports direction, aged 6-10 years, in a state of relative rest (control group), 5 horses with colic syndrome, and 5 horses with symptoms of laminitis were examined. 5 clinically healthy ponies (control group) and 5 ponies with metabolic syndrome on the background of obesity were used for the study. Thus, a total of 30 animals were examined. Studies on these farms were conducted during 2021-2023. The research scheme included a minimal number of animals for statistical processing using non-parametric methods of statistical analysis, allowing the determination of the informativeness of indicators according to the stated research purpose.

Feeding and housing conditions corresponded to the physiological needs of the animals. The ration of animals was balanced with essential nutrients. All animals had free access to water and enjoyed walking. Animals underwent clinical examination according to generally accepted methods, including the determination of basic physiological indicators, the examination of major organs and systems using inspection, percussion, palpation, and auscultation methods. Only clinically healthy animals were selected for the control group. The diagnosis of metabolic syndrome, colic symptom complex, and laminitis was made comprehensively, considering the data of the medical history, clinical picture, and based on the conducted laboratory studies. In addition, for the diagnosis of metabolic syndrome, an oral glucose tolerance test with insulin was applied. Horses in the control group were examined during show jumping competitions, when their bodies were subjected to significant physical and emotional stress. Blood samples from the animals were obtained in a state of relative rest and 15 minutes after a entertainment show performance with a large audience and loud music accompaniment. Blood samples were taken from the jugular vein into Vacuette vacuum tubes with a volume of 10 $cm^3$ for further obtaining native blood, plasma,

and serum depending on the research methods for its biochemical analysis, which were conducted at the Research Institute of Experimental and Clinical Medicine, Kharkiv.

The research on biochemical indicators in the blood serum, including total cholesterol, triacylglycerols, high-density lipoproteins (HDL), low-density lipoproteins (LDL), and very low-density lipoproteins (VLDL), was conducted using the photometric system COBAS C 311 (Germany) with the corresponding ion-selective electrodes. Total cholesterol was determined using an enzymatic colorimetric method, where cholesterol esters are hydrolysed by cholesterol esterase into cholesterol and fatty acids. Cholesterol oxidase catalyses the oxidation of cholesterol to form hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide acts on the oxidative coupling of phenol and 4-aminophenazone to form a red-coloured compound, the intensity of which determines the concentration of cholesterol. Cholesterol in lipoproteins was determined using a homogeneous colorimetric method based on the use of cholesterol esterase and cholesterol oxidase in the presence of surfactants that selectively absorb certain types of lipoproteins.

During the experimental research, all manipulations with the horses involved in the studies were conducted considering the basic principles of bioethics, in accordance with the Law of Ukraine No. 3447-IV (2006, February), the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986), and the "General Ethical Principles of Experiments on Animals" adopted by the First National Congress on Bioethics (Procedure for conducting..., 2012).

Statistical analysis of the data was performed using the Minitab 19 program by Minitab Inc. The results of the statistical processing present non-parametric indicators in the tables, such as Median, Quartiles $Q_1$ and $Q_3$. The significant difference between the study groups was established based on the calculation of the Mann-Whitney criterion ($P<0.05$).

## Results and Discussion

The clinical condition of the horses before the start of the study corresponded to physiological parameters. The body temperature, pulse, and respiratory rate in horses were within the range of Me = quartile $Q_1$ and $Q_3$, respectively. Cardiac auscultation revealed no changes in heart tones and murmurs, and lung auscultation detected vesicular breath sounds throughout the projection of the lungs on the chest wall. Therefore, these animals were selected into the control group for further investigation.

According to the results of H. Gehlen *et al.* (2020), the composition of the blood lipid profile in animals of different species shows significant differences. For instance, in pigs, the quantitative distribution of cholesterol fractions is similar to that in humans, namely, the share of low-density lipoprotein cholesterol accounts for about 50-60% of the total serum cholesterol. In rabbits and mice, on the contrary, almost all serum cholesterol is part of very low-density and high-density lipoproteins. According to P.G. Xenoulis *et al.* (2020) and F.H. Alonso *et al.* (2022), in the blood serum of dogs, cholesterol in high-density lipoproteins is 5-7 times higher than in low-density lipoproteins. In horses, as reported by E.M. Norton *et al.* (2019), similar to dogs and unlike humans, the highest proportion of cholesterol is noted in high-density lipoproteins, preventing the development of atherosclerosis in animals of these species. These data indicate significant variability in lipid metabolism indicators in the blood serum of different animal species, necessitating further examination. It can be assumed that such variability may result from the influence of different diets and genetically

determined differences in the mechanisms of lipoprotein and cholesterol metabolism in animals of different species.

Table 1 presents the results of the study of the lipid profile in the blood serum of clinically healthy resting horses.

**Table 1**. Lipid profile indicators in the blood serum of clinically healthy horses, n=10 (mmol/L)

| Indicator | Total cholesterol | Triglycerides | HDL Cholesterol | LDL Cholesterol | VLDL Cholesterol |
|---|---|---|---|---|---|
| Median | 1.85 | 0.36 | 1.39 | 0.29 | 0.17 |
| $Q_1$ | 1.81 | 0.34 | 1.37 | 0.28 | 0.16 |
| $Q_3$ | 1.89 | 0.38 | 1.40 | 0.31 | 0.18 |

***Source**: developed by the author*

According to the results shown in Table 1, it was found that in clinically healthy horses, the share of HDL cholesterol was 75.1%, while LDL cholesterol was 15.7%, and associated with VLDL cholesterol was 9.2%. This indicator is consistently unchanging in all examined animals, both in terms of the median value and when calculating individual quartiles. This aligns with the findings of most other researchers. For example, J.J. Kaneko *et al.* (2008) indicated that in the bodies of clinically healthy horses, high-density lipoproteins play a crucial role in lipid metabolism. This is explained by their ability to transport cholesterol alongside other representatives of this class through the circulatory system's organ network. According to their model, high-density lipoproteins exhibit an anti-atherogenic effect through their role in the reverse transport of cholesterol by removing it from macrophages and incorporating it into the early forms of these lipoproteins, followed by the transformation of free cholesterol into esterified form (cholesterol esters). Esterified cholesterol is then extracted from the plasma by the liver through high-density lipoprotein receptors and transported to the bile for subsequent intestinal excretion. Thus, high-density lipoproteins can perform functions related to transporting cholesterol from tissues and the intima of blood vessels to the liver for the synthesis of bile acids and the elimination of excess lipids from the body. J. Delarocque *et al.* (2021) indicated that this mechanism provides regulation of cholesterol levels in the body. In addition, high-density lipoproteins exhibit antioxidant properties positively impacting the endothelium and the vascular system's condition. They also transport cholesterol for the synthesis of not only bile acids but also steroid hormones and vitamin D. This underscores the substantial functional aspects of lipid metabolism in horses, indicating the importance of high-density lipoproteins in maintaining effective cholesterol and other lipid exchange. Understanding these metabolic processes in clinically healthy animals can shed light on anomalies that occur in horses, especially those suffering from laminitis, where considerable changes in lipid metabolism and serum lipid profile are observed. Such studies open prospects for developing targeted therapeutic strategies to overcome characteristic lipid metabolism disorders affecting the development of internal diseases in horses.

It can be asserted that determining lipid metabolism indicators, along with other biochemical tests, should be applied not only in diagnosing internal diseases in horses but also

in assessing the condition of clinically healthy animals under physical or emotional stress, particularly during sports competitions or other stressful factors. In the current environment where demands on sports horses are steadily increasing, their bodies are not always able to withstand intense training and stress loads during competitions. Therefore, veterinary professionals need rapid and effective methods to assess the functional state and training capabilities of horses' bodies to maximise their adaptation to stress factors, adequately and timely correct possible disorders, and thus support their ability to endure intense loads and demonstrate high performance in competitions. This is precisely what prompted the determination of lipid metabolism indicators in sports horses during show jumping competitions.

The increase in the content of triglycerides in the blood serum is a specific indicator for exercising horses, as the primary source for working muscles during loading is the oxidation of free fatty acids released by their breakdown. Despite the consumption of free fatty acids by the muscles, the triglyceride content in the blood serum increases, and their synthesis in the liver also increases. Therefore, this increase is a specific sign for a horse undergoing emotional and physical stress during training. This elevation is a basis for a more in-depth analysis of lipid metabolism in sports horses and should be considered when assessing lipid exchange indicators in horses. Therefore, an examination of lipid exchange indicators in major pathologies associated with its disturbances has been conducted.

Among the pathologies in horses that manifest substantial changes in lipid metabolism, researchers prefer a group of diseases accompanied by a colic symptom complex, caused by a sharp change in the diet when transitioning animals from stable to pasture. The diagnosis of the colic symptom complex was established comprehensively. According to the history, it is known that the animals were transferred to green fodder, which included mown meadow grass, replacing hay. They eagerly consumed the green mass of mown grass, and in the evening, clinical signs of digestive disorders in the form of colic were observed. These animals showed restlessness, constantly lying down and getting up, hitting their abdomen with their limbs, making pendulum-like movements with their bodies. At the same time, an increase in pulse and respiration rates was noted. Upon palpation of the abdominal wall, tension and tenderness were detected. Auscultation revealed the absence of bowel peristalsis sounds, and percussion indicated excessive gas accumulation. After blood sampling, the animals were given symptomatic treatment and transferred to a different diet. The lipid profile indicators in the blood serum of horses with the colic symptom complex are presented in Table 2.

**Table 2**. Lipid profile indicators in the blood serum of horses with the colic symptom complex, n=5 (mmol/L)

| Indicator | Total cholesterol | Triacylglycerols | HDL Cholesterol | LDL Cholesterol | VLDL Cholesterol |
|---|---|---|---|---|---|
| Median | 2.55* | 0.38 | 1.19 | 1.15* | 0.21 |
| $Q_1$ | 2.32 | 0.36 | 1.09 | 1.05 | 0.18 |
| $Q_3$ | 2.93 | 0.46 | 1.32 | 1.29 | 0.32 |

***Note:*** ** P<0.05, compared to clinically healthy horses (Table 1)*

***Source****: developed by the author*

From the indicators presented in Table 2, it can be inferred that with the clinical manifestation of colic in the blood serum of horses, there was an increase in the concentration of total cholesterol by 37.8% (P<0.05) compared to clinically healthy animals (Table 1), mainly due to a 3.9-fold increase (P<0.05) in the concentration of low-density lipoprotein cholesterol. Therefore, with a sharp change in the diet of these animals, the reverse cycle of cholesterol metabolism is likely to be disrupted. As a result, the intensity of cholesterol entering tissues and vessels increases, further deposition under the intima occurs, and the functioning of internal organs is disrupted. However, for colic symptoms caused by a sharp change in the diet, there were no noteworthy changes in the blood serum level of triglycerides and, accordingly, very low-density lipoprotein cholesterol. Thus, hypercholesterolemia developed in this pathological condition due to low-density lipoprotein cholesterol against the background of a relatively stable level of triglycerides.

Another fairly common pathology among all breeds of horses is laminitis. Its pathophysiology is not yet fully understood and is contradictory. Chronic laminitis is accompanied by persistent lameness, breakdown of anatomical structures of the hoof, including changes in the laminae, prolapse of the sole, and disruption of hoof horn growth. There is a separation of the basal membrane of the hoof bone and the epidermal lamellae lining the inner surface of the hoof capsule. Only a very limited number of horses restore their athletic condition after experiencing chronic laminitis and can return to a normal training regimen. Laminitis more often occurs as a complication of pathologies of internal organs that seem unrelated to the limbs, among which the most common are gastrointestinal pathologies, septic metritis, pneumonia, pleuritis, insulin resistance, and others. According to A.C. Ericsson *et al.* (2021), laminitis can also occur against the background of hyperlipidemia. However, this mechanism in the pathogenesis of this pathology in horses is not sufficiently studied. Presumably, hyperlipidemia induces insulin resistance, suppressing the biological action of the hormone and reducing glucose intake into all cells of the body. This includes the lamellar keratinocytes of the horse's hoof, which have an extremely high glucose requirement.

Perhaps it is associated with hyperinsulinemia and "glucose starvation" of the hoof tissues, or perhaps with an increase in capillary pressure in the hoof vessels. Nevertheless, the final mechanism of laminitis development is not yet established. Therefore, five horses with clinical symptoms of laminitis were investigated, and a complex of biochemical indicators, including a lipid profile, was determined in their blood serum. The animals showed a change in gait and short steps, changes in body position, limb swapping to alleviate pain. Some animals had swelling in the hoof wall area, increased temperature in the hoof area. Unnatural positions were also noted; horses tried to reduce the load on the limbs, standing on the back of the hoof or even lying down. Difficulty in standing up was observed because horses with laminitis may have difficulty rising or even refuse to stand due to pain.

Considering this, the lipid exchange indicators in the blood serum of horses with laminitis were determined (Table 3). According to the research results, horses with laminitis showed a considerable increase in the serum concentration of total cholesterol by 34.1% (P<0.05) and low-density lipoprotein (LDL) cholesterol by 51.7% (P<0.05) compared to clinically healthy animals (Table 1). It is noteworthy that lipoproteins of this fraction transport cholesterol to tissues, and in normal circumstances, their level in the blood of animals is insignificant, unlike in humans, where this indicator depends particularly on dietary fat intake. The

elevation of cholesterol concentration in this fraction is a pathogenic factor in the development of internal diseases in animals, particularly laminitis in horses.

**Table 3.** Indicators of the lipid profile in the blood serum of horses with laminitis, n=5 (mmol/L)

| Indicator | Total cholesterol | Triacylglycerols | HDL Cholesterol | LDL Cholesterol | VLDL Cholesterol |
|---|---|---|---|---|---|
| Median | 2.48* | 1.02* | 1.56 | 0.44* | 0.48* |
| $Q_1$ | 2.27 | 0.83 | 1.49 | 0.39 | 0.39 |
| $Q_3$ | 2.77 | 1.07 | 1.68 | 0.55 | 0.54 |

***Notes:*** ** P<0.05, compared to clinically healthy horses (Table 1)*

***Source***: *developed by the author*

However, an increase in the level of high-density lipoprotein (HDL) cholesterol, where cholesterol concentration is usually highest in clinically healthy animals compared to other lipoprotein fractions, is not observed in horses. High-density lipoproteins are known to facilitate the reverse transport of cholesterol from tissues to the liver, where it serves as a source for bile acid formation, vitamin D, and steroid hormones. Horses with laminitis experienced a substantial increase in the concentration of triglycerides and very low-density lipoproteins by 2.8 times (P<0.05), indicating the development of hyperlipidemia (Table 3). This condition arises due to the increase in cholesterol concentration, which is a cyclic alcohol by chemical structure and a neutral fat represented by triglycerides. This phenomenon is not observed in colic symptoms. However, this hyperlipidemia does not lead to the direct deposition of fats on the epidermal lamellae covering the inner surface of the hoof capsule in horses, as is characteristic in large ruminants.

Thus, in horses, hypercholesterolemia likely leads to microvessel damage in the distal part of the limb, accelerating dystrophic and destructive disturbances in hoof tissues and contributing negatively to the development of the pathological process. However, all the pathogenetic links of this process are currently not definitively revealed. It is worth noting that direct deposition of triglycerides on the lamellae did not occur.

In horses, especially in ponies as their variety, metabolic disorders, primarily associated with obesity, are highlighted as a separate metabolic syndrome, often accompanied by laminitis, making it particularly important to inspect pathologies in horses. The existence of metabolic syndrome is considered established only in horses and ponies, and its presence in animals of other species remains an open question. For instance, N. Heliczer *et al.* (2022) noted that ponies with excess body weight exhibit large local fat deposits, especially in the withers and on the back, often accompanied by insulin resistance, a leading factor in the development of laminitis in horses, as described earlier. Moreover, the authors acknowledge that ponies, like horses, with metabolic syndrome are less prone to vascular complications, including the development of ischemic disease and atherosclerosis, compared to humans. This is primarily attributed to differences in lipoprotein metabolism, dietary habits, and different lifespans. The pathophysiological mechanisms linking metabolic syndrome to impaired insulin regulation and the onset of laminitis in these animals require further investigation. Various mechanisms are considered, including

the impact of reduced intensity of peripheral glucose metabolism on lamellar bone tissue, pro-inflammatory status associated with insulin resistance, accumulation of end products of glycation, effects mediated by insulin-like growth factor, vasoactive properties of insulin, and subsequent development of endothelial damage. Endothelial dysfunction likely plays a primary role in the development of laminitis. Nevertheless, whether laminitis in the presence of metabolic syndrome should be interpreted as a metabolic, cardiovascular, or inflammatory condition or as their combination remains a contentious issue.

Metabolic syndrome in ponies was diagnosed based on the analysis of anamnesis data, clinical presentation, morphometric indicators, and the results of biochemical blood serum studies. Insulin resistance in ponies was diagnosed using an oral sugar test, which is increasingly used by practicing veterinarians to assess insulin regulation disorders. Although this test quantitatively determines hyperinsulinemia and insulin regulation disorders in response to oral glucose levels, its results were used to assess insulin sensitivity in horses and ponies. In these animals, pancreatic exhaustion is rarely observed. Conversely, it begins to produce significant amounts of insulin. The peculiarity lies in the fact that insulin toxicity may play a key role in triggering laminitis when exceeding its maximum norm values (100 μIU/mL) by more than 3 times.

This aspect opens up new possibilities for further investigation and the development of treatment and prevention strategies for laminitis in ponies, considering the importance of metabolic syndrome in this process. Further exploration of the relationships between metabolic syndrome and the development of laminitis in these animals will contribute to the understanding of its mechanisms and allow the development of effective measures to preserve their health.

Table 4 provides the results of the examination of two groups of ponies – those without signs of obesity and those with massive fat deposits.

**Table 4**. Serum lipid profile indicators in ponies under normal conditions and with metabolic syndrome, n=5 (mmol/L)

| group No. | Total cholesterol | Triacylglycerols | Cholesterol LDL | Cholesterol HDL | Cholesterol VLDL |
|---|---|---|---|---|---|
| 1.Median | 2.09 | 0.39 | 0.55 | 1.36 | 0.18 |
| $Q_1$ | 1.96 | 0.37 | 0.49 | 1.31 | 0.16 |
| $Q_3$ | 2.24 | 0.42 | 0.60 | 1.45 | 0.19 |
| 2.Median | 4.57 | 0.62* | 1.98* | 2.30* | 0.29* |
| $Q_1$ | 4.31 | 0.58 | 1.85 | 2.21 | 0.25 |
| $Q_3$ | 4.81 | 0.69 | 2.09 | 2.39 | 0.33 |

***Notes:*** *1. – clinically healthy animals; 2. – obesity, metabolic syndrome; *P<0.05 compared between groups*
***Source****: developed by the author*

According to the data in Table 4, in the blood serum of clinically healthy ponies, the content of total cholesterol and triglycerides did not significantly differ from the indicators in clinically healthy horses (Table 1). However, unlike the latter, in animals without signs of obesity, a reverse correlation between the fractions of low-density lipoprotein cholesterol and high-density lipoproteins was observed – the amount of low-density lipoproteins exceeded

the content of high-density lipoproteins by 2.5 times. In healthy horses, on the contrary, the amount of high-density lipoproteins is 4.8 times higher than that of low-density lipoproteins. This indicates the existence of metabolic syndrome in animals, which may be analogous to that in humans. Defining metabolic syndrome in animals requires specifying their species affiliation, for example, metabolic syndrome in cats, dogs, horses, etc. In cases where there is insufficient objective evidence of the development of metabolic syndrome in animals, it is considered appropriate to use the term "metabolic dysfunction" to describe the condition of representatives of a certain species.

In the group of ponies with pronounced signs of obesity, significant hyperlipidemia was observed, confirming the presence of metabolic syndrome and being a risk factor for the development of laminitis. The increase in the levels of all lipid profile indicators in the blood serum of animals in this group confirms its importance in the formation of the specified pathological condition. The directionality of the ratio of low-density lipoprotein cholesterol to high-density lipoproteins was maintained, but an increase in the amount of low-density lipoprotein cholesterol by 1.2 times compared to high-density lipoproteins was noted (Table 4). This highlights the complex metabolic changes that occurred in the bodies of ponies with obesity and emphasises the need for further examination to uncover the molecular mechanisms of these changes and develop effective strategies for managing metabolic disorders in horses with obesity. Considering the complexity of the impact of these changes on the organism, further investigation will be a key stage in the development of therapeutic approaches and preventive measures for correcting metabolic disorders in the studied species. The obtained results on determining lipid metabolism indicators in horses and ponies with colic symptoms, laminitis, and obesity suggest the feasibility of a more in-depth investigation of the role of lipids and lipoproteins in the development of internal diseases in horses as informative diagnostic tests.

## Conclusions

The conducted study disclosed that the serum lipid profile in clinically healthy Ukrainian Hutsul horses was characterised by the following composition: the share of high-density lipoprotein cholesterol was 75.1%, low-density lipoproteins were 15.7%, and very low-density lipoproteins were 9.2%. In cases of colic symptom complex induced by a sharp change in the diet, horses experienced an acute inflammatory process accompanied by hypercholesterolemia, due to an increase in the content of low-density lipoprotein cholesterol, compared to clinically healthy animals, with no changes in the content of triglycerides, very low-density, and high-density lipoproteins.

For laminitis as a chronic inflammatory process, horses exhibited hyperlipidemia due to an increase in the concentration of triglycerides, total cholesterol, very low-density lipoprotein cholesterol, and low-density lipoproteins, compared to clinically healthy animals, with no changes in the content of high-density lipoprotein cholesterol. In clinically healthy ponies, the content of total cholesterol and triglycerides in serum did not significantly differ from the indicators in clinically healthy horses. However, in horses, quantitative indicators of high-density lipoprotein cholesterol were 4.8 times higher than those for low-density lipoproteins; in ponies, on the contrary, the amount of low-density lipoprotein cholesterol was 2.5 times higher than high-density lipoproteins. This supports the idea of a tendency to develop metabolic syndrome in ponies, similar in course to that in humans. Ponies with metabolic syndrome showed hyperlipidemia due

to an increase in both serum triglyceride and cholesterol content in all types of lipoprotein complexes of the lipid profile. In diseased animals, the content of low-density lipoprotein cholesterol exceeded high-density lipoprotein cholesterol by 1.2 times.

The results of determining lipid metabolism indicators, as informative diagnostic tests, for colic symptoms, laminitis, and metabolic syndrome in horses and ponies suggest the expediency of more in-depth further investigation of the role of lipids and lipoprotein complexes in the development of internal animal diseases.

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## Conflict of Interest

None.

## References

[1] Alonso, F.H., Behling-Kelly, E., & Borjesson, D.L. (2022). Lipoprotein profile of pleural and peritoneal transudates in dogs and cats. *Journal of Veterinary Internal Medicine*, 36(2), 464-472. doi: 10.1111/jvim.16369.

[2] Andriichuk, A., Tkachenko, H., Kurhaluk, N., Tkachova, I., & Vartovnyk, M. (2014). Blood biochemical parameters in horses involved in eventing under the influence of exercise. *The Animal Biology*, 16(1), 9-20.

[3] Cantarelli, C., Dau, S.L., Stefanello, S., Azevedo, M.S., De Bastiani, G.R., Palma, H.E., & De La Côrte, F.D. (2018). Evaluation of oral sugar test response for detection of equine metabolic syndrome in obese Crioulo horses. *Domestic Animal Endocrinology*, 63, 31-37. doi: 10.1016/j.domaniend.2017.10.006.

[4] Daradics, Z., Crecan, C.M., Rus, M.A., Morar, I.A., Mircean, M.V., Cătoi, A.F., Cecan, A.D., & Cătoi, C. (2021). Obesity-related metabolic dysfunction in dairy cows and horses: Comparison to human metabolic syndrome. *Life (Basel, Switzerland)*, 11(12), article number 1406. doi: 10.3390/life11121406.

[5] de Laat, M.A., & Fitzgerald, D.M. (2023). Equine metabolic syndrome: Role of the enteroinsular axis in the insulin response to oral carbohydrate. *The Veterinary Journal*, 294, article number 105967. doi: 10.1016/j.tvjl.2023.105967.

[6] Delarocque, J., Frers, F., Feige, K., Huber, K., Jung, K., & Warnken, T. (2021). Metabolic changes induced by oral glucose tests in horses and their diagnostic use. *Journal of Veterinary Internal Medicine*, 35(1), 597-605. doi: 10.1111/jvim.15992.

[7] Ericsson, A.C., Johnson, P.J., Gieche, L.M., Zobrist, C., Bucy, K., Townsend, K.S., Martin, L.M., & LaCarrubba, A.M. (2021). The influence of diet change and oral metformin on blood glucose regulation and the fecal microbiota of healthy horses. *Animals*, 11(4), article number 976. doi: 10.3390/ani11040976.

[8] European convention for the protection of vertebrate animals used for research and other scientific purposes. (1986). Retrieved from https://zakon.rada.gov.ua/laws/show/994_137#Text.

[9] Gehlen, H., Schwarz, B., Bartmann, C., Gernhardt, J., & Stöckle, S.D. (2020). Pituitary pars intermedia dysfunction and metabolic syndrome in donkeys. *Animals*, 10(12), article number 2335. doi: 10.3390/ani10122335.

[10] Heliczer, N., Gerber, V., Bruckmaier, R., van der Kolk, J.H., & de Solis, C.N. (2017). Cardiovascular findings in ponies with equine metabolic syndrome. *Journal of the American Veterinary Medical Association*, 250(9), 1027-1035. doi: 10.2460/javma.250.9.1027.

[11] Kaneko, J.J., Harvey, J.W., & Bruss, M.L. (2008). *Clinical biochemistry of domestic animals*. California: Academic Press. doi: 10.1016/B978-0-12-370491-7.X0001-3.

[12] Karikoski, N.P., Box, J.R., Mykkänen, A.K., Kotiranta, V.V., & Raekallio, M.R. (2022). Variation in insulin response to oral sugar test in a cohort of horses throughout the year and evaluation of risk factors for insulin dysregulation. *Equine Veterinary Journal*, 54(5), 905-913. doi:10.1111/evj.13529.

[13] Law of Ukraine No. 3447-IV "On Protection of Animals from Cruelty". (2006, February). Retrieved from https://zakon.rada.gov.ua/laws/show/3447-15#Text.

[14] Loos, C.M.M., Dorsch, S.C., Elzinga, S.E., Brewster-Barnes, T., Vanzant, E.S., Adams, A.A., & Urschel, K.L. (2019). A high protein meal affects plasma insulin concentrations and amino acid metabolism in horses with equine metabolic syndrome. *The Veterinary Journal*, 251, article number 105341. doi: 10.1016/j.tvjl.2019.105341.

[15] Lu, R., Yuan, T., Wang, Y., Zhang, T., Yuan, Y., Wu, D., Zhou, M., He, Zh., Lu, Y., Che, Y., Fan, J., Liang, J., & Cheng, Y. (2018). Spontaneous severe hypercholesterolemia and atherosclerosis lesions in rabbits with deficiency of low-density lipoprotein receptor (*LDLR*) on exon 7. *EBioMedicine*, 36, 29-38. doi: 10.1016/j.ebiom.2018.09.020.

[16] Marycz, K., Michalak, I., & Kornicka, K. (2018a). Advanced nutritional and stem cells approaches to prevent equine metabolic syndrome. *Research in Veterinary Science*, 118, 115-125. doi: 10.1016/j.rvsc.2018.01.015.

[17] Marycz, K., Weiss, C., Śmieszek, A., & Kornicka, K. (2018b). Evaluation of Oxidative Stress and mitophagy during adipogenic differentiation of adipose-derived stem cells isolated from equine metabolic syndrome (EMS) Horses. *Stem Cells International*, 2018, article number 5340756. doi: 10.1155/2018/5340756.

[18] Norton, E.M., Schultz, N.E., Rendahl, A.K., Mcfarlane, D., Geor, R.J., Mickelson, J.R., & McCue, M.E. (2019). *Heritability of metabolic traits associated with equine metabolic syndrome in Welsh ponies and Morgan horses*. Retrieved from https://beva.onlinelibrary.wiley.com/doi/10.1111/evj.13053.

[19] Olley, R.B., Carslake, H.B., Ireland, J.L., & McGowan, C.M. (2019). *Comparison of fasted basal insulin with the combined glucose-insulin test in horses and ponies with suspected insulin dysregulation*. Retrieved from https://www.sciencedirect.com/science/article/abs/pii/S1090023319300905?via%3Dihub.

[20] Posternak, L.I. (2017). Prospects and criteria of horse-breeding in Ukraine. *Agricultural Science and Food Technology,* 2(96), 230-236.

[21] Procedure for conducting research and experiments on animals by scientific institutions. (2012). Retrieved from https://zakon.rada.gov.ua/laws/card/z0416-12.

[22] Reynolds, A., Keen, J.A., Fordham, T., & Morgan, R.A. (2019). Adipose tissue dysfunction in obese horses with equine metabolic syndrome. *Equine Veterinary Journal*, 51(6), 760-766. doi: 10.1111/evj.13097.

[23] Stetsiuk, O. (2020). *Development of equestrian tourism infrastructure in Ukraine*. Retrieved from https://sportscience.ldufk.edu.ua/index.php/trends/article/view/989/959.

[24] Xenoulis, P.G., Cammarata, P.J., Walzem, R.L., Suchodolski, J.S., & Steiner, J.M. (2020). *Serum triglyceride and cholesterol concentrations and lipoprotein profiles in dogs with naturally occurring pancreatitis and healthy control dogs*. Retrieved from http://surl.li/rktya.

[25] Yusiuk-Omelnytska, T.A. (2023). Current state of the equine industry in state ownership. Development of the livestock industry: Innovations, issues, prospects. In *All-Ukrainian scientific-practical conference of scientists, teachers, and postgraduates* (pp. 54-56). Kharkiv: NBTU.

[26] Zhukova, I.A. (2014). A dynamics of the physiology state of sporting horse is at physical activities. *Scientific Messenger of Lviv National University of Veterinary Medicine and Biotechnologies named after S.Z. Gzhytskyj*, 16(2), 120-124.

## Маркерні зміни ліпідограми крові у патогенезі деяких внутрішніх хвороб коней

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**Анотація.** Порушення метаболізму ліпідів в організмі коней (рід кінь, *Equus*) за дії різноманітних чинників часто призводить до виникнення захворювань, у тому числі з розвитком метаболічного синдрому. Тому визначення маркерних змін ліпідограми сироватки крові за метаболічних розладів є актуальним питанням у дослідженні патогенезу найпоширеніших хвороб у цього виду тварин. Мета роботи полягала у визначенні особливостей показників обміну ліпідів в сироватці крові коней за фізіологічного стану та у разі виникнення симптомокомплексу кольок, ламініту і метаболічного синдрому. Дослідження показників обміну ліпідів у сироватці крові цих тварин здійснювали ензиматичним колориметричним методом із використанням біохімічного автоматичного аналізатора COBAS C 311 (“Roche Diagnostics GmbH”, Німеччина). Встановлено, що у

ліпідограмі сироватки крові клінічно здорових коней української верхової породи частка холестеролу ліпопротеїнів високої щільності становила 75,1 %, ліпопротеїнів низької щільності – 15,7 %, ліпопротеїнів дуже низької щільності – 9,2 %. За патології з симптомокомплексом кольок у сироватці крові коней частка холестеролу ліпопротеїнів високої щільності істотно зменшувалася, а ліпопротеїнів низької щільності підвищувалася у 3,9 раза порівняно із здоровими тваринами. При цьому, в ліпідограмі сироватки крові клінічно здорових поні на частку холестеролу ліпопротеїнів високої щільності доводилось 26,3 %, ліпопротеїнів низької щільності – 65,1 %, ліпопротеїнів дуже низької щільності – 8,61 %. У разі виникнення в поні ламініту, динаміка показників ліпідного складу сироватки крові відповідала такій у коней. Встановлені закономірності щодо змін ліпідограми сироватки крові у різних представників роду коней дозволили уточнити їх роль у патогенезі окремих найпоширеніших патологій. Результати дослідження мають практичну цінність для застосування у клінічній ветеринарній медицині та сприятимуть проведенню якісної лабораторної діагностики найпоширеніших патологій в коней (за симптомокомплексу кольок, ламініту та метаболічного синдрому), у патогенезі яких відмічаються порушення метаболізму ліпідів

**Ключові слова:** поні; Українська верхова порода; холестерол; ліпопротеїни; симптомокомплекс колік; ламініт; метаболічний синдром

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# Microstructural analysis of meat and internal organs of broiler chickens using a probiotic biological product

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**Abstract.** Probiotic preparation containing bacteria of the genus *Bacillus subtilis and Bacillus licheniformis* used for feeding broiler chickens to improve feed digestion, nutrient absorption, increase immune status and productivity, and for the prevention and treatment of various poultry diseases. The purpose of the study is to conduct histological tests of broiler chicken slaughter products when they were administered a probiotic biologic medical product in doses of: 0.5 g, 2.0, and 4.0 g per 10 $dm^3$ of water. The material was examined by the histological method. It was found that the muscle fibres in the pectoralis major are of the same type, evenly directed, the cytoplasm of muscle fibres is moderately eosinophilic, uniformly light pink, and minor layers of adipose tissue are found between the bundles of muscle fibres. The morphological architectonics of the heart muscle are preserved, cardiomyocytes are homogeneous and have a clear orientation. The microstructure of the liver of broiler chickens is unchanged: hepatocytes are collected in the same type of groups; the central veins are desolate; the cytoplasm of these cells is homogeneous, clear, and pink; the nuclei are weakly basophilic. In the spleen, the follicular structure is formed, leukocytes are diffusely placed at different stages of differentiation; vessels in significant numbers, thickened, of different calibre. The cuticle of the muscular part of the stomach contains the epithelial layer, the volume part of the connective tissue base layer is revealed; muscle fibres

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are located under the mesenchymal base of the cuticle. Lungs by morphological structure have bronchial tubes throughout the structure, which contain blood cells. According to the results of the conducted studies, a beneficial effect of a probiotic biological product at a dose of 4.0 g/10 $dm^3$ of water on the morphology of the pectoralis major and internal organs of broiler chickens was established. Therefore, a probiotic at a dose of 4.0 g/10 $dm^3$ of water during the drinking of broiler chickens can be recommended to increase productivity and produce safe slaughter products. The practical significance of the results obtained is to determine the features of the effect of feeding poultry with different doses of probiotics on the microstructure of its slaughter products, which is important for obtaining the best effect from its use

**Keywords:** poultry; pectoralis major; slaughter products; histological studies; morphology; probiotic

## Introduction

The poultry industry uses modern intensive technologies for the production of poultry slaughter products, in particular, more than 120 million tonnes of chicken, turkey, duck, and quail meat are produced per year. However, due to the intensive nature of modern poultry farming, there are problems with the well-being of poultry, the impact on the environment, and the economic stability of the country. Therefore, it is necessary to develop modern systems for monitoring and controlling the maintenance of poultry, and well-being, in particular, improving the diet of feeding with the use of probiotic drugs (excluding antibiotics), and monitoring the production of poultry slaughter products and their safety. A.S. George & A.S.H. George (2023) pointed out that a modern monitoring and control system – the HACCP system – has been widely implemented in accordance with the requirements of the National Food Management Standard ISO 22000:2018 and the international food standard *Global Food Safety Initiative* (*GFSI*).

Probiotics, prebiotics, and synbiotics have had significant positive effects not only on human health, but also on animal health, according to A. Rousseaux *et al.* (2023), in particular on the functions of internal organs, muscle tissue, and intestinal mucosa, where the composition of beneficial microorganisms was modulated – *Bifidobacteria* and *Firmicute*. The use of probiotics has led to positive results in the prevention and treatment of a significant number of diseases, such as infectious diseases, malignant tumours, allergies, etc. When planning the development of poultry processing enterprises, it is necessary to consider the impact of their functioning on the environment. R. Kler *et al.* (2022) found that during the production of high-quality and safe meat of broiler chickens, it is necessary to ensure proper sanitary and hygienic conditions for their maintenance, feeding, and well-being. S. Tavaniello *et al.* (2023) proved a significant effect of the synbiotic drug "PoultryStar", when fed to broiler chickens, on increasing body weight, carcass yield, improving the quality indicators of meat and offal, in particular morphological ones.

J. Lei *et al.* (2022) noted a significant effect of the gut microbiota of broiler chickens on some indicators of meat quality, in particular, moisture retention capacity and structure. Therefore, it is necessary to use probiotic drugs during poultry feeding, which will affect the state of the intestinal microflora. Y. Zhao *et al.* (2020) claimed that when feeding broiler

chickens a dietary probiotic *Bacillus licheniformis* H2 was noted to increase their growth, improve the morphological characteristics of the intestine, liver, and other internal organs. Safety and quality of poultry meat, as noted by D. Shalginbayev *et al.* (2022), can be determined not only by the content of moisture, fat, dry matter, etc., but also confirmed by histological studies during meat production and storage. The researchers established morphological changes in poultry meat when it was frozen at a temperature of negative (18±2)°C, which occurred during transportation. Researchers also developed a colorimetric method for identifying defrosted poultry meat by processing it with current and gas, which was used to establish falsification of the thermal state of poultry meat due to repeated freezing. F. Abdulkhaliq & A.D. Sabow (2023) evaluated the morphological characteristics of poultry slaughter products, considering the effectiveness of the pre-slaughter state of poultry, the degree of exsanguination of carcasses, the composition of feed for feeding and the quality of poultry meat.

A.P. Paliy *et al.* (2020) pointed out the importance of conducting histological studies of meat raw materials and poultry slaughter products, since this method can confirm the safety and quality of meat products. V.G. Stoyanovskyy *et al.* (2020) claimed that the addition of humic acids and the probiotic "Laktin" with plant-based preparations to the diet of broiler chickens increased the immunological status of the body, increased the safety of poultry, and the level of productivity. The morphological structure of the poultry intestinal tissue was characterised by the presence of immune structures, which affected the growth and development of broiler chickens during the rearing period. The Regulation of the European Union No. 1381 (2019) stipulates that monitoring the safety of meat and slaughtered products should be carried out transparently, considering the sustainability of risk assessment in the food and feed chains. Currently, the use of probiotic biological product "Subtiform" is relevant in the production of broiler chicken meat.

The purpose of the study is to conduct a microstructural analysis of the pectoralis major muscle of broiler chickens and internal organs (heart, spleen, liver, stomach muscle tissue, lungs) after feeding probiotic biologics to broiler chickens in different doses.

## Literature Review

The use of antibiotics in the rearing of broiler chickens is harmful to their health, due to the irrational use and accumulation of residues in poultry meat and the appearance of antibiotic-resistant strains of microorganisms. Q. Zhu *et al.* (2021) searched for feed additives that are functional and beneficial for the health of broiler chickens and affect their growth and productivity. The researchers preferred to develop diets containing probiotics, prebiotics, emulsifiers, organic acids, essential oils, enzymes, etc.

Use of enzymatic products, probiotics, and prebiotics as indicated by G. Vinderola *et al.* (2023) and J. Choi *et al.* (2023) brought health benefits to animals and humans, considering their microbiological and chemical properties, and also had a positive effect on the microstructure of internal organs and muscle tissue. M.A. Arain *et al.* (2022) showed that probiotics added to feeding broiler chickens affected weight gain, organ growth, and development of digestive organs, muscle tissue, and their morphological composition. F.U. Memon *et al.* (2022) claimed that probiotic preparations were easily mixed with feed and water, improved feed intake by broiler chickens and their immunity, digestive process, increased nutrient absorption, had antimicrobial effects, and did not negatively affect the morphological parameters of the carcass and internal organs.

R. Jha *et al.* (2023) pointed out that improving the efficiency of feeding broiler chickens, maintaining the physiological health of poultry, and the morphological structure of meat and internal organs was influenced by the state of the gastrointestinal tract and immune system. Therefore, to regulate the process of feed digestion, it is necessary to use probiotics in the poultry diet, which positively affected the microstructural integrity of the intestine and the stability of the microbiota. O.M. Chechet *et al.* (2022) argued that the use of probiotic drugs in broiler chickens is a promising area for increasing poultry resistance and productivity, providing prevention of infectious diseases in poultry. Researchers also pointed out the positive effect of the synbiotic drug "Biomagn" on the productivity and quality of poultry meat, in particular, on its morphological structure.

When feeding broiler chickens vitamin supplements containing tocopherol acetate and ascorbic acid, as indicated by O.I. Vishchur *et al.* (2020), there was an improvement in lipid synthesis in the liver and their deposition in skeletal muscle, and the preservation of liver and muscle structure. M.A.M. Shaufi *et al.* (2023) and G.M. Suliman *et al.* (2023) noted a positive effect of probiotics and their combinations on the gut microbiota of broiler chickens and the structure of muscle tissue and internal organs that did not change. Z. Zhang *et al.* (2023) proved that a probiotic *Lactobacillus johnsonii* had therapeutic properties in various diseases of poultry, had a positive effect on their performance, structure of internal organs, intestines, and muscle tissue. S.L. Weimer *et al.* (2018) noted a positive effects of probiotic preparation and yeast *Saccharomyces cerevisiae* on the structure of internal organs of broiler chickens. S. Aslam *et al.* (2020) found that the use of probiotic drugs in the diet of broiler chickens not only increased productivity, but also mobilised all processes in the body at the cellular level, improved the physical and chemical parameters of meat, and the structure of muscle tissue and slaughter products. R.N. Soomro *et al.* (2019) proved that the probiotic mixture "Protexin" as a growth stimulator of poultry increased productivity, improved health and reduced mortality rate for raising meat-type quails, and improved the qualitative and morphological parameters of muscle tissue and internal organs.

## Materials and Methods

The research was conducted in 2022-2023 in the accredited research laboratory of the Department of the Veterinary and Sanitary Expertise, Hygiene of Animal Husbandry Products and Pathological Anatomy called after J.S. Zahaievskyi and accredited research laboratory of the Department of Veterinary and Sanitary Expertise of Institute of Postgraduated Education for managers and specialists of Veterinary medicine of the Bila Tserkva National Agrarian University.

Samples of the pectoralis major muscle of broiler chickens and internal organs of the bird – the heart, spleen, liver, the muscular part of the stomach and lungs – served as material for the study. A control group of broiler chickens was formed in the amount of 20 animals, who were not given probiotic biologics "Subtiform", and three experimental groups (each with 20 animals) – who were given probiotic biologics with water as follows: chickens of the experimental group 1-0.5 g/10 $dm^3$; experimental group 2-2.0 g/10 $dm^3$; experimental group 3-4.0 g/10 $dm^3$. The drug was given to broiler chickens from 28 to 42 days of rearing.

According to the methodology of L.P. Horalskyi *et al.* (2015) and the national standard DSTU 7353:2013 (2014) a microstructural analysis of the pectoralis major and internal organs (heart, spleen, liver, stomach muscles, and lungs) of broiler chickens was performed. At

least three pieces of the pectoralis major muscle and internal organs, 0.2-0.4 cm thick, were taken from each sample and placed in disposable labelled plastic cassettes, which were then placed in a container for fixation with a 10% aqueous formalin solution.

Subsequently, histological preparations were made from the selected material and stained with Carazzi's hematoxylin and eosin to identify the main structural elements of tissues and pathological changes. The histological specimens were microscoped using an Axioskop laboratory microscope (Poland) on a "light field" contrast at 10x, 20x, 40x magnification and a colour digital camera Industrial Digital Camera 8.OMP 1/2.5 Color USB 2.0 with a resolution of 8.0 MP (Poland), which reflected the actual magnification of the objects in the field of view. ToupView software was used to analyse the image. When stained with Carazzi's hematoxylin and eosin, the cell nuclei were stained blue-purple, the cytoplasm – pink-red. Microstructural analysis of the pectoralis major, heart, spleen, liver, stomach, and lung muscles was performed in accordance with the requirements of the regulatory document (DSTU 7353:2013, 2014).

Scientific research was conducted in accordance with requirements of DSTU ISO/IEC 17025:2005 (2006), EUs Directive 2010/63/EU on the protection of animals used for scientific purposes (2010) and Law of Ukraine No. 3447-IV (2006, February).

## Results and Discussion

According to histological studies of the pectoralis major muscle and internal organs of broiler chickens: heart, spleen, liver, muscle part of the stomach and lungs, it was found that the microstructure of the pectoralis major muscle did not change, but revealed muscles consisting of relatively large fibres, and others – of smaller fibres. The nuclei had an elongated-oval shape. In the striated muscles, the nuclei were located on the periphery of the cytoplasm, the fibers – near the sarcolemma and are oriented parallel to it by their long axis. Transverse straight stripes are visible on clearly longitudinal sections. But on sections that run slightly obliquely, these stripes are arched (Fig. 1-14).

Along the longitudinal section, the muscle fibres of the pectoralis major muscle of broiler chickens of the control group are the same thickness and evenly directed (Fig. 1).

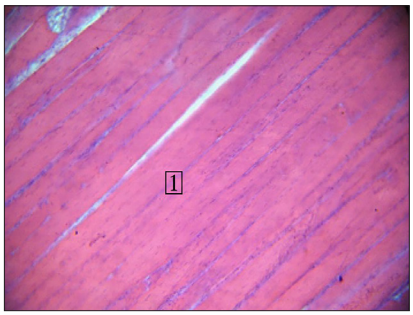


**Figure 1.** Morphologically unchanged structure of the muscle tissue of the pectoralis major muscle in the control group

***Notes:*** *1 – muscle fibres are the same thickness, evenly directed. Staining with Carazzi's hematoxylin and eosin, x200*

***Source:*** *developed by the authors*

A uniform pale pink cytoplasm and empty blood vessels were noted. Rather large nuclei of elongated-oval shape, light, weakly basophilic, located closer to the sarcolemma containing nucleoli were observed. It was also noted that individual muscles had constrictions, which preceded their fragmentation. Minor fat layers were found between the bundles of muscle fibres (Fig. 2).

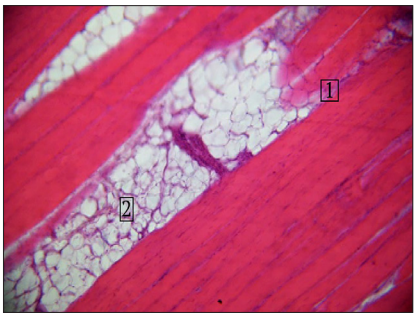


**Figure 2.** Morphologically unchanged structure of the muscle tissue of the pectoralis major muscle in the control group
***Notes:*** *1 – constrictions in the muscles; 2 – fat layers. Staining with Carazzi's hematoxylin and eosin, x200*
***Source:*** *developed by the authors*

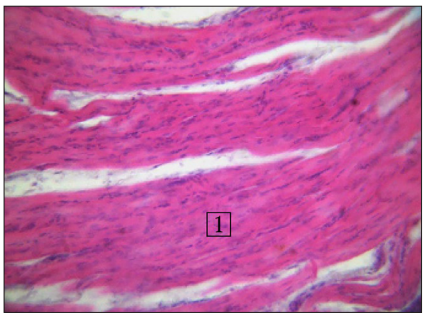


**Figure 3.** Microscopic structure of the heart muscle of broiler chickens from the control group
***Notes:*** *1 –cardiomyocytes (in longitudinal section) are the same thickness. Staining with Carazzi's hematoxylin and eosin, x200*
***Source:*** *developed by the authors*

Figure 3 shows the microscopic structure of the heart muscle of broiler chickens in the control group. Morphologically unchanged structure of the heart muscle was noted. The heart muscle tissue looked like a mass of branching and anastomosing fibres separated by slit-like spaces. These fibres were made up of individual cells connected end-to-end. The slit-like spaces between the anastomosing fibres were filled by the endomysium, where capillaries and lymphatic vessels passed closely. The fibres of the heart muscle are characterised by transverse striation. Individual heart muscle fibre cells usually contained a single nucleus, but sometimes binucleated cells were also found. It was noted that the nuclei are slightly larger and lighter than in skeletal muscle fibres and are usually located closer to the central axis of the fibre.

Thus, Figure 3 shows that the structure of the heart muscle is preserved: cardiomyocytes (in longitudinal section) are the same thickness, pale pink with homogeneous cytoplasm. The nuclei are cigar-shaped, basophilic, with a moderate chromatin content. The vessels are moderately dilated and thickened.

From the cross-section of the muscles, a uniform pale pink cytoplasm was noted, the nuclei in which are moderately basophilic. The vessels are moderately dilated (Fig. 4).

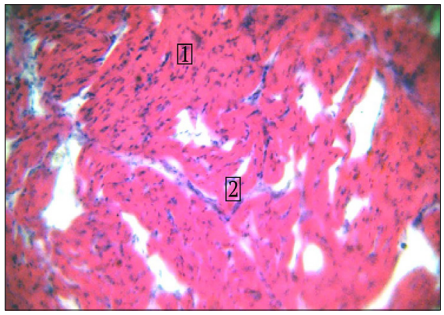


**Figure 4.** Microscopic structure of the heart muscle of broiler chickens from the control group
***Notes:*** *1 – in a cross-section of the muscle – the structure is preserved, the cytoplasm is uniformly pale pink; 2 – vessels are moderately dilated. Staining with Carazzi's hematoxylin and eosin, x200*
***Source:*** *developed by the authors*

Histological examination of the spleen of broiler chickens from the control group revealed that the structure of the organ is represented

by a diffuse accumulation of leukocytes at varying stages of differentiation. The vessels are desolate and moderately dilated (Fig. 5).

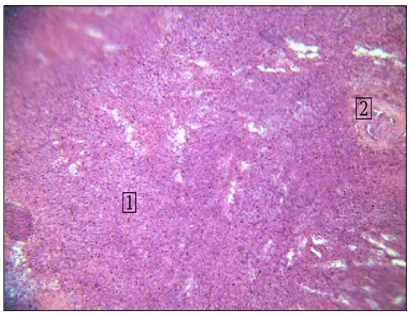


**Figure 5.** Microscopic structure of the spleen of broiler chickens from the control group

***Notes:*** *1 – diffuse accumulation of leukocytes; 2 – vessels are moderately dilated. Staining with Carazzi's hematoxylin and eosin, x200*

***Source:*** *developed by the authors*

The trabecular base of the spleen is poorly defined (Fig. 6). Lymphoid nodules do not have a clear structure.

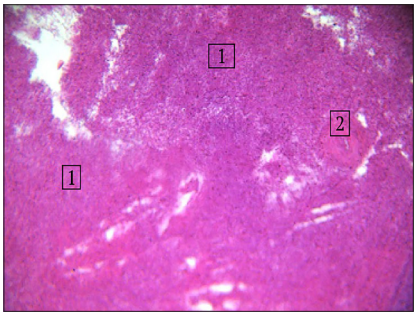


**Figure 6.** Microscopic structure of the spleen of broiler chickens from the control group

***Notes:*** *1 – leukocytes in a state of differentiation; 2 – vessel. Staining with Carazzi's hematoxylin and eosin, x200.*

***Source:*** *developed by the authors*

According to the conducted studies, it was found that the morphological structure of the liver of broiler chickens of the control group did not undergo pathological changes. The liver lobules are usually separated from each other by layers of loose connective tissue called interlobular septa or interstices. In the liver of broiler chickens, interparticle layers were not visible. Figure 7, 1 show that hepatocytes are collected in groups of the same type. The central veins are dessolated (Fig. 7, 2), of moderate size. The cytoplasm of hepatocytes is uniform, clear, and pink. The nuclei are weakly basophilic. Interstitial vessels are desolate (Fig. 7, 3) or contain isolated blood cells.

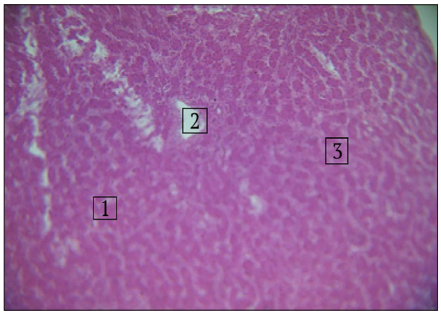


**Figure 7.** Microstructure of liver hepatocytes in broiler chickens from the control group

***Notes:*** *1 – hepatocytes; 2 – central vein is desolate; 3 – interstitial vessels are desolate. Staining with Carazzi's hematoxylin and eosin, x200*

***Source:*** *developed by the authors*

Histological examination of the muscular part of the stomach of broiler chickens was observed (Fig. 8) that the cuticle is represented by a formed epithelial layer (Fig. 8, 1) under which a sufficiently wide layer is occupied by the connective tissue base.

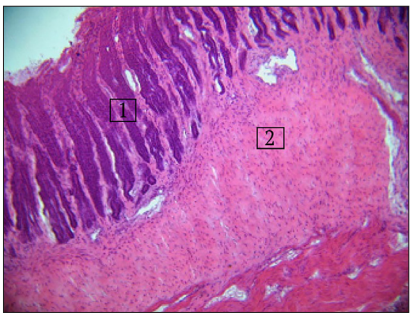


**Figure 8.** Formed epithelial layer of the muscle cuticle parts of the stomach of broiler chickens from the control group

***Notes:*** *1 – epithelial layer; 2 – submucosal base. Staining with Carazzi's hematoxylin and eosin, x200*

***Source:*** *developed by the authors*

Under the stromal base there are muscle fibres that are loosened by rather voluminous layers of the mesenchyme (Fig. 9, 1). The vessels are dilated, partially filled with blood cells (Fig. 9; 10).

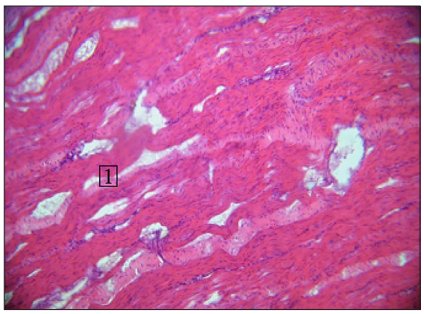


**Figure 9.** Microscopic structure of the muscular part of the stomach of broiler chickens of broiler chickens from the control group

***Notes:*** *1 – loose muscle fibres. Staining with Carazzi's hematoxylin and eosin, x200*

***Source:*** *developed by the authors*

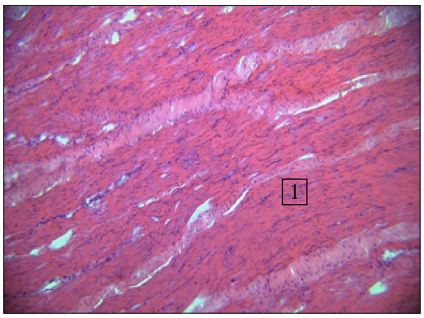


**Figure 10.** Microscopic structure of the muscular part of the stomach of broiler chickens from the control group

***Notes:*** *1 –longitudinal section of the muscles. Staining with Ca*razzi's hematoxylin and eosin, x200

***Source:*** *developed by the authors*

The lower layers of muscles (relative to the cuticle) are characterised by uniformity, clear orientation (Fig. 11, 1). The nuclei are elongated-oval in shape and moderately basophilic (Fig. 11).

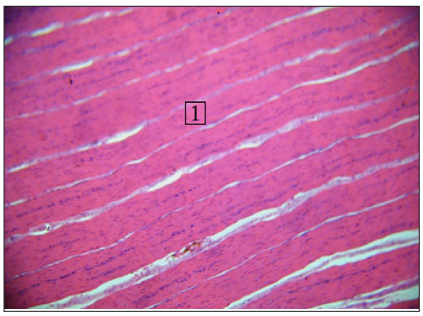


**Figure 11.** Microscopic structure of the muscular part of the stomach of broiler chickens from the control group

***Notes:*** *1 – longitudinal direction of muscle fibres. Staining with Carazzi's hematoxylin and eosin, x200*

***Source:*** *developed by the authors*

Histological examination of the lungs of broiler chickens revealed that the bronchial tubes largely contained blood cells (possibly post-slaughter artefacts) (Fig. 12).

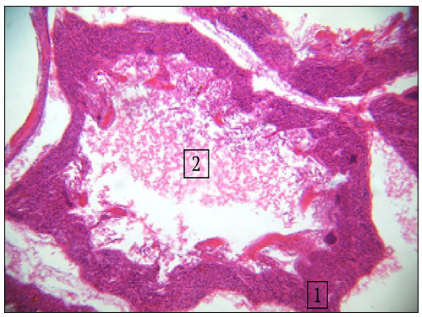


**Figure 12.** Microscopic structure of the lungs of broiler chicken from the control group
***Notes:*** *1 – bronchial tubes; 2 – shaped elements. Carazzi's hematoxylin and eosin, x200*
***Source:*** *developed by the authors*

The structure of the lung tissue was in a state of compression deformation (Fig. 13).

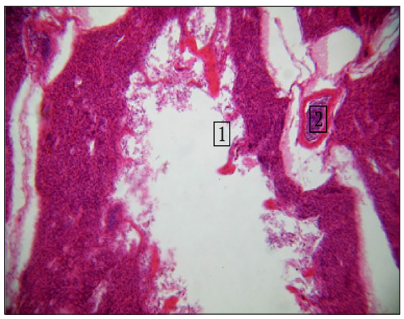


**Figure 13.** Compression deformity in the lungs of broiler chicken from the control group
***Notes:*** *1 – deformed wall of the bronchial tube; 2 – a vessel containing blood cells. Staining with Carazzi's hematoxylin and eosin, x200.*
***Source:*** *developed by the author*

The vessels of the organ contained a significant amount of blood cells (possibly poor exsanguination of the carcass) (Fig. 14, 1).

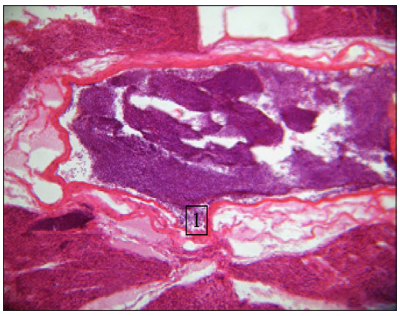


**Figure 14.** Microscopic structure of the lungs of broiler chicken from the control group
***Notes:*** *1 – blood cells in the vessels of the lungs. Staining with Carazzi's hematoxylin and eosin, x200*
***Source:*** *developed by the authors*

Figures 15 and 16 show the microscopic structure of the pectoralis major muscle of broiler chickens from experimental group 1. It was found that according to the longitudinal projection, the pectoralis major muscles are uniform, striation was not determined, they have different thickness, single – minor flask-shaped thickenings. Most muscle groups are divided into fairly large fragments (Fig. 15, 1). The cytoplasm of muscle fibres is weakly eosinophilic and has different intensity. The nuclei, in most cases, are located closer to sarcolemes, elongated, of which single ones have acquired a rounded shape, weakly basophilic.

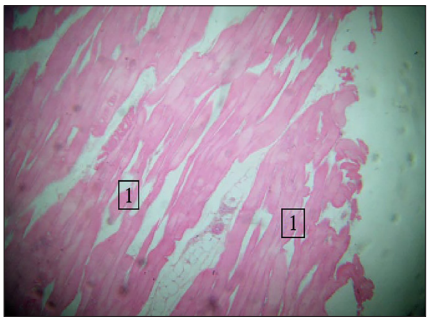


**Figure 15.** Pectoralis major of broiler chickens from experimental group 1
***Notes:*** *1 – muscle fragmentation. Staining with Carazzi's hematoxylin and eosin, x200*
***Source:*** *developed by the authors*

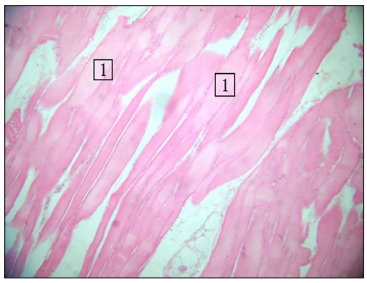


**Figure 16.** Pectoralis major of broiler chickens from experimental group 1

***Notes:*** *1 – muscle cytoplasm is weakly basophilic. Staining with Carazzi's hematoxylin and eosin, x400*

***Source:*** *developed by the authors*

Figure 17 presents the preserved morphological architectonics of the heart (epicardium, myocardium, endocardium) of broiler chickens from experimental group 1. Cardiomyocytes, in longitudinal section, are homogeneous, had a clear direction, the latter anastomosed with each other, forming specific branched formations. Intermuscular connective tissue is moderately loose (Fig. 17, 2), along the course of the fibres, there are blood capillaries located in the endomysium. The cytoplasm of cardiomyocytes is pale pink, uniformly homogeneously coloured. The nuclei are elongated (cigar-shaped), moderately basophilic, and the vessels are desolate.

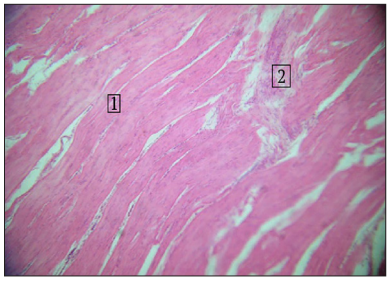


**Figure 17.** Microscopic structure of the heart muscle of broiler chickens from experimental group 1

***Notes:*** *1 – bundles of muscle fibres; 2 – intermuscular connective tissue. Staining with Carazzi's hematoxylin and eosin, x200*

***Source:*** *developed by the authors*

According to histological examination of the spleen (Fig. 18) it was found that leukocytes are diffusely placed throughout the entire structure of the specimen under study (Fig. 18, 1) at different stages of differentiation. Lymphoid nodules are single, clearly defined. Vessels of various calibre, desolate. The trabecular base is poorly developed.

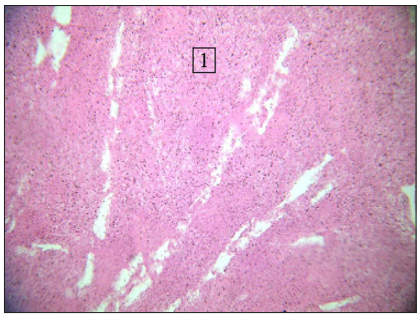


**Figure 18.** Microscopic structure of the spleen of broiler chickens from experimental group 1

***Notes:*** *1 – diffuse placement of leukocytes. Staining with Carazzi's hematoxylin and eosin, x200*

***Source:*** *developed by the authors*

The liver microstructure of broiler chickens from experimental group 1 is represented by unformed groups of hepatocytes (Fig. 19). Hepatocytes are slightly enlarged and displaced relative to the basement membrane (Fig. 19, 1), contained clear eosinophilic cytoplasm of varying intensity. The nuclei of such hepatocytes are enlarged, the karyoplasm is clear. Interstitial vessels (Fig. 19, 2) and the central veins are desolate. The bile ducts are clearly developed. The latter do not have an exosecret.

Microscopic structure of the stomach of broiler chickens from experimental group 1 (Fig. 20) is represented by the developed epithelial layer of the cuticle (Fig. 20, 1) under which a sufficiently wide layer is occupied by a connective tissue base (Fig. 20, 2). Under the latter are muscle fibres (Fig. 20, 3), which are separated by rather voluminous layers of the mesenchyme.

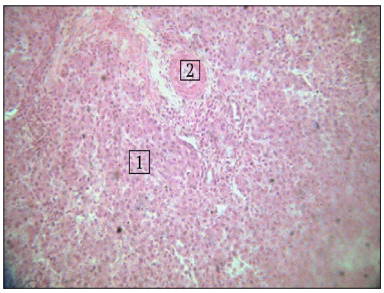


**Figure 19.** Microscopic structure of the liver of broiler chickens from experimental group 1

***Notes:*** *1 – liver plates; 2 – vessel. Staining with Carazzi's hematoxylin and eosin, x400*

***Source:*** *developed by the authors*

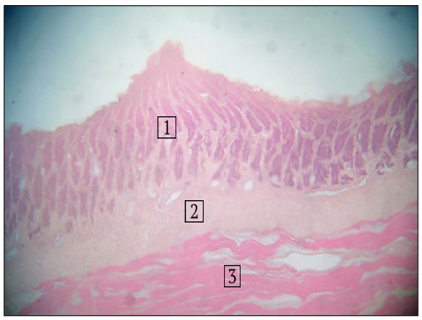


**Figure 20.** Microscopic structure of the muscular part of the stomach of broiler chickens from experimental group 1

***Notes:*** *1 – epithelial layer; 2 – submucosal base; 3 – muscles. Staining with Carazzi's hematoxylin and eosin, x200*

***Source:*** *developed by the authors*

The vessels are intensively dilated, partially filled with blood cells. Figure 21 shows that the muscle layers below (relative to the cuticle) are uniform and loose (Fig. 21, 1), have a certain orientation. The nuclei are elongated, moderately basophilic.

The structure of the lungs was characterised by the fact that the bronchial tubes (Fig. 22, 1) largely contain blood cells, possibly a post-slaughter artefact. The structure of lung tissue in a state of compression deformation. The vessels of the organ contain a significant amount of blood cells (poor exsanguination of the carcass is possible) (Fig. 22).

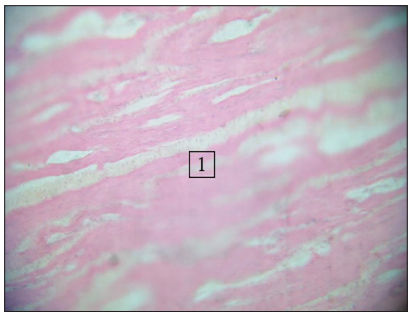


**Figure 21.** Microscopic structure of the muscular part of the stomach of broiler chickens from experimental group 1

***Notes:*** *1 – loose muscle fibres. Staining with Carazzi's hematoxylin and eosin, x200*

***Source:*** *developed by the authors*

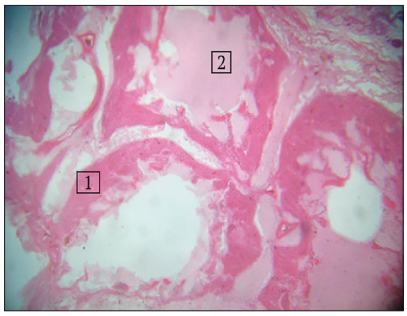


**Figure 22.** Microscopic structure of the lungs of broiler chickens from experimental group 1

*Notes: 1 – bronchial tubes; 2 – hemolised erythrocytes. Staining with Carazzi's hematoxylin and eosin, x200*

*Source: developed by the authors*

Figure 23 shows the microscopic structure of the pectoralis major muscle of broiler chickens from experimental group 2 – part of the pectoralis major muscle is thickened (Fig. 23, 1), contained pale pink cytoplasm; the nuclei are weakly basophilic, elongated. Between the fragments of muscle fibres – layers of adipose tissue (Fig. 23, 2)

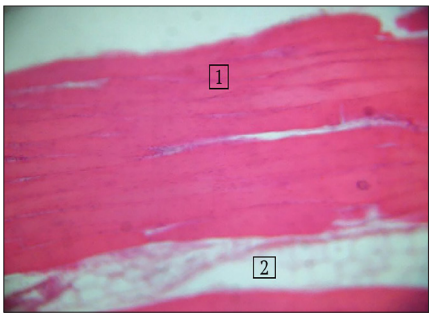


**Figure 23.** Microscopic structure of the pectoralis major muscle of broiler chickens from experimental group 2

***Notes:*** *1 – muscle fibres; 2 – layers of adipose tissue. Staining with Carazzi's hematoxylin and eosin, x200*

***Source:*** *developed by the authors*

Thickened, partially fragmented muscle fibres of the pectoralis major (Fig. 24, 1) with a uniform pale pink cytoplasm, oval-elongated nuclei with a low chromatin content. Adipose tissue is placed between individual fragments of muscle fibres (Fig. 24, 3).

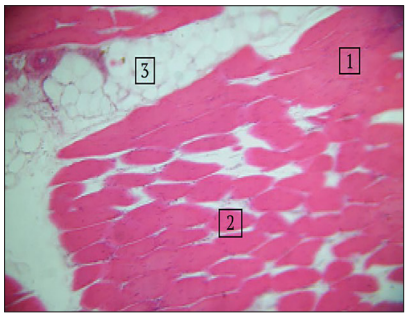


**Figure 24.** Microscopic structure of the pectoralis major muscle of broiler chickens from experimental group 2

***Notes:*** *1 – muscle fibres; 2 – muscle fragmentation; 3 – adipose tissue. Staining with Carazzi's hematoxylin and eosin, x200*

***Source:*** *developed by the authors*

Figure 25 shows that the structure of the myocardium is preserved: cardiomyocytes (in longitudinal section) are of the same thickness, have a pale pink cytoplasm of uniform density (Fig. 25, 1). The vessels are moderately dilated and desolate. According to the cross-section of the muscles, the structure is preserved (Fig. 25, 2), the cytoplasm is uniformly pale pink, the nuclei are moderately basophilic.

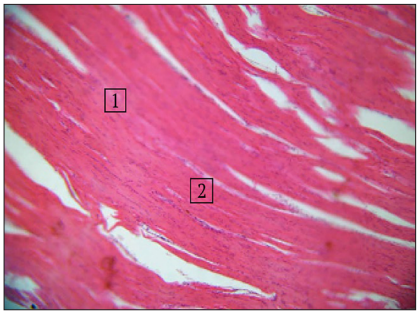


**Figure 25.** Microscopic structure of the heart muscle of broiler chickens from experimental group 2

***Notes:*** *1 – cardiomyocytes; 2 – the muscle structure is preserved. Staining with Carazzi's hematoxylin and eosin, x200*

***Source:*** *developed by the authors*

Individual muscle fibres are clear and contain an enlarged, rounded nucleus with a low chromatin content (Fig. 26, 2).

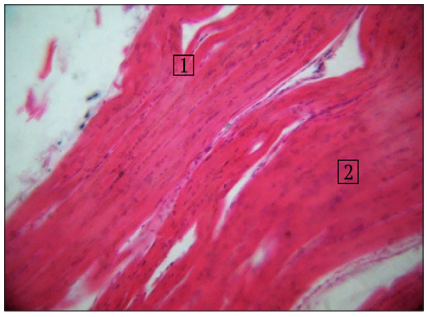


**Figure 26.** Microscopic structure of the heart muscle of broiler chickens from experimental group 2: preservation of muscle structure

***Notes:*** *1 – clear muscle karyoplasm; 2 – muscle fibres. Staining with Carazzi's hematoxylin and eosin, x400*

***Source:*** *developed by the authors*

In the spleen, the white pulp was based on lymphoid nodules, and diffusely placed leukocytes were recorded at different stages of differentiation (Fig. 27).

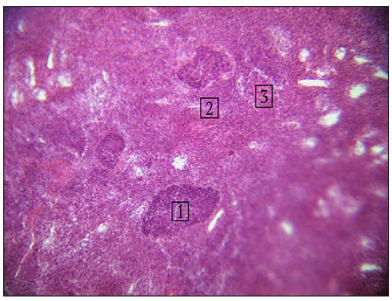


**Figure 27.** Microscopic structure of the spleen of broiler chickens from experimental group 2
***Notes:*** *1 – lymphoid nodules; 2 – red pulp; 3 – blood vessels. Staining with Carazzi's hematoxylin and eosin, x200*
***Source:*** *developed by the authors*

Histological studies of the liver showed that the groups of hepatocytes are slightly enlarged with a uniform pale pink cytoplasm (Fig. 28, 1, 2). The nuclei of such hepatocytes are rounded and enlarged. They contained a small amount of chromatin (Fig. 28).

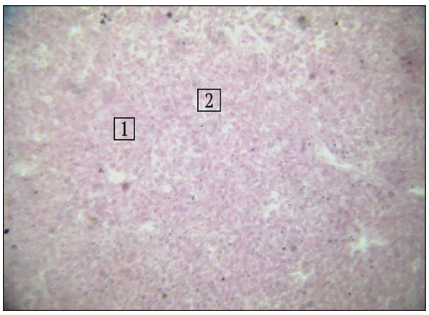


**Figure 28.** Follicular structure of the liver of broiler chickens from experimental group 2
***Notes:*** *1 – hepatocytes; 2 – hypsochromic hepatocytes with clear cytoplasm. Staining with Carazzi's hematoxylin and eosin, x400*
***Source:*** *developed by the authors*

The apical part of the gastric cuticle of broiler chickens from experimental group 2 is presented in a state of desquamation (peeling epithelium) (Fig. 29, 1). The cuticle is thinned (part of the epithelium is replaced by reticular cells and fibrous structures) (Fig. 29, 2).

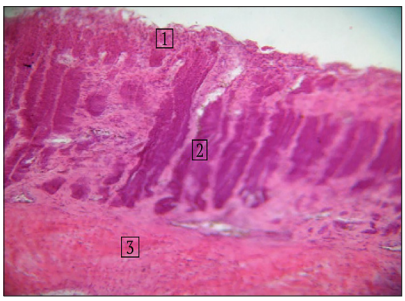


**Figure 29.** Desquamation of the cuticle of the muscular part of the stomach of broiler chickens from experimental group 2
***Notes:*** *1 – cuticle desquamation; 2 – epithelium; 3 – submucosal base. Staining with Carazzi's hematoxylin and eosin, x200*
***Source:*** *developed by the authors*

The muscles under the subquicular layer are clearly divided into bundles, and the muscle structure is somewhat blurred (some bundles are thicker, others are thinner), the intermuscular tissue is loosened. By the longitudinal section of the muscles – the latter are swollen, the structure is uniform, and the nuclei are mostly rounded, although they contain enough chromatin (Fig. 30; 31).

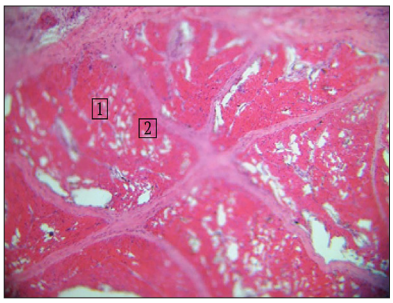


**Figure 30.** Microscopic structure of the muscular part of the stomach of broiler chickens from experimental group 2
***Notes:*** *1 – muscles; 2 – bundles of muscle fibres. Staining with Carazzi's hematoxylin and eosin, x200*
***Source:*** *developed by the authors*

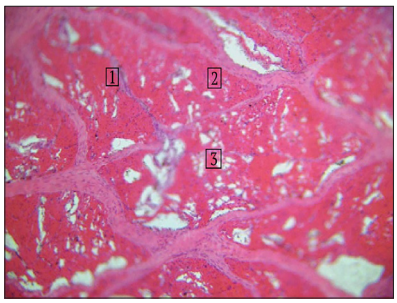


**Figure 31.** Microscopic structure of the muscular part of the stomach of broiler chickens from experimental group 2

***Notes:*** *1 – muscles; 2 – bundles of muscle fibres; 3 – loosening of muscles. Staining with Carazzi's hematoxylin and eosin, x200*

***Source:*** *developed by the authors*

Figure 32 shows the microscopic structure of the lungs of broiler chickens from experimental group 2 in a state of compression deformation. The bronchial tubes largely contain blood cells (possibly a post-slaughter artefact). The vessels of the organ contain a significant amount of blood cells (poor exsanguination of the carcass is possible).

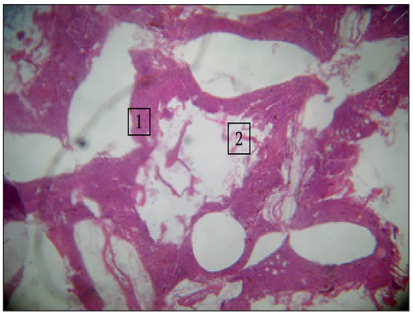


**Figure 32.** Compression deformity of the lung structure of broiler chickens from experimental group 2

***Notes:*** *1 – bronchial tubes; 2 – compression deformation of the lung structure. Staining with Carazzi's hematoxylin and eosin, x200*

***Source:*** *developed by the authors*

Fig. 33, 34 show the microstructure of the pectoralis major muscle of broiler chickens from experimental group 3. According to the longitudinal section of the muscles, the following was recorded: muscle fibres of the same type, identical in thickness, evenly directed (Fig. 33, 1, 2). The vessels are desolate. The cytoplasm of muscle fibres is moderately eosinophilic, uniformly light pink. The nuclei are elongated-oval in shape, weakly basophilic, located closer to the sarcolemma, and contain nucleoli. Minor layers of adipose tissue were found between the bundles of muscle fibres (Fig. 33, 3).

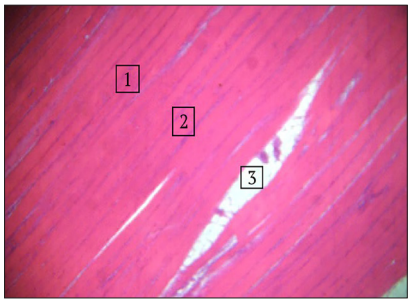


**Figure 33.** Microstructure of the pectoralis major muscle of broiler chickens from experimental group 3

***Notes:*** *1 – muscle; 2 – longitudinal muscle fibres; 3 – adipose tissue. Staining with Carazzi's hematoxylin and eosin, x200*

***Source:*** *developed by the authors*

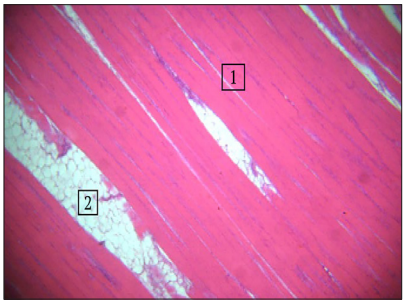


**Figure 34.** Microstructure of the pectoralis major muscle of broiler chickens from experimental group 3

***Notes:*** *1 – longitudinal direction of muscle fibres; 2 – layers of fat. Staining with Carazzi's hematoxylin and eosin, x200*

***Source:*** *developed by the authors*

Figure 35 shows the microstructure of the heart muscle of broiler chickens from experimental group 3. It was found that the morphological architectonics of the heart were preserved, cardiomyocytes of longitudinal shape, uniform, clearly directed, anastomosed with each other, forming specific branched formations. Intermuscular connective tissue is moderately loose. Blood capillaries in the endomysium are located longitudinally, along the course of the fibres; the cytoplasm of cardiomyocytes is pale pink, uniformly coloured; the nuclei are elongated (cigar-shaped), moderately basophilic, the vessels are desolate.

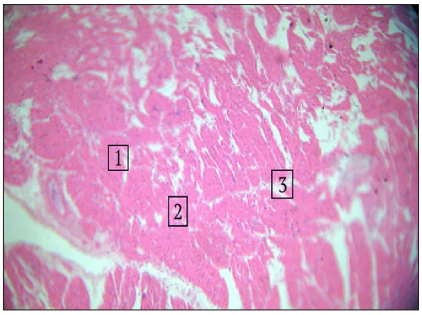


**Figure 35.** Morphological architectonics of the heart muscle broiler chickens from experimental group 3

***Notes:*** *1 – cross-section of muscles; 2 – homogeneous eosinophilic karyoplasm; 3 – anastomoses of muscle fibres. Staining with Carazzi's hematoxylin and eosin, x200*

***Source:*** *developed by the authors*

Figure 36 shows the microstructure of the spleen of broiler chickens from experimental group 3. It was found that the follicular structure is formed with diffusely placed leukocytes at different stages of differentiation (Fig. 36, 2). A significant number of thickened vessels of various calibre were observed.

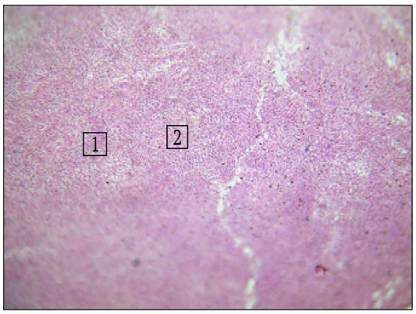


**Figure 36.** Microscopic structure of the spleen of broiler chickens from experimental group 3

***Notes:*** *1 – homogeneous structure of red pulp; 2 – leukocytes in a state of differentiation. Staining with Carazzi's hematoxylin and eosin, x200*

***Source:*** *developed by the authors*

The microstructure of the liver is represented by hepatocytes collected in homogeneous groups (Fig. 37, 1). The central veins are desolate and moderate in size. The cytoplasm of hepatocytes is homogeneous, clear, and pink, their nuclei are weakly basophilic. The interstitial vessels were desolate or contained isolated blood cells.

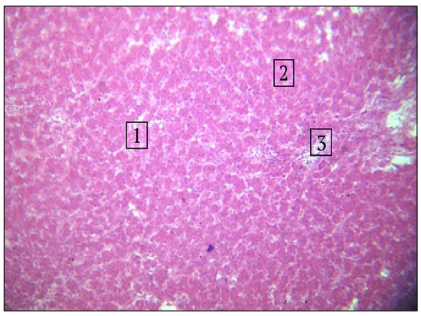


**Figure 37.** Uniformity of the microscopic structure of the liver of broiler chickens from experimental group 3

***Notes:*** *1 – hepatocytes; 2 – preserved structure; 3 – central vein. Staining with Carazzi's hematoxylin and eosin, x200*

***Source:*** *developed by the authors*

Figure 38 shows the microscopic structure of the muscular part of the stomach of broiler chickens from experimental group 3.

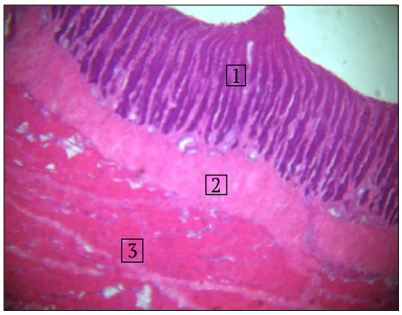


**Figure 38.** Microscopic structure of the muscular part of the stomach of broiler chickens from experimental group 3

***Notes:*** *1 – cuticle; 2 – connective tissue base; 3 – homogeneous muscles. Staining with Carazzi's hematoxylin and eosin, x200*

***Source:*** *developed by the authors*

The cuticle of the muscular part of the stomach of broiler chickens contained a formed epithelial layer (Fig. 38, 1) under which a sufficiently voluminous part of the connective tissue base layer is placed (Fig. 38, 2). Both layers of the muscular part of the stomach of broiler chickens form single, well-defined folds. Under the mesenchymal base of the cuticle are muscle fibres, which in some places are loosened by insignificant layers of the stroma. The vessels are dilated, partially filled with blood cells. Below (relative to the cuticle), the muscle layers are uniform and have a certain orientation. The nuclei are elongated-oval in shape, moderately basophilic (Fig. 39).

The microscopic structure of the lungs of broiler chickens from experimental group 3 is shown in Figures 40, 41. The bronchial tubes of the lungs contain blood cells, possibly as a post-slaughter artefact (Fig. 40; 41, 1). The vessels of the organ contained a significant amount of blood cells (possibly poor exsanguination of the carcass) (Fig. 40, 2).

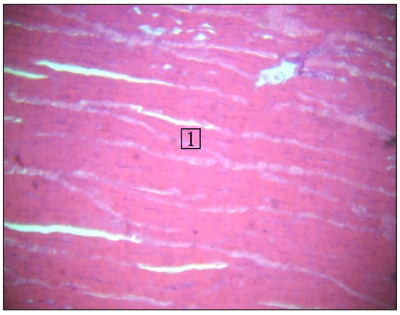


**Figure 39.** Uniformity of the microscopic structure of the muscle part of the stomach of broiler chickens from experimental group 3

***Notes:*** *1 – longitudinal muscle section. Staining with Carazzi's hematoxylin and eosin, x200*

***Source:*** *developed by the authors*

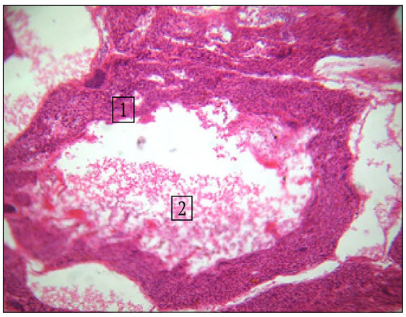


**Figure 40.** Microscopic structure of the lungs of broiler chicken from experimental group 3

***Notes:*** *1 – bronchial tubes of the lungs; 2 –blood cells. Staining with Carazzi's hematoxylin and eosin, x200*

***Source:*** *developed by the authors*

The structure of the lung tissue was in a state of compression deformation (Fig. 41, 2).

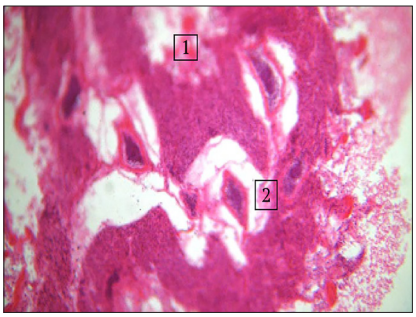


**Figure 41.** Microscopic structure of the lungs of broiler chicken from experimental group 3
***Notes:*** *1 – bronchial tubes of the lungs; 2 – structure of the lungs in a state of compression deformation. Staining with Carazzi's hematoxylin and eosin, x200*
***Source:*** *developed by the authors*

Researchers M. Angelovičová *et al.* (2016) argue that due to the use of phytogenic additives and nutraceuticals in feeding broiler chickens, the chemical composition of the pectoralis major and femoral muscles of carcasses improved. In particular, a significant increase in protein content by 3.05% (P<0.001) and fat content by 2.9% (P<0.001) was observed in the meat of this bird, which positively affected the morphological parameters of muscle tissue. The researchers did not note pathological changes in the form of granular dystrophy in the muscle tissue of broiler chickens and degeneration of internal organ tissues. In general, based on the conducted histological studies, they noted a positive effect of phytogenic additives and nutraceuticals on the development of internal organs of broiler chickens. Thus, the structure of the heart muscle was preserved. Cardiomyocytes had the same thickness, pale pink colour with uniform cytoplasm, basophilic nuclei with moderate chromatin content, slightly dilated and thickened vessels. In the muscular part of the stomach, the cuticle was represented by a developed epithelial layer, below which a significant layer of connective tissue base was found. No pathological changes were detected in the liver, namely: the hepatic lobules of the gland were separated from each other by layers of connective tissue, including no interparticle layers. The structure of the spleen was marked by a diffuse accumulation of leukocytes at different stages of differentiation. Thus, the data of researchers correlated with the results obtained experimentally, according to Fig. 35-39.

The results obtained from this study coincide with the results of V.G. Stoyanovskyy *et al.* (2020), who found that when the probiotic "Laktin" was added to the diet with plant-based preparations, the morphological structure of the pectoralis major muscle of broiler chickens was characterised by the same type of muscle fibres, uniform orientation and looseness of the intermuscular connective tissue, which contained layers of adipose tissue. These morphological parameters corresponded to the author's results of experimental tests in the control group (Fig. 1, 2) and experimental groups of broiler chickens: 1 – Fig. 15, 16; 2 – Fig. 23, 24; 3 – Fig. 33, 34. The researchers also established a morphologically unchanged structure of the heart muscle, the same size of cardiomyocytes with a uniform pink cytoplasm and basophilic nuclei. Histological examination of the liver revealed that the hepatocytes were gathered in similar groups, the interstitial vessels and central veins were desolate, and in some places single blood cells were found, which confirmed the results of the tests with the probiotic. Thus, Figure 7, 1 shows that hepatocytes in the liver are collected in the same type of groups, the central veins are desolate (Fig. 7, 2) and of moderate size. The cytoplasm of hepatocytes is homogeneous, clear, and pink, the nuclei are weakly basophilic, the interstitial vessels are desolate. The vessels of the lung tissue contained a significant amount of blood cells, which indicated non-compliance with veterinary and sanitary requirements

when slaughtering broiler chickens and ,as a result, poor exsanguination of poultry carcasses. According to the results of the study, similar data were obtained, in particular, a significant number of blood cells were found in the vessels of the organ, which may be due to poor exsanguination of the carcass (Fig. 14,1; 22; 32; 40). The structure of the lung tissue was in a state of compression deformation (Fig. 41, 2), which is also confirmed by researchers.

As noted by G. Vinderola *et al.* (2023), the use of probiotic drugs in the poultry diet had a positive effect on the morphological structure of the muscle tissue of the pectoralis major, femoral and cardiac muscles, and desolate vessels were also detected in the structure of the spleen. It was found that in the structure of the spleen of the control group of birds, the vessels are desolate and moderately dilated, the trabecular base of the spleen is poorly developed (Fig. 6). Figure 36 shows the uniformity of the red pulp structure in the spleen of the experimental group 3 of broiler chickens (Fig. 36, 1) and leukocytes in a state of differentiation (Fig. 36, 2).

But the microstructure of the liver did not change, but the interstitial vessels contained a small amount of blood cells. The researchers found that the microstructure of the muscular part of the stomach for the use of probiotic drugs in broiler chickens is represented by the formed epithelial layer of the cuticle, a wide layer of intermuscular connective tissue without pathological changes, which coincides with the results obtained in this study. J. Choi *et al.* (2023) indicated that probiotic drugs had a fairly positive effect on the development of the pectoralis major muscle of broiler chickens in the intermuscular connective tissue of which small layers of adipose tissue with muscle fibres of the same size were found. No pathological changes were detected in the heart muscle, and microstructural analysis of the lungs revealed that the bronchial tubes contained blood cells. These data are also confirmed by the study – there are 3 broiler chickens in the experimental group, as shown in Figures 40; 41, 1, blood cells were found in the bronchial tubes of the lungs (possibly a post-slaughter artefact). Moreover, the vessels of the organ contained a significant amount of blood cells, probably due to poor exsanguination of the carcass (Fig. 40, 2). Researchers have noted that in the cross-section of the spleen, there were no small accumulations of leukocytes, and the microstructure of the liver remained unchanged.

The results of the study presented in this paper are also consistent with the results obtained by Y. Zhao *et al.* (2020), who claimed that when feeding broiler chickens a dietary probiotic *Bacillus licheniformis* H2 an increase in poultry productivity, improved immune status, and also had a positive effect on the microstructure of skeletal muscles and other internal organs, in particular the liver, heart muscle, and lungs, were observed. T. Yang *et al.* (2023) proved that the addition of probiotic drugs to the diet of broiler chickens led to rational digestion of feed and improved nutrient absorption. In turn, this affected the improvement of the quality indicators of meat and offal – an increase in water content by 2.9% ($P<0.001$), fat by 3.2% ($P<0.001$), protein by 3.2% ($P<0.001$), and their micromorphological characteristics – the development of the pectoralis major muscle with layers of adipose tissue, which is consistent with the results of microstructural anilisation described in this paper (Fig. 34, 1; 34, 2); in the liver, hepatocytes were collected in groups of the same type, their cytoplasm looked uniform, clear and pink, the nuclei had weak basophilicity and empty interstitial vessels, which correlates with the data of this study (Fig. 37, 1; 37, 2); the spleen also had empty blood vessels, and its trabecular base looked underdeveloped, as shown in Fig. 36, 1; 36, 2; the bronchial tubes of the lungs contained blood cells, as shown

in Fig. 40, 1, 40, 2; the muscular part of the stomach was well developed, had a sufficiently formed epithelial layer and a voluminous layer of connective tissue base, which corresponded to the results of this study (Fig. 38, 1; 38, 2).

To achieve an optimal effect, it is necessary to make a choice in favour of probiotic strains of microorganisms *Bacillus subtilis* and *Bacillus licheniformis*. J. Karavolias *et al.* (2018) noted that the national policy of most European countries is aimed at growing animals and poultry are free of antibiotics, and therefore, the use of probiotics is an alternative in animal husbandry, in particular in poultry farming. Researchers claimed a pronounced positive effect of probiotics on the well-being of broiler chickens, preventing the occurrence of infectious diseases, and improving the safety and quality of slaughter products.

A. Sharma *et al.* (2019) emphasised that, guided by European regulations, in particular Regulation of the European Union No. 625 (2017), veterinary inspectors should identify the risks associated with production, in particular, the inclusion of probiotic drugs in the poultry diet that affect their health and productivity, and control the circulation of meat and slaughter products of broiler chickens. For the implementation of the traceability system, which is an integral part of the functioning of the HACCP system, at the poultry meat production facility, it is necessary to follow the basic requirements for establishing dangerous risks at all stages of production and sale of broiler chicken slaughter products in accordance with the requirements of Regulation of the European Union No. 765 (2008).

The Rapid Alert System for Food and Feed (RASFF) has provided the competent control authorities with an effective tool for exchanging information on sanitary and hygienic measures to ensure the safety of feed and feed additives that have affected the safety of broiler chicken slaughter products in accordance with the requirements of the regulatory document Regulation of the European Union No. 1020 (2019). The results obtained were consistent with the requirements of this document. Thus, having studied the effect of the probiotic "Subtiform" in different doses on the microstructure of the pectoralis major muscle and internal organs of broiler chickens, certain structural and morphological differences were found in the organs, which led to the following conclusions.

## Conclusions

According to microstructural changes in the pectoralis major muscle and internal organs (heart, spleen, liver, stomach muscle, lungs) of broiler chickens, the most favourable effect of the probiotic biological product at a dose of 4.0 g/10 $dm^3$ of water was found (experimental group 3). The microscopic structure of the pectoralis major muscle of broiler chickens did not change, in particular, the muscle fibres did not differ in thickness, they were evenly directed, the cytoplasm was uniformly pale pink and fat layers were visible; the intermuscular connective tissue was somewhat loosened, and the blood capillaries in the endomysium followed the fibres. The structure of the heart muscle has been preserved, its cytoplasm is uniformly pale pink, the nuclei are moderately basophilic, cardiomyocytes in the longitudinal section are of the same size, and the vessels are desolate. Histological examination of the spleen revealed that the microstructure of the organ is represented by a diffuse accumulation of leukocytes at different stages of differentiation. At the same time, thickened vessels of various calibres were found in significant quantities. It was also proved that the microstructure of the liver of broiler chickens did not change after feeding the probiotic biological product "Subtiform". It was found that the hepatocytes were gathered in groups of the same type, the

central veins and interstitial vessels were desolate or contained single blood cells. The microstructure of the muscular part of the stomach of broiler chickens is formed by the epithelial layer of the cuticle, under which a fairly wide layer of connective tissue base is placed, forming single, clearly formed folds. The vessels are dilated, slightly filled with blood cells, muscle fibres are located under the mesenchymal base of the cuticle, which are somewhat loosened by insignificant stroma layers. Histological examination of the lungs of broiler chickens revealed that the bronchial tubes of the lungs contain blood cells. The structure of the lung tissue is in a state of compression deformation, the vessels of the organ contain a significant amount of blood cells (poor exsanguination of the carcass is possible). It is suggested that histological examination is an important method for identifying possible pathological changes in the organs of broiler chickens, which can lead to a decrease in their productivity, product quality, and associated risks. In the future study, it is planned to conduct microbiological tests of broiler chicken slaughter products with the use of a probiotic biological product.

## Acknowledgements

None.

## Conflict of Interest

None.

## References

[1] Abdulkhaliq, F., & Sabow, A.D. (2023). Carcass characteristics and meat quality assessments in broiler chickens subject to different pre-slaughter restraining methods. *Tikrit Journal for Agricultural Sciences*, 23(2), 42-61. doi: 10.25130/tjas.23.2.4.

[2] Angelovičová, M., Mellen, M., Bučko, O., Tkáčová, J., Čapla, J., Zajác, P., & Angelovič, M. (2016). Relation between selected nutrients in the chicken meat depending on phytogenic feed additives. *Potravinarstvo Slovak Journal of Food Sciences*, 10(1), 215-222. doi :10.5219/573.

[3] Arain, M.A., Nabi, F., Marghazani, I.B., & Hassan, F., Soomro, H., Kalhoro, H., Soomro, F., & Buzdar, J.A. (2022). In *ovo* delivery of nutraceuticals improves health status and production performance of poultry birds: A review. *World's Poultry Science Journal*, 78(3), 765-788. doi: 10.1080/00439339.2022.2091501.

[4] Aslam, S., Shukat, R., Khan, M.I., & Shahid, M. (2020). Effect of dietary supplementation of bioactive peptides on antioxidant potential of broiler breast meat and physicochemical characteristics of nuggets. *Food Science Animal Resource*, 40(1), 55-73. doi: 10.5851/kosfa.2019.e82.

[5] Chechet, O.M., Kovalenko, V.L., Vishchur, O.I., Haidei, O.S., Liniichuk, N.V., Gutyj, B.V., & Krushelnytska, O.V. (2022). The activity of T- and B-cell links of specific protection of chicken-broilers under the influence of synbiotic preparation "Biomagn" and "Diolide" disinfectant. *Ukrainian Journal of Veterinary and Agricultural Sciences*, 5(1), 46-52. doi: 10.32718/ujvas5-1.08.

[6] Choi, J., Kong, B., Bowker, B.C., Zhuang, H., & Kim, W.K. (2023). Nutritional strategies to improve meat quality and composition in the challenging conditions of broiler production: A review. *Animals*, 13(8), article number 1386. doi: 10.3390/ani13081386.

[7] DSTU 7353:2013. (2014). *"Meat. Methodology of histological determination of freshness and degree of ripening"*. Retrieved from https://online.budstandart.com/ua/catalog/doc-page?id_doc=89373.

[8] DSTU ISO/IEC 17025:2006. (2006). *General requirements for the competence of testing and calibration laboratories*. Retrieved from http://online.budstandart.com/ua/catalog/doc-page.html?id_doc=50873.

[9] EUs Directive 2010/63/EU on the protection of animals used for scientific purposes. (2010). Retrieved from https://norecopa.no/legislation/eu-directive-201063/.

[10] George, A.S., & George, A.S.H. (2023). Optimizing poultry production through advanced monitoring and control systems. *Partners Universal International Innovation Journal (PUIIJ),* 1(5), 77-97. doi: 10.5281/zenodo.10050352.

[11] Horalskyi, L.P., Khomych, V.T., & Kononskyi, O.I. (2015). *Fundamentals of histological technique and morphofunctional research methods in normal and pathological conditions: A study guide*. Zhytomyr: Polissya.

[12] Jha, R., Singh, A.K., Yadav, S., Berrocoso, J.F.D., & Mishra, B. (2019). Early nutrition programming (*in ovo* and post-hatch feeding) as a strategy to modulate gut health of poultry. *Frontiers in Veterinary Science*, 6, article number 82. doi: 10.3389/fvets.2019.00082.

[13] Karavolias, J., Salois, M.J., Baker, K.T., & Watkins, K. (2018). Raised without antibiotics: Impact on animal welfare and implications for food policy. *Animal Science*, 2(4), 337-348. doi: 10.1093/tas/txy016.

[14] Kler, R., Gangurde, R., Elmirzaev, S., Hossain, M.S., Vo, N.V., Nguyen, T.V., & Kumar, P.N. (2022). Optimization of meat and poultry farm inventory stock using data analytics for green supply chain network. *Hindawi Discrete Dynamics in Nature and Society*, 2022, article number 8970549. doi: 10.1155/2022/8970549.

[15] Law of Ukraine No. 3447-IV "On Protection of Animals from Cruelty". (2006, February). Retrieved from https://zakon.rada.gov.ua/laws/show/3447-15#Text.

[16] Lei, J., Dong, Y., Hou, Q., He, Y., Lai, Y., Liao, C., Kawamura, Y., Li, J., & Zhang, B. (2022). Intestinal microbiota regulate certain meat quality parameters in chicken. *Frontiers in Nutrition*, 9, article number 747705. doi: 10.3389/fnut.2022.747705.

[17] Memon, F.U., Yang, Y., Zhang, G., Leghari, I.H., Lv, F., Wang, Y., Laghari, F., Khushk, F.A., & Si, H. (2022). Chicken gut microbiota responses to dietary *Bacillus subtilis* probiotic in the presence and absence of *Eimeria* infection. *Microorganisms*, 10(8), article number 1548. doi: 10.3390/microorganisms10081548.

[18] Paliy, A.P., Stegniy, B.T., Palii, A.P., Rodionova, A.O., Bogatko, N.M., Vashchyk, Ye.V., Sakhniuk, N.I., Ovcharenko, H.V., Dudus, T.V., Ihnatieva, T.M., & Kovalenko, L.V. (2020). Microstructural alanalysis of sausage quality. *Ukrainian Journal of Ecology*, 10(2), 404-409. doi: 10.15421/2020_115.

[19] Regulation (EU) 2019/1020 of the European Parliament and of the Council of 20 June 2019 "On Market Surveillance and Compliance of Products and Amending Directive 2004/42/EC and Regulations (EC) No. 765/2008 and (EU) No. 305/2011". (2019, June). Retrieved from https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex:32019R1020.

[20] Regulation (EU) 2019/1020 of the European Parliament and of the Council of 20 June 2019 "On Market Surveillance and Compliance of Products and Amending Directive 2004/42/EC and Regulations (EC) No. 765/2008 and (EU) No. 305/2011". (2019, June). Retrieved from https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex:32019R1020.

[21] Regulation of the European Parliament and of the Council (EU) No. 765/2008 "On the Establishment of Requirements for Accreditation and Market Surveillance Related to the Sale of Products and on the Repeal of Regulation (EEC) No. 339/93. (2008, July). Retrieved from https://zakon.rada.gov.ua/laws/show/994_938#Text.

[22] Regulation of the European Parliament and of the Council (EU) No. 1381/2019 "Regarding the Transparency and Sustainability of Risk Assessment in the Food Chain". (2019, June). Retrieved from https://zakon.rada.gov.ua/laws/show/984_032-09#Text.

[23] Rousseaux, A., Brosseau, C., & Bodinier, M. (2023). Immunomodulation of B- lymphocytes by prebiotics, probiotics and synbiotics: Application in pathologies. *Nutrients*, 15(2), article number 269. doi: 10.3390/nu15020269.

[24] Shalginbayev, D., Uazhanova, R., & Mateyeva, A. (2022). Development of a laboratory method for determination of the quality and freshness of frozen poultry meat. *Potravinarstvo Slovak Journal of Food Sciences*, 16, 271-278. doi: 10.5219/1757.

[25] Sharma, A., Motta, V., & Martinez, L. (2019). Effectiveness of short videos to enhance HACCP information for consumers. *Journal of Foodservice Business Research*, 22(1), 1-14. doi: 10.1080/15378020.2019.1663104.

[26] Shaufi, M.A.M., Sieo, C.C., Chong, C.W., Geok Hun, T., Omar, A.R., Han Ming, G., & Wan Ho, Y. (2023). Effects of phage cocktail, probiotics, and their combination on growth performance and gut vicrobiota of broiler chickens. *Animals*, 13(8), article number 1328. doi: 10.3390/ani13081328.

[27] Soomro, R.N., Abd El-Hack, M.E., Shah, S.S., Taha, A.E., Alagawany, M., Swelum, A.A., Hussein, E.O.S., Ba-Aawdh, H.A., Saadeldin, I., El-Edel, M.A., & Tufarelli, V. (2019). Impact of restricting feed and probiotic supplementation on growth performance, mortality and carcass traits of meat-type quails. *Animal Science*, 90(10), 1388-1395. doi: 10.1111/asj.13290.

[28] Stoyanovskyy, V.G., Shevchuk, M.O., & Kolomiiets, I. A. (2020). Physiological state of the body of broiler chickens against the background of combined stress when humic acids and probiotics are included in the diet. *Scientific Bulletin of the Lviv National University of Veterinary Medicine and Biotechnology named after S.Z. Gzhitskyi (Veterinary Sciences),* 22(99), 157-161. doi: 10.32718/nvlvet9725.

[29] Suliman, G.M., Hussein, E.O.S., Alsagan, A., Al-Owaimer, A.N., Alhotan, R., Al-Baadani, H.H., Ba-Awadh, H.A., Qaid, M.M., & Swelum, A.A. (2023). Effects of adding nano-emulsified plant oil and probiotics to drinking water during different periods besides sex on processing characteristics, physicochemical properties, and meat quality traits of broiler chickens. *Frontiers in Veterinary Science*, 10, article number 1133605. doi: 10.3389/fvets.2023.1133605.

[30] Tavaniello, S., De Marzo, D., Bednarczyk, M., Palazzo, M., Zejnelhoxha, S., Wu, M., Peng, M., Stadnicka, K., & Maiorano, G. (2023). Influence of a commercial synbiotic administered in ovo and in-water on broiler chicken performance and meat quality. *Foods*, 12(13), article number 2470. doi: 10.3390/foods12132470.

[31] Vinderola, G., Cotter, P.D., Freitas, M., Gueimonde, M., Holscher, H.D., Ruas-Madiedo, P., Salminen, S., Swanson, K.S., Sanders, M.E., & Cifelli, C.J. (2023). Fermented foods: A perspective on their role in delivering biotics. *Frontiers in Microbiology*, 14, article number 1196239. doi: 10.3389/fmicb.2023.1196239.

[32] Vishchur, O.I., Romanovych, L.V., Smolyaninov, K.B., Masyuk, M.B., & Romanovych, M.M. (2020). The effects of vitamins E and C on individual lipides in the liver and skeletal muscles of chicken broilers'. *Journal for Veterinary Medicine, Biotechnology and Biosafety*, 6(1), 11-14. doi: 10.36016/JVMBBS-2020-6-1-2.

[33] Weimer, S.L., Wideman, R.F., Scanes, C.G., Mauromoustakos, A., Christensen K.D., & Vizzier-Thaxton, Y. (2018). An evaluation of methods for measuring stress in broiler chickens. *Poultry Science*, 97(10), 3381-3389. doi: 10.3382/ps/pey204.

[34] Yang, T., Du, M., Zhang, J., Ahmad, B., Cheng, Q., Wang, X., Abbas, Z., Tong, Y., Li, J., Zhou, Y., Zhang, R., & Si, D. (2023). Effects of *Clostridium butyricum* as an antibiotic alternative on growth performance, intestinal morphology, serum biochemical response, and immunity of broilers. *Antibiotics*, 12(3), article number 433. doi: 10.3390/antibiotics12030433.

[35] Zhang, Z., Zhao, L., Wu, J., Pan, Y., Zhao, G., Li, Z., & Zhang, L. (2023). The effects of *Lactobacillus johnsonii* on diseases and its potential applications. *Microorganisms*, 11(10), article number 2580. doi: 10.3390/microorganisms11102580.

[36] Zhao, Y., Zeng, D., Wang, H., Qing, X., Sun, N., Xin, J., Luo, M., Khalique, A., Pan, K., Shu, G., Jing, B., & Ni, X. (2020). Dietary probiotic *Bacillus licheniformis H2* enhanced growth performance, morphology of small intestine and liver, and antioxidant capacity of broiler chickens against *Clostridium perfringens*-induced subclinical necrotic enteritis. *Probiotics and Antimicrobial Proteins*, 12(3), 883-895. doi: 10.1007/s12602-019-09597-8.

[37] Zhu, Q., Sun, P., Zhang, B., Kong, L., Xiao, C., & Song, Z. (2021). Progress on gut health maintenance and antibiotic alternatives in broiler chicken production. *Frontiers in Nutrition,* 8, article number 692839. doi: 10.3389/fnut.2021.692839.

# Мікроструктурний аналіз м'яса та внутрішніх органів курчат-бройлерів за використання пробіотичного біопрепарату

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**Анотація.** Пробіотичний препарат із умістом бактерій роду *Bacillus subtilis і Bacillus licheniformis* застосовується при годівлі курчат-бройлерів для покращення перетравлення корму, засвоюваності поживних речовин, підвищення імунного статусу та продуктивності, а також для профілактики і лікування різноманітних хвороб птиці. Мета роботи – провести гістологічні дослідження продуктів забою курчат-бройлерів при застосуванні їм пробіотичного біопрепарату в дозах: 0,5 г, 2,0, і 4,0 г на 10 дм$^3$ води. Матеріал досліджували гістологічним методом. Встановлено, що м'язові волокна у великому грудному м'язі однотипові, рівномірно направлені, цитоплазма м'язових волокон помірно еозинофільна, однорідно-світло-рожева, між пучками м'язових волокон виявлено незначні прошарки жирової тканини. Морфологічна архітектоніка серцевого м'яза збережена, кардіоміоцити однорідні, мають чітку спрямованість. При цьому, незмінена мікроструктура печінки курчат-бройлерів: гепатоцити зібрані в однотипні групи; центральні вени запустілі; цитоплазма цих клітин однорідна, просвітлена, рожева, ядра слабо базофільні. У селезінці фолікулярна структура оформлена, дифузно розміщені лейкоцити на різних етапах диференціювання; судини у значній кількості, загустілі, різного калібру. Кутикула м'язової частини шлунка містить сформований епітеліальний шар, виявлена об'ємна частина прошарку сполучнотканинної основи; під мезенхімальною основою кутикули розташовані м'язові волокна. Легені за морфологічною структурою мають бронхіальні трубочки по всій структурі, які містять формені елементи крові. За результатами проведених досліджень встановлено сприятливий вплив пробіотичного біопрепарату в дозі 4,0 г/10 дм$^3$ води на морфологію великого грудного м'яза та внутрішніх органів курчат-бройлерів. Тому, пробіотик у дозі 4,0 г/10 дм$^3$ води під час випоювання курчат-бройлерів можна рекомендувати для підвищення продуктивності та отримання безпечних продуктів забою. Практична значимість отриманих результатів полягає у визначенні особливостей впливу випоювання птиці різних доз пробіотику на мікроструктуру продуктів її забою, що важливо для отримання найкращого ефекту від його застосування

**Ключові слова:** птиця; великий грудний м'яз; продукти забою; гістологічні дослідження; морфологія; пробіотик

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# Effect of lactic acid bacteria ferment cultures on pork freshness

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**Abstract.** During the primary pork processing at the stage of cooling half-carcasses, their mass is lost, leading to economic losses. One promising way to solve this problem is to wash half-carcasses with chilled water. This requires decontaminating meat with microflora, which causes

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its spoilage. The purpose of the study is to determine the effectiveness of the final treatment of pork half-carcasses with ferment cultures of SafePro® B-SF-443 (*Leuconostoc carnosum*) and SafePro® B-2 (*Lactobacillus sakei*) strains for their bioconservation and preservation of freshness. The paper uses organoleptic, chemical, and microbiological research methods. It was established that cooling half-carcasses of pork in the refrigerator using cold water washing contributes to the appearance of signs of meat spoilage in terms of microbial contamination and pH value already on the 4th day of storage. Surface treatment of half-carcasses with suspensions of lactic acid microorganisms of SafePro® B-SF-43 (*Leuconostoc carnosum*) and SafePro® B-2 (*Lactobacillus sakei*) strains at a dose of $10^7$/cm$^2$ provides proper organoleptic parameters and the pH value of pork up to 7 days of storage, which correspond to high-quality meat. Both ferment cultures of lactic acid microorganisms reduce microbial contamination of meat due to the number of mesophilic aerobic and facultative anaerobic microorganisms in paired carcasses by 1.25 and 0.65 lg CFU/cm$^2$ and increase the number of lactic acid microorganisms by 3.47 and 3.43 lg CFU/cm$^2$ accordingly, this allows extending the shelf life of meat in the chilled form to at least 7 days. The most suitable culture for the final processing of half-carcasses of pork, which are cooled in the refrigerator in combination with washing with chilled water, is the SafePro® B-2 (*Lactobacillus sakei*) strain. The results obtained are promising for improving the technology of primary pork processing at the cooling stage using ferment cultures of lactic acid microorganisms as natural preservatives, which will increase the shelf life of meat, considering its quality and safety

**Keywords**: meat; cooling; washing; storage; microbial contamination

## Introduction

Post-slaughter changes that occur during the maturation process of slaughtered animal meat play a crucial role in developing its qualitative characteristics and the overall perception of a fresh product. After animal slaughter, muscle glycogen, together with high-energy phosphate compounds, is anaerobically mobilised to maintain homeostasis by synthesising adenosine triphosphate (ATP). As a consequence of post-slaughter glycolysis, lactate and $H^+$ accumulate in the muscles and cause a decrease in pH. In the end, post-slaughter metabolism in the tissues of slaughtered animals stops due to substrate depletion or inactivation of phosphofructokinase-1, which leads to ATP deficiency and the beginning of meat freezing processes. Over time, animal muscles undergo proteolysis of structural proteins that change their properties. As noted by J.C. Wicks *et al.* (2022), during the breakdown of individual peptide bonds, the structure of proteins changes its configuration, which further improves the tenderness of muscle tissue.

Usually, muscle tissue is considered practically sterile before slaughter, but the environment during animal slaughter is not sterile, which causes a certain degree of microbial contamination, which leads to spoilage of meat. The sources of microbial contamination in this process can be generalised as endogenous and exogenous. K. Klaharn *et al.* (2021) report that the microbiological quality of fresh animal meat largely depends on its type, processing conditions, handling, and storage. In addition, according to this author, improper hygiene of slaughtering equipment, personnel and environmental factors (for example, water, air, and soil) lead to cross-contamination with

microorganisms associated with spoilage of meat and processed products. During storage, the process of microbial contamination of meat is influenced by various internal and external factors, in particular, the presence of oxygen, pH level, temperature, and competing microorganisms in the environment. The diversity of these ecophysiological factors affects microbial growth dynamics, including the composition and ratio of the microbiota, which ultimately affects the type and rate of meat spoilage processes. K. Koutsoumanis *et al.* (2023) indicate that the main bacteria that cause meat spoilage include *Pseudomonas spp., Lactobacillus spp. Enterococcus spp., Weissella spp., Brochothrix spp., Leuconostoc spp., Lactobacillus spp., Shewanella spp., and Clostridium spp*. According to the traditional practice of primary processing of carcasses, microbiological risks that cause spoilage can affect the maturation of meat. Keeping meat under controlled conditions can reduce the burden of microbiological hazards to a certain extent.

In a study by A. Nakamura *et al.* (2023), it is noted that a fast decrease in the temperature of the meat before it reaches the proper level of acidification can lead to weight loss in the carcass. Shrinkage during rapid cooling can particularly affect carcasses obtained from high-performance meat breeds of pigs due to the lower subcutaneous fat content and faster heat release. A substantial number of technologies for their primary processing are used to reduce the weight loss of slaughtered animal carcasses, in particular, the use of washing, which allows quickly reducing the temperature of the half-carcass and the intensity of moisture evaporation. Therewith, this does not allow avoiding contamination of carcasses with microflora, which occurs during the time of slaughter of animals and subsequent operations of their primary processing. There is exogenous and endogenous contamination with microorganisms, the sources of which can be the skin, the contents of the digestive system, the air environment, technological equipment, vehicles, tools, hands, clothing, and shoes of workers who have contact with meat, and water used for processing carcasses. The air microflora in animal slaughtering and primary processing of carcasses is most often represented by various spore microflora, in particular, aerobic and anaerobic bacteria, and gram-negative non-spore rods, actinomycetes, mould fungi, and yeast. They get to the surface of meat from the external environment during skinning and subsequent stages of primary processing of slaughtered animals.

V. Vovkotrub *et al.* (2023) proved that one of the promising ways to reduce meat contamination are biotechnologies based on using lactic acid microorganisms of various strains that can show antagonism to putrefactive and pathogenic microflora. These cultures include Safe-Pro® B-SF-43 (*Leuconostoc carnosum*) and Safe-Pro® B-2 (*Lactobacillus sakei*) strains, the use of which in a dose of $10^7$/cm$^2$ in half-carcasses of pork helped to reduce the intensity of autolysis of muscle tissue and, thus, extend the shelf life of meat to 7 days in a chilled form.

In most studies, lactic acid microorganisms are used in the technology of boiled or dried meat products, but for fresh meat, in particular, pork, they have not become widespread, which is due to the lack of scientifically based data on the effect of ferment cultures on the quality and safety of meat for storage in a chilled state. The purpose of the study is to determine the quality and safety indicators of pork in half-carcasses during cooling in the refrigerator compartment using washing and final treatment with suspensions *Lactobacillus sakei* and *Leuconostoc carnosum*.

## Literature Review

Meat is a source of protein in the human diet, but simultaneously – a source of spread of

pathogens of food origin around the world. Microbial contamination of meat can cause food infections, toxicoinfections, and toxicosis in humans. K. Klaharn *et al.* (2022) and Z. Rani *et al.* (2023) note that the risks associated with the threat to consumers of pathogenic microorganisms transmitted through meat require not only the examination of their distribution chains but also measures to prevent potential human diseases.

Under such conditions, there is a need to develop and implement scientifically based ways to reduce microbial contamination of animal carcasses using various means with an antimicrobial spectrum of action, in particular, organic acids, chlorine compounds, bacteriocins, essential oils, as described by F. Soyer (2020), Z.A. Ellatif *et al.* (2020), D.F. Benítez-Chao *et al.* (2021), and combining them with physical methods of meat decontamination. Because the above methods of reducing microbial contamination of meat contribute to the emergence of changes in the sensory properties of both fresh meat and products made from it, technologies that guarantee the safety of meat and improve its organoleptic characteristics come to the fore. S. Soltani *et al.* (2022) believe that one of the most promising ways to address this issue, along with good hygiene practices, is the treatment of the surface of half-carcasses of slaughtered animals with natural preservatives, in particular, suspensions of lactic acid bacteria, which can show antagonistic activity against undesirable and pathogenic microorganisms.

Bioconservation is a promising way to control the growth of unwanted bacteria on fresh meat. It is based on the ability to inhibit the growth of bacteria that spoil meat and thus increase the shelf life of meat. This aspect is much less studied compared to using an approach aimed at eliminating pathogenic bacteria in meat and processed products. Equally important is the influence of these microorganisms on the sensory characteristics of meat that attract the consumer. Studies have shown that microbial ferment cultures can delay the growth of bacteria that spoil meat, in particular, *Brochothrix thermosphacta*, *Pseudomonas* spp., and *Enterobacteriaceae*. However, despite the fact that the examination results give grounds for the introduction of such a technology in meat production, the concept of bioconservation using microorganisms for fresh meat is still limited. This is due to a lack of research in this area, especially in relation to the primary processing of pork. In addition, according to M.M. Xu *et al.* (2022), bioconservatives based on microbial ferment cultures require additional testing under conditions typical of specific meat processing plants.

The most promising microorganisms that can be used for primary processing and storage of pork include lactic acid bacteria of specialised strains that can form bacteriocins, have an antagonistic effect against pathogenic and conditionally pathogenic microorganisms, and improve the organoleptic properties of meat, especially during its storage in a chilled state. These microorganisms include bacteria of the *Lactobacillus* genus, in particular, *Lactobacillus sakei* (Costa *et al.*, 2019) and *Leuconostoc carnosum* (Raimondi *et al.,* 2019). As noted by A. Najjari *et al.* (2020), *Lactobacillus sakei* can withstand a substantial temperature range, pH level, and table salt content in the medium up to 8% and dominate among other microflora. In addition, this lactic acid microorganism forms a number of organic acids and aromatic compounds that give flavour to meat products, which makes it promising for use in meat production technology. *Leuconostoc carnosum* can grow at temperatures compatible with cooling and in the presence of up to 60 g/L of sodium chloride (NaCl) in many meat-based products.

## Materials and Methods

The experiment used half-carcasses of pork, which were obtained after slaughter and primary processing in the conditions of LLC "Antonivsky meat processing plant", Kyiv region, during May 2021. 30 fattening pigs aged 6 months were used for slaughter. The slaughter of pigs was conducted in compliance with the current Order No. 28 "Rules of Pre-Slaughter Veterinary Inspection of Animals and Veterinary-Sanitary Examination of Meat and Meat Products" (2002), good hygiene practices (GMP). After slaughter, half-carcasses of pigs were transported to specialised cold storage rooms for cooling and further storage. The resulting pork half-carcasses were divided into 3 groups of 10 half-carcasses each, which were processed according to the scheme shown in Table 1. Suspensions of lactic acid microorganisms *Leuconostoc carnosum* and *Lactobacillus sakei* produced by *Chr. Hansen* (LLC "Chr. Hansen Ukraine") were used to treat the surface of pork half-carcasses.

**Table 1**. Scheme of the experiment to determine the effect of ferment cultures of lactic acid microorganisms on the freshness of pork during storage in a chilled state

| Group | Experiment conditions |
|---|---|
| Control | Half-carcasses of pork are subjected to a wet toilet and washing |
| Experimental 1 | Half-carcasses of pork are subjected to wet toilet and washing with the final treatment with ferment culture of the SafePro® B-SF-43 (*Leuconostoc carnosum*) strain at a dose of $10^7$/cm$^2$ |
| Experimental 2 | Half-carcasses of pork are subjected to wet toilet and washing with the final treatment with ferment culture of the SafePro® B-2 (*Lactobacillus sakei*) at a dose of $10^7$/cm$^2$ |

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

Ferment cultures of lactic acid microorganisms were applied to the surface of the half-carcasses after the washing process ended. All half-carcasses of pork were stored in the industrial refrigerator of the meat processing plant at a temperature of 3±1°C. The results of the study were recorded 1 hour after cooling, on the 4$^{th}$ and 7$^{th}$ days of storage of half-carcasses. The mass of half-carcasses of pork was determined in a meat processing plant using industrial scales TV4-1500 with an accuracy of 0.1 kg. The temperature and pH of the meat were examined using EZODO MP-103M pH meter (Taiwan, GOnDO'Electronic Co., Ltd).

All microbiological studies were conducted in the Bila Tserkva city state laboratory of the State Food Safety and Consumer Protection Service of Ukraine. For determining the number of mesophilic aerobic and facultative anaerobic microorganisms (MAFAM), bacteria of the *Salmonella spp., S. aureus*, *Lactobacillus*, *L. monocytogenes* genera, fungi, and mould were washed off from the surface of half-carcasses in the area of the collar. Consecutive ninefold dilutions using sterile saline solution were prepared for the study. The number of microorganisms in meat samples was determined in colony-forming units (CFU), and the results are presented in lg CFU/cm$^2$ of the surface. Generic and specific identification of microorganisms isolated from the meat of half-carcasses of the control and both experimental groups was conducted in accordance with the current methods.

The Plate count agar M091 medium (HiMedia, India) was used to determine the number of MAFAM. For isolation and quantitative counting of bacteria of the *Lactobacillus* genus – Lactobacillus MRS Agar M641 (HiMedia, India), for isolation of pathogenic and non-pathogenic staphylococci – Baird Parker, Agar M043

(HiMedia, India), for *Salmonella* – Bismuth Sulfite Agar M027 (HiMedia, India) and Xylose Lysine Deoxycholate Agar M031 (HiMedia, India), for *L. monocytogenes* – Agar Palcam, Agar Oxford (HiMedia, India). Organoleptic assessment of pork (colour, smell, consistency, cooking sample) was determined according to DSTU 7992:2015 (2017). Microscopic analysis of meat freshness was conducted in accordance with GOST 23392:2016 (2017). Moisture-retaining ability of meat was determined by pressing. Meat sampling of slaughtered animals and their preparation for microbiological tests were conducted in accordance with the requirements of DSTU 8381:2015 (2017). The selection of washes from the surface of half-carcasses of meat stored in the cold storage rooms of the meat processing plant was conducted in the collar area in accordance with the requirements of DSTU ISO 17604:2014 (2015). Statistical processing of the examination results was performed using the ANOVA programme, and the data in the tables are presented as x±SD (mean ± standard deviation). The difference between the groups was considered likely using the Tukey Test at $P<0.05$ (considering the Bonferroni correction).

## Results and Discussion

Cooling pork in the refrigerator in combination with washing affected the appearance of the paired half-carcasses – the surface is wet, pale, and without a drying crust. When processing pork with microbial ferment cultures of SafePro® B-SF-443 (*Leuconostoc carnosum*) and SafePro® B-2 (*Lactobacillus sakei*) strains at a dose of $10^7/cm^2$, it was determined that on the fourth day of storage, organoleptic indicators: the appearance, colour, consistency, and smell of meat of half-carcasses of the control and experimental groups did not differ. The meat had a pink colour, inherent in pork, on the cut – quite dense and elastic, and the smell was specific, characteristic of fresh meat. Organoleptic parameters of meat on the 4th day of storage of half-carcasses in these groups, compared with the initial data immediately after their washing (in paired half-carcasses) and ferment treatment, substantially improved. They have acquired an attractive pink colour, and their appearance is characteristic of pork.

As noted by S. Ali & A.F. Alsayeqh (2022), the degree of microbial contamination is one of the important indicators of meat safety, especially when storing it in a chilled state. Microscopic analysis of prints of pork samples from both the control and experimental groups on the 4th day of storage revealed that the number of microorganisms in the field of view did not depend on the method of processing half-carcasses (Table 2) and was within the limits characteristic of Information of N. Bogatko (2019) for chilled meat.

**Table 2**. Indicators of microscopic analysis of smears and moisture capacity of pork on the 4th day of storage, x±SD, n=5

| Group | Number of microorganisms in the field of view | Moisture capacity, mg |
|---|---|---|
| Control | 24.60±4.15[a] | 32.44±5.31[a] |
| Experimental 1 | 9.20±4.21[a] | 32.44±3.87[a] |
| Experimental 2 | 17.75±3.69[a] | 52.28±9.67[a] |

***Note:*** *different superscript letters a, b indicate the values that considerably differed in the same row of the table (P<0.05) based on the results of comparison using the Tukey test*

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

Half-carcass washing, in this case, did not affect the microbial contamination of pork, which is consistent with the results obtained by I.F. Jaja *et al.* (2018), before and after washing beef, lamb, and pork. In particular, as noted in this study, the most contaminated areas were the neck, sides, and chest, and washing carcasses did not change the amount of sanitary microflora in the neck and sternum of pork and beef carcasses. The largest number of *E. coli* colonies was identified after washing the samples in the neck area, and the highest number of *S. aureus* colonies was isolated from the washes on the sides. One of the main technological characteristics of meat is its water retention capacity since it substantially affects the yield of the finished product. Water retention capacity of meat, according to the data, obtained by B. Lebret & M. Čandek-Potokar (2022), is mainly determined by the changes that occur in it after slaughter, especially the rate and degree of pH decrease. The moisture capacity of pork in half-carcasses in the chilled form on the 4$^{th}$ day of storage did not substantially differ between the control and experimental groups. Therewith, the final treatment of half-carcasses of pork with ferment culture of the SafePro® B-2 (*Lactobacillus sakei*) strain at a dose of $10^7$/cm$^2$ contributed to the tendency to increase the moisture capacity of meat, which indicates some improvement in its technological properties. The temperature in the thickness of half-carcasses of pork in both the control group and both experimental groups on the 7$^{th}$ day of storage did not substantially differ, which is associated with their storage at a stable temperature in one refrigerator at a temperature of 3±1°C (Table 3).

**Table 3**. Temperature and pH value of pork in half-carcasses on the 7$^{th}$ day of storage, x±SD, n=5

| Group | Temperature in the thickness of the half-carcass, °C | pH of meat, units |
|---|---|---|
| Control | 4.41±0.17$^a$ | 6.71±0.16$^a$ |
| Experimental 1 | 4.39±0.23$^a$ | 5.90±0.12$^b$ |
| Experimental 2 | 4.30±0.10$^a$ | 5.86±0.14$^b$ |

***Note:*** *different superscript letters a, b indicate the values that considerably differed in the same row of the table (P<0.05) based on the results of comparison using the Tukey test*

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

One of the critical indicators that indicate the freshness of meat is its pH value, which, in the case of cooling half-carcasses of pork in the refrigerator using washing and final treatment with ferment SafePro® B-SF-43 (*Leuconostoc carnosum*) and SafePro® B-2 (*Lactobacillus sakei*) strains at a dose of $10^7$/cm$^2$ on the 7$^{th}$ day of storage was lower (P<0.05) by 0.81 and 0.85 units compared to the control. Therewith, on the 7$^{th}$ day of storage, the pH value of pork in both experimental groups was within the limits characteristic of fresh meat, and in the control group – higher and indicated the beginning of its spoilage. Notably, unlike live animals, which have a muscle tissue pH value in the range of 7.0-7.2, after slaughter, as a result of oxygen deficiency, anaerobic processes begin to prevail over aerobic ones, which are accompanied by the accumulation of lactic acid and rapid acidification of muscle tissue. Although carbohydrates make up a small percentage of muscle tissue (0.5-1.5% of muscle mass), they play an essential role in converting muscle into meat. In particular, glucose and glycogen are important

for muscle metabolism and can be used in both aerobic and anaerobic environments. C. Álvarez *et al.* (2019) report that glycogen is converted to ATP with the accumulation of lactic acid until the pH value of meat reaches a 5.6-5.3.

The optimal pH value of pork is considered to be 5.6-5.9 units, which, according to A. Zmudzińska *et al.* (2020) and H. Jankowiak *et al.* (2021), indicates its freshness and proper quality. All properties of meat are directly or indirectly determined by the intensity of biochemical processes that occur in the muscle tissue of the animal during life, after slaughter, and during the maturation of meat, and the pH value of the medium largely regulates their course. The level of acidification (pH value) is associated with changes in muscle protein hydration and in the binding and retention of water in meat, which ultimately determines its tenderness. In addition, the changes in the pH values in pork during chilled storage reflect to a certain extent the ratio, total number, and metabolic activity of the dominant microorganisms that cause meat spoilage, in particular, species belonging to the *Pseudomonas* genus which are known for their proteolytic activity. This, in turn, can lead to the formation of alkaline bacterial metabolites, which lead to a shift in the pH value of meat to the alkaline side (Fengou *et al.,* 2019). Such changes were identified in the experiment in pork of the control group.

The obtained data on changes in the pH value of pork cooled in a refrigerator using washing and treatment of half-carcasses with ferment cultures of lactic acid microorganisms are consistent with the indicators of organoleptic research of meat of the control and both experimental groups (Table 4).

**Table 4**. Organoleptic parameters of pork meat on the 7th day of storage, n=10

| Group | Appearance, colour | Consistency | Smell |
|---|---|---|---|
| Control | the surface is moistened, slightly sticky, darkened | the meat is not dense and elastic enough, the hole is made slowly during pressing (within 1 min.), the fat is soft. | slightly sour or with a hint of mustiness |
| Experimental 1<br>Experimental 2 | the meat is pink or pale pink in colour, the surface is shiny, without pronounced stickiness | on the cut, the meat is dense, elastic, the hole is quickly filled when pressed. | specific, characteristic of pork that is ripe |

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

Thus, pork meat in half-carcasses of the first and second experimental groups showed signs of fresh. The meat of half-carcasses of pork in the control group was classified as questionable freshness according to the main organoleptic parameters since it showed signs of spoilage. Signs of meat spoilage that were observed during the storage of half-carcasses of pork in the control group include, according to information of Y. Zhu *et al*. (2022), substantial discolouration, unpleasant odours, and mucus formation, which define the main criteria for consumers' rejection of this food product. Considering the data of organoleptic studies, pork samples from half-carcasses of experimental groups were taken for further analysis of smear-preparations. The number of microorganisms in smears-preparation of pork that was cooled in a refrigerator using washing and a final treatment with ferment culture of the SafePro® B-SF-43 (*Leuconostoc carnosum*) strain, on the 7th day of storage was 2 times higher ($P<0.05$), than when treating the culture of SafePro® B-2 (*Lactobacillus sakei*) strain with ferment (Table 5).

**Table 5**. Indicators of microscopic analysis of smears-impressions and moisture capacity of pork for 7 days of storage of half-carcasses, x±SD, n=5

| Group | Number of microorganisms in the field of view | Moisture capacity, mg |
|---|---|---|
| Experimental 1 | 505.67±25.00$^{b}$ | 40.63±9.94$^{a}$ |
| Experimental 2 | 251.67±16.42$^{a}$ | 46.53±4.25$^{a}$ |

***Note:*** *different superscript letters a, b indicate the values that considerably differed in the same row of the table (P<0.05) based on the results of comparison using the Tukey test*

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

An increase in the number of microorganisms in smears-prints indicates the questionable freshness of meat, but the reason for such a large number of bacteria in the field of view of the microscope, in this case, was the processing of pork in half-carcasses with cultures of lactic acid bacteria that suppress putrefactive microflora and can penetrate the thickness for 7 days of its storage. Therewith, the absence of tissue breakdown, the presence of delineation of muscle fibres and organoleptic research data indicate the proper freshness of this meat. The moisture content of pork during this period did not substantially differ between the experimental groups (Table 5) and was in the optimal range characteristic of the meat of young pigs, which, according to O.M. Iakubchak *et al.* (2005), ranges from 38 to 48%. This indicator can be influenced by a number of factors: growing conditions, fattening, breed, primary processing, meat storage, and microflora contamination.

Although the surface of half-carcasses of pork of experimental groups was treated with suspensions of lactic acid bacteria of SafePro® B-2 (*Lactobacillus sakei*) and SafePro® B-SF-43 (*Leuconostoc carnosum*) strains at a dose of $10^7$/cm$^2$, the number of MAFAM on the meat surface decreased (P<0.05) by 1.25 lg CFU/cm$^2$ and 0.65 lg CFU/cm$^2$, respectively, compared to the control. Therewith, the number of MAFAM on the surface of the meat of half carcasses of pork, which was subject to the final surface treatment with ferment culture of the SafePro® B-2 (*Lactobacillus sakei*) strain, increased (P<0.05) by 0.6 lg CFU/cm$^2$ compared to the same indicator of half-carcasses that were treated with ferment culture of the SafePro® B-SF-43 (*Leuconostoc carnosum*) strain (Table 6).

**Table 6**. Microbiological parameters of pork in paired half-carcasses, x±SD, n=5, lg CFU/cm$^2$

| Group | MAFAM | Lactic acid bacteria |
|---|---|---|
| Control | 5.29±0.91$^{a}$ | 2.51±0.94$^{a}$ |
| Experimental 1 | 4.04±0.67$^{b}$ | 5.98±0.32$^{b}$ |
| Experimental 2 | 4.64±0.23$^{c}$ | 5.94±0.51$^{b}$ |

***Note:*** *different superscript letters a, b indicate the values that considerably differed in the same table row (P<0.05) based on the results of comparison using the Tukey test*

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

The number of lactic acid bacteria on the surface of paired pork half-carcasses, which were treated with suspensions of lactic acid bacteria of SafePro® B-2 (*Lactobacillus sakei*) and SafePro® B-SF-43 (*Leuconostoc carnosum*) strains during cooling at a dose of $10^7$/cm$^2$, increased (P<0.05) by 3.47 lg CFU/cm$^2$ and 3.43 lg CFU/cm$^2$, respectively, compared to the control group (Table 6). This is due to the ability of lactic acid microorganisms to penetrate

the thickness of meat and show an antagonistic effect on other types of microbiota. The most common approach to producing quality meat is to use agents that have antimicrobial effects against bacteria responsible for food spoilage. The ideal bioconservation agent should exhibit only specific antimicrobial activity against targeted pathogens or microorganisms that cause spoilage and should not negatively affect the consumer's own gut microbiome.Lactic acid microorganisms *L. carnosum* and *L. sakei* correspond to these characteristics Therewith, an important property of *L. carnosum* and *L. sakei* is the ability to rapidly increase the number of colonies in food products, in particular, meat, acidify the environment in combination with antibacterial, bacteriostatic, and bactericidal properties due to the formation of organic acids, hydrogen peroxide, and bacteriocins (Barcenilla *et al.*, 2022). High adaptation of *Leuconostoc carnosum* and *Lactobacillus sakei*, as noted by M. Zagorec & M.C. Champomier-Vergès (2017) and F. Candeliere *et al.* (2021), also contributes to a nitrogen-rich environment, such as meat. Colonisation of meat with individual strains of these lactic acid microorganisms avoids changes in its sensory characteristics during storage in a chilled form.

In addition, meat, as noted by O.A. Odeyemi *et al.* (2020), is an ideal substrate for microbes due to its rich nutrient density, favourable water activity and pH value. On the 4$^{th}$ day of storage of pork, which was treated with suspensions of lactic acid bacteria of SafePro® B-2 (*Lactobacillus sakei*) and SafePro® B-SF-43 (*Leuconostoc carnosum*) strains during cooling at a dose of $10^7$/cm$^2$, a lower number of MAFAM was identified at 0.82 lg CFU/cm$^2$ and 0.53 lg CFU/cm$^2$ compared to control. Simultaneously, in pork of both experimental groups, which was] subjected to final treatment with suspensions of lactic acid microorganisms, an increase ($P<0.05$) in the number of lactic acid bacteria was observed by 1.65 lg Kuo/cm$^2$ and 1.17 lg Kuo/cm$^2$ compared to the control (Table 7).

**Table 7**. Microbiological parameters of pork in half-carcasses on the 4$^{th}$ day of storage, x±SD, n=5, lg CFU/cm$^2$

| Group | MAFAM | Lactic acid bacteria |
|---|---|---|
| Control | 5.34±0.87$^a$ | 4.08±0.16$^a$ |
| Experimental 1 | 4.52±0.36$^b$ | 5.73±0.23$^b$ |
| Experimental 2 | 4.51±0.07$^b$ | 5.25±0.18$^b$ |

***Note:*** *different superscript letters a, b indicate the values that considerably differed in the same row of the table (P<0.05) based on the results of comparison using the Tukey test*

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

It was proved that in pork that was cooled in a refrigerator using washing without treatment with suspensions of lactic acid microorganisms (control group), lactic acid microorganisms dominated on the 4$^{th}$ day of storage. The same pattern was found by C. Braley *et al.* (2023) for storing pork in vacuum packaging on days 15 and 29 at a temperature regime of -1.5°C, which was changed according to production risks at 2°C or 10°C for several hours. However, in this case, the researchers note that in contrast to SafePro® B-2 (*Lactobacillus sakei*) and SafePro® B-SF-43 (*Leuconostoc carnosum*) strain cultures, most types of lactic acid microorganisms belong to the microflora that cause spoilage of meat (EFSA Panel on Biological Hazards (BIOHAZ) (2023). Therewith, it is proved that after applying cultures of lactic acid microorganisms

of the above-mentioned strains to half-carcasses for 4 days of storage in a chilled state, their number practically does not increase. This is regarded as a positive fact, provided that these lactic acid microorganisms, to some extent, restrain the development of other microflora, especially mould fungi and yeast, and do not form mucus and fermentation products with an unpleasant smell.

According to C.H. Jeong *et al.* (2022) and S.G. Kim & S.Y. Kim (2023), the property of individual strains of lactic acid microorganisms has been used for quite a long time for the production of various meat products, in particular, sausages and dried ham. However, in this case, it is desirable to achieve a substantial increase in the number of lactic acid bacteria that are involved in the fermentation of the food product. Thus, it not only ensures its preservation but also the maturation and acquisition of specific sensory properties.In the case of processing half-carcasses of pork with suspensions of lactic acid microorganisms SafePro® B-2 (*Lactobacillus sakei*) and SafePro® B-SF-43 (*Leuconostoc carnosum*) as bioconservants, the goal is to achieve a stable number of them in the product, which guarantees the suppression of the development and reproduction of unwanted microflora, including other types of lactic acid microorganisms that cause meat spoilage, but does not provide for their further rapid growth. This method of bioconservation of pork half-carcasses during primary processing and cooling has not yet become widespread in meat processing enterprises.

Considering the fact that the pork of the control group, which was subjected to cooling in a refrigerator using washing, according to organoleptic parameters, is not suitable for further storage, microbiological studies on the 7$^{th}$ day of storage were conducted on only half of the experimental groups. The data obtained in the course of the study are consistent with similar results of M. Kukhtyn *et al.* (2020), noted for storing beef chilled. Therewith, the authors established that storage of beef meat with an initial amount of mesophilic bacteria of about 4.88 log CFU/cm$^2$ and surface and psychrotrophic bacteria – 3.79 log CFU/cm$^2$ at a temperature of 0°C is possible only for 8 days. Further, microbiological indicators exceed the permissible norms and such meat is considered unsuitable. The number of MAFAM per 7 days of storage in pork during the final ferment treatment of the SafePro® B-SF-43 (*Leuconostoc carnosum*) strain culture increased (P<0.05) compared to similar indicators of pork during the final treatment of half-carcasses with a suspension of lactic acid microorganisms of the SafePro® B-2 (*Lactobacillus sakei*) strain (Table 8).

**Table 8**. Microbiological indicators of pork in half-carcasses by 7 storage day, x±SD, n=5, lg CFU/cm$^2$

| Group | MAFAM | Lactic acid bacteria |
|---|---|---|
| Experimental 1 | 5.62±0.35$^b$ | 4.80±0.88$^b$ |
| Experimental 2 | 4.82±0.51$^a$ | 5.47±0.08$^a$ |

***Note:*** *different superscript letters a, b indicate the values that considerably differed in the same row of the table (P<0.05) based on the results of comparison using the Tukey test*

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

Therewith, the number of lactic acid microorganisms in pork treated with ferment culture of the SafePro® B-SF-43 (*Leuconostoc carnosum*) strain decreased by 0.67 lg CFU/cm$^2$ (P<0.05) compared to pork treated with a suspension of lactic acid microorganisms of the SafePro® B-2

(*Lactobacillus sakei*) strain. It is difficult to compare the research results obtained with similar data from other researchers since they were performed on meat processing products and using different packaging options. In this case, S. Raimondi *et al.* (2019) established that the number of *Leuconostoc carnosum* in packaged meat products, in particular, in boiled ham, increases from the first days of the shelf life to several weeks, reaching its peak by $10^8$ cells/g and dominates the microflora until the end of the product's shelf life. In an experiment on pork in half-carcasses treated with a spray suspension of the SafePro® B-SF-43 (*Leuconostoc carnosum*), this effect was not observed during the meat storage period.

This fact indicates the specificity of various strains of this microorganism and selective suitability for processing raw meat, in particular, half-carcasses of pork. Although the microorganisms of the SafePro® B-2 (*Lactobacillus sakei*) strain were not characterised by rapid reproduction in meat stored in a refrigerator, their numbers were fairly stable throughout the entire study period (Table 8). Furthermore, A. Najjari *et al.* (2020) indicate that microorganisms of this type, can substantially improve the organoleptic characteristics of meat products by creating an attractive aroma, in particular, during the fermentation of dry sausages. Therewith, the resistance of these microorganisms to bile acids, which, against the background of their entry into the small intestine and the ability to form bacteriocins, ensures the performance of a probiotic function and prevents colonisation by pathogenic microflora of the consumer's body. Therefore, it is necessary to focus further research on using ferment cultures of lactic acid microorganisms for preserving pork in half-carcasses after eliminating the risks of meat contamination at this meat processing enterprise and improving good hygiene practices.

Colonies of *S. aureus, Salmonella spp*. and *L. monocytogenes* were not identified on the surface of paired half-carcasses of pork of the control group and both experimental groups during the entire storage period, which indicates the safety of meat according to these pathogens. The data obtained do not contradict the results of the paper by T. Jacobsen *et al.* (2003), which indicate the advantages of using live lactic acid microorganisms in meat product technology over white blood cells or bacteriocins to suppress pathogenic microorganisms, especially *L. monocytogenes*. This is due to ensuring the stability of the number of colonies of lactic acid microorganisms in the food product, in contrast to the use of biotechnological synthesis products that can be destroyed during storage. Notably, the latest studies of a number of researchers, in particular, D.-M. Meng *et al.* (2020; 2021), are devoted to the use of impure cultures of living microorganisms and preservatives of microbial synthesis for storing pork in chilled, packaged form, in particular, Hispidalin and Mitihitin-A. These results indicate a reduction in meat weight loss during storage and suppression of pathogenic microflora, preventing an increase in pH and meat spoilage. However, the effectiveness of these preservatives for processing whole carcasses or half-carcasses of slaughtered animals during chilled storage is not fully understood.

Notably, each meat processing enterprise has its own microbial association, which must be considered when developing measures to improve hygiene practices. A comprehensive approach is needed to reduce the risks of contamination of meat with microorganisms, especially pork in half-carcasses that are stored in chilled form, which will allow combining measures to reduce air contamination of meat processing shops, equipment, and the pork itself during primary processing and, thus, increase the duration of its storage in fresh form.

## Conclusions

Cooling half-carcasses using washing and final treatment with suspensions of lactic acid microorganisms of SafePro® B-SF-43 (*Leuconostoc carnosum*) and SafePro® B-2 (*Lactobacillus sakei*) strains at a dose of $10^7$/cm$^2$ to extend the shelf life of pork in half-carcasses in chilled form up to 7 days and preserve its freshness is promising.

Cooling half-carcasses of pork in the refrigerator using washing without additional treatment with bioconservants contributes to meat spoilage already on the 4$^{th}$ day of storage. The use of washing to cool half-carcasses of pork in the refrigerator in combination with final treatment with suspensions of bacterial ferment cultures of SafePro® B-SF-43 (*Leuconostoc carnosum*) and SafePro® B-2 (*Lactobacillus sakei*) strains at a dose of $10^7$/cm$^2$ contributed to a decrease in the number of MAFAM in fresh pork meat by 1.25 and 0.65 lg CFU/cm$^2$, respectively, with a simultaneous increase in the number of lactic acid bacteria by 3.47 and 3.43 lg CFU/cm$^2$, respectively, compared to the control. Storage of pork that was cooled using washing caused an increase in the number of MAFAM on day 4, which led to signs of meat spoilage. Pork in half-carcasses, which was subjected to final treatment with suspensions of bacterial ferment cultures of SafePro® B-SF-43 *(Leuconostoc carnosum*) and SafePro® B-2 (*Lactobacillus sakei*) strains, was characterised by a lower number of MAFAM by 0.82 and 0.53 lg CFU/cm$^2$ with the increasing number of lactic acid bacteria by 1.65 and 1.17 lg CFU/cm$^2$, respectively, compared to the control. Surface treatment of pork half carcasses with suspensions of bacterial ferment cultures of SafePro® B-SF-43 (*Leuconostoc carnosum*) and SafePro® B-2 (*Lactobacillus sakei*) strains at a dose of $10^7$/cm$^2$ provided a stable number and dominance of lactic acid microorganisms in meat during 7 days of storage in a chilled state. Cooling of half-carcasses of pork in the refrigerator using washing and final treatment of the surface of half-carcasses with suspensions of lactic acid microorganisms of SafePro® B-SF-43 (*Leuconostoc carnosum*) and SafePro® B-2 (*Lactobacillus sakei*) strains at a dose of $10^7$/cm$^2$ created conditions for ensuring the pH value of meat on the 7$^{th}$ day of storage at a level characteristic of high-quality meat and did not affect its moisture-retaining ability. The use of processing half-carcasses of pork with suspensions of lactic acid microorganisms will allow increasing the shelf life of chilled pork while maintaining its quality and ensuring safety. A promising area of research is to determine the effectiveness of cold water washing of pork in half-carcasses in combination with treatment with suspensions of lactic acid microorganisms and physical methods of meat bioconservation aimed at improving organoleptic parameters and preserving its freshness and biological value.

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None.

## Conflict of Interest

None.

## References

[1] Ali, S., & Alsayeqh, A.F. (2022). Review of major meat-borne zoonotic bacterial pathogens. *Frontiers in Public Health*, 10, article number 1045599. doi: 10.3389/fpubh.2022.1045599.

[2] Álvarez, C., Morán, L., Keenan, D.F., Mullen, A.M., & Delgado-Pando, G. (2019). Mechanical and biochemical methods for rigor measurement: Relationship with eating quality. *Journal of Food Quality,* 2019, article number 1894543. doi: 10.1155/2019/1894543.

[3] Barcenilla, C., Ducic, M., López, M., Prieto, M., & Álvarez-Ordóñez, A. (2022). Application of lactic acid bacteria for the biopreservation of meat products: A systematic review. *Meat Science*, 183, article number 108661. doi: 10.1016/j.meatsci.2021.108661.

[4] Benítez-Chao, D.F., León-Buitimea, A., Lerma-Escalera, J.A., & Morones-Ramírez, J.R. (2021). *Bacteriocins*: An overview of antimicrobial, toxicity, and biosafety assessment by *in vivo* models. *Frontiers in Microbiology*, 12, article number 630695. doi: 10.3389/fmicb.2021.630695.

[5] Bogatko, N. (2019). The effects of falsification of meat of slaughtered animals with sodium hydrocarbonate on their quality and safety. *Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies. Series: Veterinary Sciences*, 21(95), 66-74. doi: 10.32718/nvlvet9512.

[6] Braley, C., Gaucher, M.L., Fravalo, P., Shedleur-Bourguignon, F., Longpré, J., & Thibodeau, A. (2023). Slight temperature deviation during a 56-day storage period does not affect the microbiota of fresh vacuum-packed pork loins. *Foods (Basel, Switzerland)*, 12(8), article number 1695. doi: 10.3390/foods12081695.

[7] Candeliere, F., Raimondi, S., Spampinato, G., Tay, M.Y.F., Amaretti, A., Schlundt, J., & Rossi, M. (2021). Comparative genomics of *Leuconostoc carnosum*. *Frontiers in Microbiology*, 11, article number 605127. doi: 10.3389/fmicb.2020.605127.

[8] Costa, J., Bover-Cid, S., Bolívar, A., Zurera, G., & Pérez-Rodríguez, F. (2019). Modelling the interaction of the sakacin-producing *Lactobacillus sakei* CTC494 and *Listeria monocytogenes* in filleted gilthead sea bream under modified atmosphere packaging at isothermal and non-isothermal conditions. *International Journal of Food Microbiology*, 297, 72-84. doi: 10.1016/j.ijfoodmicro.2019.03.002.

[9] DSTU 7992:2015. (2017). *Meat and meat raw materials. Methods of sampling and organoleptic assessment of freshness*. Retrieved from http://surl.li/qoiio.

[10] DSTU 8381:2015. (2017). *Meat and meat products. Organization and methods of microbiological research*. Retrieved from http://online.budstandart.com/ua/catalog/doc-page?id_doc=84538.

[11] DSTU ISO 17604:2014. (2015). *Microbiology of food and animal feeding stuffs. Carcass sampling for microbiological analysis*. Retrieved from http://surl.li/qmyso.

[12] Ellatif, Z.A., Saad, S., Hassanin, F., Salem, A., & Saleh, E. (2020). Efficiency of some organic acids as decontaminants in sheep carcasses. *Benha Veterinary Medical Journal*, 38(2), 116-119. doi: 10.21608/bvmj.2020.27504.1197.

[13] Fengou, L.C., Spyrelli, E., Lianou, A., Tsakanikas, P., Panagou, E.Z., & Nychas, G.J.E. (2019). Estimation of minced pork microbiological spoilage through fourier transform infrared and visible spectroscopy and multispectral vision technology. *Foods (Basel, Switzerland)*, 8(7), article number 238. doi: 10.3390/foods8070238.

[14] GOST 23392:2016. (2017). *Meat. Methods for chemical and microscopic analysis of freshness*. Retrieved from https://files.stroyinf.ru/Data2/1/4293747/4293747512.pdf.

[15] Iakubchak, O.M., Khomenko, V.I., Melnychuk, S.D., Kovbasenko, V.M., Kravtsiv, R.Y., Mykytyuk, P.V., Kozak, M.V., & Oliynyk, L.V. (2005). *Veterinary-sanitary examination with the basics of technology and standardization of animal origin*. Kyiv: Bioprom.

[ 16] Jacobsen, T., Budde, B.B., & Koch, A.G. (2003). Application of *Leuconostoc carnosum* for biopreservation of cooked meat products. *Journal of Applied Microbiology*, 95(2), 242-249. doi: 10.1046/j.1365-2672.2003.01970.x.

[17] Jaja, I.F., Green, E., & Muchenje, V. (2018). Aerobic mesophilic, coliform, *Escherichia coli*, and *Staphylococcus aureus* counts of raw meat from the formal and informal meat sectors in South Africa. *International Journal of Environmental Research and Public Health*, 15(4), article number 819. doi: 10.3390/ijerph15040819.

[18] Jankowiak, H., Cebulska, A., & Bocian, M. (2021). The relationship between acidification (pH) and meat quality traits of polish white breed pigs. *European Food Research and Technology*, 247, 2813-2820. doi: 10.1007/s00217-021-03837-4.

[19] Jeong, C.H., Lee, S.H., & Kim, H.Y. (2022). Microbiological composition and sensory characterization analysis of fermented sausage using strains isolated from Korean fermented foods. *Food Science of Animal Resources*, 42(6), 928-941. doi: 10.5851/kosfa.2022.e56.

[20] Kim, S.G., & Kim, H.Y. (2023). Effect of the types of starter on microbiological and physicochemical properties of dry-cured ham. *Food Science of Animal Resources*, 43(3), 454-470. doi: 10.5851/kosfa.2023.e90.

[21] Klaharn, K., Pichpol, D., Meeyam, T., Harintharanon, T., Lohaanukul, P., & Punyapornwithaya, V. (2022). Bacterial contamination of chicken meat in slaughterhouses and the associated risk factors: A nationwide study in Thailand. *PLoS ONE*, 17(6), article number e0269416. doi: 10.1371/journal.pone.0269416.

[22] Klaharn, K., Pichpol, D., Meeyam, T., Pfeiffer, D., Moomon, A., Lohaanukul, P., & Punyapornwithaya, V. (2021). Analysis of nationwide survey data to determine bacterial contamination levels in meat from pig slaughterhouses in Thailand. *Food Control*, 126, article number 108005. doi: 10.1016/j.foodcont.2021.108005.

[23] Koutsoumanis, K., Allende, A., Alvarez-Ordóñez, A., Bover-Cid, S., Chemaly, M., De Cesare, A., Herman, L., Hilbert, F., Lindqvist, R., Nauta, M., Peixe, L., Ru, G., Simmons, M., Skandamis, P., Suffredini, E., Blagojevic, B., Van Damme, I., Hempen, M., Messens, W., & Bolton, D. (2023). Microbiological safety of aged meat. *EFSA Journal European Food Safety Authority*, 21(1), article number e07745. doi: 10.2903/j.efsa.2023.7745.

[24] Kukhtyn, M., Salata, V., Berhilevych, O., Malimon, Z., Tsvihun, A., Gutyj, B., & Horiuk, Y. (2020). Evaluation of storage methods of beef by microbiological and chemical indicators. *Potravinarstvo Slovak Journal of Food Sciences*, 14, 602-611. doi: 10.5219/1381.

[25] Lebret, B., & Čandek-Potokar, M. (2022). Review: Pork quality attributes from farm to fork. Part I. Carcass and fresh meat. *Animal*, 16(1), article number 100402. doi: 10.1016/j.animal.2021.100402.

[26] Meng, D.M., Sun, X.Q., Sun, S.N., Li, W.J., Lv, Y.J., & Fan, Z.C. (2020). The potential of antimicrobial peptide Hispidalin application in pork preservation during cold storage. *Journal of Food Processing and Preservation*, 44(6), article number e14443. doi: 10.1111/jfpp.14443.

[27] Meng, D.M., Yang, X.M., Sun, X.Q., Cheng, L., & Fan, Z.C. (2021). Application of antimicrobial peptide Mytichitin-A in pork preservation during refrigerated storage. *Journal of Food Processing and Preservation,* 45(5), article number e15404. doi: 10.1111/jfpp.15404.

[28] Najjari, A., Boumaiza, M., Jaballah, S., Boudabous, A., & Ouzari, H.I. (2020). Application of isolated *Lactobacillus sakei* and *Staphylococcus xylosus* strains as a probiotic starter culture during the industrial manufacture of Tunisian dry-fermented sausages. *Food Science & Nutrition*, 8(8), 4172-4184. doi: 10.1002/fsn3.1711.

[29] Nakamura, A., Takahashi, H., Koike, F., Kuda, T., & Kobayashi, M. (2023). Transition of microbial contamination on the surface of carcass during the cattle slaughter process. *Food Microbiology*, 112, article number 104245. doi: 10.1016/j.fm.2023.104245.

[30] Odeyemi, O.A., Alegbeleye, O.O., Strateva, M., & Stratev, D. (2020). Understanding spoilage microbial community and spoilage mechanisms in foods of animal origin. *Comprehensive Reviews in Food Science and Food Safety*, 19(2), 311-331. doi: 10.1111/1541-4337.12526.

[31] Order No. 28 "Rules of Pre-Slaughter Veterinary Inspection of Animals and Veterinary-Sanitary Examination of Meat and Meat Products". (2002, June). Retrieved from http://surl.li/qoipf.

[32] Raimondi, S., Luciani, R., Sirangelo, T.M., Amaretti, A., Leonardi, A., Ulrici, A., Foca, G., D'Auria, G., Moya, A., Zuliani, V., Seibert, T.M., Søltoft-Jensen, J., & Rossi, M. (2019). Microbiota of sliced cooked ham packaged in modified atmosphere throughout the shelf life: Microbiota of sliced cooked ham in MAP. *International Journal of Food Microbiology*, 289, 200-208. doi: 10.1016/j.ijfoodmicro.2018.09.017.

[33] Rani, Z.T., Mhlongo, L.C., & Hugo, A. (2023). Microbial profiles of meat at different stages of the distribution chain from the abattoir to retail outlets. *International Journal of Environmental Research and Public Health*, 20(3), article number 1986. doi: 10.3390/ijerph20031986.

[34] Soltani, S., Biron, E., Ben Said, L., Subirade, M., & Fliss, I. (2022). Bacteriocin-based synergetic consortia: A promising strategy to enhance antimicrobial activity and broaden the spectrum of inhibition. *Microbiology Spectrum*, 10(1), article number e0040621. doi: 10.1128/spectrum.00406-21.

[35] Soyer, F., Keman, D., Eroğlu, E., & Türe, H. (2020). Synergistic antimicrobial effects of activated lactoferrin and rosemary extract in vitro and potential application in meat storage. *Journal of Food Science and Technology*, 57, 4395-4403. doi: 10.1007/s13197-020-04476-5.

[36] Vovkotrub, V., Iakubchak, O., Horalskyi, L., Vovkotrub, N., Shevchenko, L., Shynkaruk, N., Rozbytska, T., Slyva, Y., Tupitska, O., & Shtonda, O. (2023). The microscopic structure of pork neck after cooling with showering stiving and processing by culture Lactobacillus sakei. *Potravinarstvo Slovak Journal of Food Sciences*, 17, 759-776. doi: 10.5219/1905.

[37] Wicks, J.C., Bodmer, J.S., Yen, C.N., Zumbaugh, M.D., Matarneh, S.K., Scheffler, T.L., Silva, S.L., Shi, H., & Gerrard, D.E. (2022). Postmortem muscle metabolism and meat quality. In *New aspects of meat quality* (pp. 67-93). Cambridge: Woodhead Publishing.

[38] Xu, M.M., Kaur, M., Pillidge, C.J., & Torley, P.J. (2022). Microbial biopreservatives for controlling the spoilage of beef and lamb meat: Their application and effects on meat quality. *Critical Reviews in Food Science and Nutrition*, 2(17), 4571-4592. doi: 10.1080/10408398.2021.1877108.

[39] Zagorec, M., & Champomier-Vergès, M.C. (2017). *Lactobacillus sakei*: A starter for sausage fermentation, a protective culture for meat products. *Microorganisms*, 5(3), article number 56. doi: 10.3390/microorganisms5030056.

[40] Zhu, Y., Wang, W., Li, M., Zhang, J., Ji, L., Zhao, Z., Zhang, R., Cai, D., & Chen, L. (2022). Microbial diversity of meat products under spoilage and its controlling approaches. *Frontiers in Nutrition*, 9, article number 1078201. doi: 10.3389/fnut.2022.1078201.

[41] Zmudzińska, A., Bigorowski, B., Banaszak, M., Roślewska, A., Adamski, M., & Hejdysz, M. (2020). The effect of diet based on legume seeds and rapeseed meal on pig performance and meat quality. *Animals*, 10(6), article number 1084. doi: 10.3390/ani10061084.

# Вплив заквасок молочнокислих бактерій на свіжість свинини

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**Анотація.** Під час первинної переробки свинини на етапі охолодження півтуш відбувається втрата їх маси, що призводить до економічних збитків. Одним із перспективних шляхів вирішення цієї проблеми є використання душування півтуш охолодженою водою, що потребує застосування засобів деконтамінації м'яса від мікрофлори, яка спричиняє його псування. Мета дослідження – визначити ефективність заключної обробки півтуш свинини заквасками культур штамів SafePro® B-SF-43 (*Leuconostoc carnosum*) і SafePro® B-2 (*Lactobacillus sakei*) для їх біоконсервації та збереження свіжості. В роботі використано органолептичні, хімічні та мікробіологічні методи дослідження. Встановлено, що охолодження півтуш свинини в холодильній камері з використанням душування холодною водою сприяє виникненню ознак псування м'яса за рівнем мікробної контамінації та величиною pH вже на 4 добу зберігання. Обробка поверхні півтуш суспензіями молочнокислих мікроорганізмів штамів SafePro® B-SF-43 (*Leuconostoc carnosum*) і SafePro® B-2 (*Lactobacillus sakei*) у дозі $10^7$/см$^2$ забезпечує належні органолептичні показники і величину pH свинини до 7 доби зберігання,

які відповідають якісному м'ясу. Обидві закваски молочнокислих мікроорганізмів знижують мікробну контамінацію м'яса за рахунок чисельності мезофільних аеробних і факультативно-анаеробних мікроорганізмів у парних тушах на 1,25 та 0,65 lg КУО/см$^2$ та збільшують чисельність молочнокислих мікроорганізмів на 3.47 і 3.43 lg КУО/см$^2$ відповідно, що дозволяє продовжити термін зберігання м'яса в охолодженому вигляді не менше 7 діб. Для заключної обробки півтуш свинини за охолодження в холодильній камері у поєднанні з душуванням охолодженою водою найпридатнішою є культура штаму SafePro® B-2 (*Lactobacillus sakei*). Отримані результати досліджень є перспективними для удосконалення технології первинної переробки свинини на етапі охолодження з використанням заквасок молочнокислих мікроорганізмів в якості натуральних консервантів, що дозволить збільшити термін зберігання м'яса з урахуванням його якості та безпечності

**Ключові слова:** м'ясо; охолодження; душування; зберігання; мікробна контамінація

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# The nature of abdominal surgery for polycystic kidney disease in animals and the role of sonographic indicators at different stages of surgical intervention: A literature review

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**Abstract.** Analysis of the method of using sonography during surgery in animals with polycystic kidney disease is an urgent task since firstly, sonography is a safe and non-invasive method of examination, which allows determining the structural features of the kidneys before, during, and after surgery. Secondly, from the standpoint of improving the results of surgery, sonography helps to clarify the localisation of cysts and determine their size. Thirdly, an important factor in the use of sonography is the reduction of pain and the risk of postoperative complications. In addition, due to this method of kidney examination, it is possible to more accurately determine the optimal route of access to cysts, which helps to reduce tissue injury and ensures rapid recovery of the animal after surgery. The purpose of the study is to analyse in detail and describe the method of using sonography during surgery in animals with polycystic kidney disease. The study focuses on the need to determine how sonography affects reducing the duration of surgery, improving the quality of cyst removal, and reducing the risk of complications during abdominal surgery. The approach in this study is based on the analysis of scientific papers on this subject, in particular on the experience of veterinarians who have already used sonography during abdominal operations

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in animals with polycystic kidney disease. Thus, special techniques of sonography and surgical treatment of kidney cysts include dopplerography, colour dopplerography, 3D and 4D sonography, elastography, introperative sonography, intraperitoneal sonography, and duplex scanning of renal arteries and veins. Surgical methods of treatment include extraction of individual cysts, drainage of cysts, resection, and nephrectomy. The use of sonography at different stages of surgical intervention helps to optimise the operation process, reduce the risk of complications, and contribute to the introduction of new approaches in the treatment of animals with polycystic kidney disease, which will substantially improve their quality of life

**Keywords:** veterinary medicine; organ degeneration; epithelial cells; diagnostics; abdominal cavity; minimally invasive surgery

## Introduction

According to F. Perondi *et al.* (2020), the problem of polycystic kidney disease is important in the field of veterinary medicine, as its prevalence in animals is increasing. This is a challenge for veterinary specialists and requires the development of effective treatment and diagnosis methods. The importance of the problem lies in the fact that polycystic kidney disease can lead to the development of kidney failure and other serious complications.

Scientific studies on polycystic kidney disease in animals are conducted in different countries of the world. Sonography is one of the most effective methods for diagnosing polycystic kidney disease, which allows accurately determining the size, shape, and number of cysts on the surface of the kidneys. Most research on polycystic kidney disease in animals has focused on the use of sonography to diagnose and monitor kidney health during treatment. Some of them focus on analysing the results of sonography and its impact on the results of surgery, while other studies determine the effectiveness of minimally invasive surgical methods for treating animals with polycystic kidney disease. The results of the analysis of current studies of Ukrainian researchers in the context of polycystic kidney disease in animals reflect the focus of research on determining effective methods for the diagnosis and treatment of this disease. Thus, N.J. Patel *et al.* (2020) indicate the importance of sonography for determining the prevalence and monitoring the dynamics of polycystic fibrosis. The results of their study show that sonography is an important tool in the diagnosis and monitoring of kidney disease in animals with this pathology. The study of L. Schirrer *et al.* (2021) highlights the main aspects of diagnosis and approaches to treating polycystic fibrosis. Researchers emphasise the importance of timely diagnosis and choosing the optimal treatment plan, considering the age and individual characteristics of the patient. K. Debruyn *et al.* (2012) describe current methods for the diagnosis and treatment of polycystic kidney disease, mentioning sonography and focusing on the importance of early detection and thorough examination of patients to choose the optimal treatment strategy.

Studies on the effectiveness of sonography during abdominal surgery for polycystic kidney disease in animals have shown that this method is reliable and helps reduce the risk of complications during its implementation. One such recent study was conducted by B. Dekerle *et al.* (2022), analysing data from a 10-month-old dog the sonography of which accurately determined the location of a cyst in the

kidney. Subsequently, the cyst was drained by ultrasound monitoring, performed before and during surgery. The study showed that sonography helped to more accurately determine the size and location of the cyst on the surface of the kidney, which contributed to its effective removal during surgery. In addition, sonography during surgery was identified to determine the presence of additional cysts and complications, such as bleeding. The same conclusion was reached in the studies by F. Rossi *et al.* (2023), which describe the use of computed tomography and sonography to detect cysts on the kidney surface in dogs and cats with polycystic kidney disease. The researchers noted that sonography is a highly sensitive method for diagnosing the presence of cysts in the kidneys, which helps determine the degree of prevalence of the disease and decide whether to perform surgery.

A study published by C. Thanaboonnipat *et al.* (2020) examines the use of elastography (a type of sonography) in the examination of kidneys in animals. Research has shown that elastography can be useful in assessing the texture of kidney tissue and help identify kidney cancer. Other studies focus on the use of 3D and 4D sonography to determine the shape and size of cysts on the kidney surface in animals, including in real-time. For example, Y. Huang *et al.* (2021) showed the use of 3D sonography to assess the size and shape of cysts on the kidney surface in dogs with acute abdominal haemorrhage. It was established that 3D sonography can be useful for assessing the relationship of cysts with nearby organs and structures. Authors investigated the use of sonography in kidney screening and reducing invasiveness. As a result, it was argued that "sonography can be a promising imaging diagnostic tool for assessing kidney condition". Studies on the effectiveness of sonography during abdominal surgery for polycystic kidney disease in animals show that sonography is an integral part of surgical practice for such patients.

This review paper is based on an analysis of various sources and methods, including PubMed, Google Scholar, and other databases, for obtaining scientific publications, monographs, dissertations, and scientific conference reports on polycystic kidney disease in animals. The information obtained was processed, summarised, and evaluated in the context of surgical treatment. By comparative analysis of various methods of diagnosis and treatment of polycystic kidney disease, the most effective approach was identified. The results of previous studies and publications, including data from well-known specialists related to the surgical treatment of animals with this disease, were reviewed. The data obtained helped determine the best approaches for the diagnosis and treatment of animals with polycystic kidney disease and also confirmed the validity of using sonography in the surgical treatment of this pathology.

The purpose of the study is to analyse scientific sources regarding the nature of abdominal surgery for polycystic kidney disease in animals and the role of sonographic indicators at different stages of surgical intervention. This will lead to the enrichment of scientific knowledge, solving the problem of polycystic kidney disease in animals, and improving the practice of surgical intervention in this case. Tasks include: analysis of scientific papers covering the problem of polycystic kidney disease in animals and the nature of abdominal surgery in this case; determination of the role of sonography as a diagnostic method for polycystic kidney disease in animals; establishing a relationship between sonography indicators and surgical intervention results; evaluating the effectiveness of using sonography at different stages of abdominal surgery for polycystic kidney disease in animals.

**Methods of treatment of polycystic kidney disease in animals and basic tools**

Abdominal surgery for polycystic kidney disease in animals can be performed using various methods depending on the size and number of cysts on the surface of the kidneys and requires great care and precision on the part of the veterinary surgeon since cysts can be very thin-walled and easily torn during surgery. According to the data of I.S. Dekhnych *et al.* (2021), this may lead to the development of complications.

The studies of N.J. Patel *et al.* (2020) and B. Dekerle *et al.* (2022) indicate that abdominal surgery for polycystic kidney disease in animals can include several stages: preparation of the animal for the operation: keeping it on a starvation diet for 12-24 hours before the operation, improving the level of hygiene and additional studies, such as clinical blood tests and sonography; anaesthesia of the animal: before the operation, the animal is subjected to anaesthesia, which ensures a painless condition during its implementation and helps to reduce the risk of complications. Instruments and tools are also prepared for the operation, as various methods can be applied to the operation, such as sterilisation of the operating area and preparation of proper equipment for its implementation. After preparing the operating area, the animal is given a lying position on its side with a positional sonography lens to clarify the location of cysts and kidneys; then, an incision is made, and muscle and fascial tissues are separated to provide access to the kidneys. Once access to the kidneys is achieved, cysts are removed, which can include various methods, such as removing cysts with special tools or cutting them into smaller pieces that can be removed with minimal damage to the surrounding tissue. After that, the final stage of the operation takes place.

J. Park *et al.* (2019) argue that after opening the abdominal cavity and examining the kidneys, the surgeon can continue to compare the presence of cysts with a preliminary sonographic assessment. If a cyst is found that obscures the image, the surgeon can confirm its location and size using dopplerography and elastography. In addition, additional sonographic studies, such as colour dopplerography and 3D/4D sonography, can be performed to assess the relationship of cysts with surrounding organs and structures.

X. Zhang *et al.* (2020) in their paper note that after evaluating the results of the sonographic examination, the surgeon can apply various treatment methods:

- (extraction) of individual cysts: the surgeon removes the cyst from the kidney using a special tool;
- cyst drainage: the surgeon inserts a thin hose through the kidney cyst and applies drainage to remove fluid from the cysts;
- kidney resection (partial removal): the surgeon can perform resection of the part of the kidney where the cysts are located;
- nephrectomy (complete removal) of the kidney: if the cysts are located in the area of the renal branches, the surgeon performs a nephrectomy – complete removal of the kidney.

After removing cysts that could cause complications, according to E. Stock *et al.* (2018), a final examination of the kidneys and surrounding tissues is performed to detect other abnormalities or complications. After removing the cysts and performing drainage, the surgeon should check all operated vessels for bleeding and ensure their hemostasis. Then, the condition of the kidneys is evaluated and it is identified whether they are working well. Special tests are usually performed to do this, for example, diuresis and blood creatinine levels are measured. If the operation is successful and no complications occur, the sick animal can be sent to the hospital for postoperative observation or allowed to be taken home, provided

that certain instructions and recommendations for care are followed. Notably, polycystic kidney disease is a chronic disease, so postoperative care and regular medical examinations by a veterinarian are necessary to monitor the functional state of the kidneys and prevent relapses of the disease.

During surgery for polycystic kidney disease in animals, a variety of tools are used to remove cysts. The main tools, according to Y. Bayazit *et al*. (2003), include:

♦ endograbler or "rake", which has very thin forceps with a hook at the end, enabling the collection and removal of small cysts from the kidneys;

♦ endoclipper or "scissors", equipped with two blades, which are usually located at the end of a flexible shaft, and designed for cutting cysts;

♦ an endodrill or "crushing drill" is used to remove large cysts, which is supplemented with a crushing head that can rotate at high speed, allowing the cysts to be crushed;

♦ an endoscope is a small device consisting of a thin tube with a camera at the end that is inserted into the body through a small incision in the skin and used to examine the surgical field and monitor the process of removing cysts;

♦ laparoscope – allows the surgeon to perform surgery through small incisions in the skin using a camera located at the end of a thin tube.

The surgeon selects instruments based on the size and location of the cysts, and the functional ability of the kidneys and the general health of the animal. It is essential to choose the right tools and techniques to reduce the risk of complications and maximise kidney function. According to K. Debruyn *et al*. (2012), the role of sonography during surgery for polycystic kidney disease in animals depends on the type of instrument used during surgery. When using traditional tools such as scissors, tweezers, and curettes, sonography can be helpful in determining the location of cysts and tracking the process of removing them. Additionally, sonography allows tracking the degree of violation of the structure of tissues during surgery.

When using endoscopic control instruments, such as laparoscopes and robotic systems, as noted by F. Rossi *et al.* (2023), sonography can be used to map the internal structure of kidney organs and cysts with high resolution. This can help reduce the risk of damage to surrounding tissues and organs during surgery. Abdominal surgery for polycystic kidney disease in animals is a serious medical intervention that should only be performed by qualified veterinary surgeons with experience in such operations. The animal should be prepared in detail for the operation, including a preliminary assessment of its health status, performing various laboratory and instrumental studies to ensure safety during the operation.

Postoperative animal care is also quite important. After the operation, the animal should be placed in a special room where it can recover under the supervision of veterinary specialists. The animal must receive sufficient food and water and the necessary amount of medicine. Signs of possible postoperative complications, such as bleeding, infections, or other health problems must be carefully monitored.

## Methods of sonographic research, disadvantages of using sonography in polycystic kidney disease in animals and evaluation of the effectiveness of using sonography

C. Thanaboonnipat *et al.* (2020) note in their paper that sonographic examination is a painless and non-invasive method that plays an essential role at various stages of abdominal surgery in the case of polycystic kidney disease in animals. Sonography allows estimating the size and number of cysts that may be present in the animal's kidneys. Before starting the operation,

sonography helps the veterinary surgeon plan the course of the operation, choosing the safest technique for removing cysts from the animal's kidneys. In addition, this method can be used to assess the extent of damage to the kidneys and other internal organs, which can help the veterinary surgeon determine the complexity of the operation (Fig. 1).

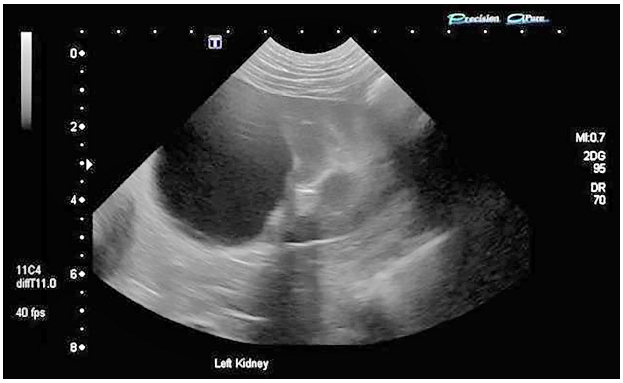


**Figure 1.** Ultrasound image along the long axis of the left kidney of an 8.8-year-old, 41.9 kg, neutered male, mixed breed with a diagnosis of intra-renal cystic lesion
***Sourse**: M. Vagias (2022)*

The method of the study consists in placing the patient on the operating table so that the cyst under study is available for scanning from different angles. The study should be conducted in B-mode, which allows getting a two-dimensional image of the object, as stated by L. Meomartino *et al.* (2021). According to N. Bilgen *et al.* (2020), to determine the size of the cyst, images are made in two projections: vertical and horizontal. Scanning from different angles, changing the position of the sensor is conducted to do this. The image measures the distance between opposite points on the periphery of the cyst. Y. Hwang *et al.* (2020) note that for a more accurate assessment of the cyst size, it is necessary to make several measurements in different projections and calculate the average value.

It is important to get images in different projections to assess the shape of the cyst. Visualisation of the cyst in a profile projection allows you to estimate its shape and length-to-width ratio. As studies by K.R. Waller *et al.* (2007) show, to determine the relationship of cysts with surrounding organs and structures, sonographic studies using various scanning modes are applied. Commonly used modes are B-mode, M-mode, and colour dopplerography. The patient should be positioned on its back to determine the relationship of the cyst with the kidneys and ureters. Firstly, the kidneys are examined and their size and shape are determined. Then, with the help of an angular sonda, examinations are performed at different levels of the kidneys and ureters. M.M. Larson *et al.* (2021) note that during this study, it is important to determine the presence of cysts in the kidneys and ureters and to determine whether cysts are able to compress or interfere with the normal flow of urine.

G. Kumar (2012) indicate that B-mode and colour dopplerography are used to determine the relationship of cysts with the abdominal cavity and individual internal organs. Firstly,

the abdominal cavity is examined to determine the presence of cysts and their location. I. Matos *et al.* (2018) report that individual internal organs and structures should then be examined using various sondas to elucidate existing relationships with cysts. According to W.M. Thaiss *et al.* (2019), in some cases, additional examinations may be required, such as computed tomography (Fig. 2) or magnetic resonance imaging, for more detailed information about the relationship of cysts with surrounding organs and structures.

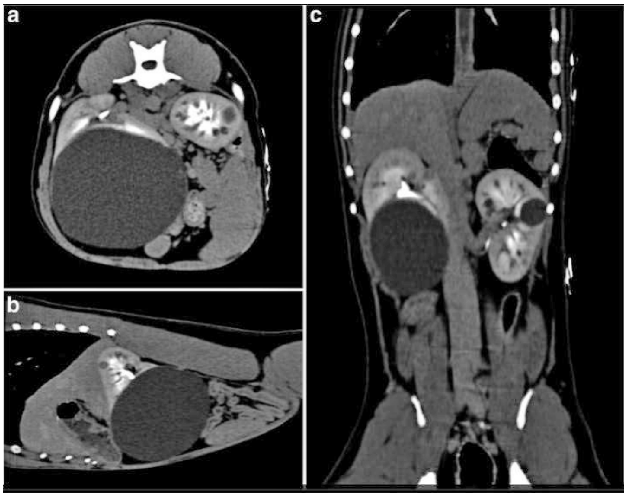


**Figure 2.** Transverse (a), sagittal (b), and dorsal (c) conventional computed tomography multiplanar reconstructions of images of the same dog diagnosed with a kidney cyst
***Notes**: numerous non-contrasting, well-defined ovoid structures of various sizes and fluid attenuation are visible in the cortical and medulla of both kidneys. The caudal pole of the right kidney contains a very large, egg-shaped, thin-walled, non-contrasting reinforcing fluid weakening structure that has replaced the normal architecture of its caudal third. In Figures (a) and (c), the left side of the dog is to the right of the images*
***Sourse**: M. Vagias (2022)*

According to C.A. McAloney *et al.* (2018), special techniques are used to determine the location of a cyst during sonographic examination. One of them is the "cross-scanning" or "perpendicular scanning" technique. It consists in the fact that the sonography device is placed parallel to the surface of the animal's body, and the probe is placed perpendicular to it in the direction that needs to be examined. During cross-scanning, the probe is placed on the animal's skin where the examination is required, and scans are performed from different angles. This allows getting more detailed information about the location of the cyst, its size, and shape.

In addition, as noted by K. Debruyn *et al.* (2012), the "real-time scanning" or "two-way scanning" technique can be used to determine the location of the cyst. In this case, the probe is placed on the animal's skin at the point that needs to be examined, and scans are performed in real-time. During the scan, not only the cyst tissue but also the surrounding space is visualised, which allows getting more detailed information about the location of the cyst, as confirmed by W.R. Widmer *et al*. (2004). A. Agut *et al.* (2020) note that in any case, to determine the topography of cyst placement, it is necessary to conduct a study in different planes, using dif-

ferent scanning techniques to get the most complete information about its size, shape, and location. During surgery, especially in organs with a rich network of blood vessels, the possibility of bleeding is a severe risk. Sonography can be useful for identifying possible sources of bleeding and estimating its volume.

In their study, L. Schirrer *et al.* (2021) note that sonography can assess the structure of tissues, which helps to identify possible bleeding sites, in particular, the separation of bleeding vessels from non-bleeding tissues, the detection of narrowing or dilation of blood vessels, and the identification of possible changes in the shape and size of organs. In addition, using colour and pulse dopplerography, the speed of blood flow in the vessels can be determined, which can help identify pathological changes in blood circulation. If a bleeding area is detected during surgery, the doctor may take the necessary measures to stop the bleeding, such as ligation or blood coagulation. Sonography can help during these procedures by providing visualisation of bleeding vessels and helping the doctor choose the most effective method to stop the bleeding.

After surgery, a sonographic examination can be used to monitor the condition of the animal's kidneys and evaluate the effectiveness of the treatment. It allows the veterinary surgeon to determine how completely cysts were removed from the animal's kidneys and assess the condition of the kidneys and surrounding tissues after surgery. D.J.X. Liu *et al.* (2019) claim that sonographic examination can be performed during surgery using a portable device with an ultrasound head. High-frequency ultrasound heads with a frequency of 7.5 to 12 MHz are usually used to ensure optimal visualisation of the kidneys during surgery.

According to the study by M.M. Larson *et al.* (2021), sonography techniques during surgery may vary depending on the type of surgery and the location of cysts in the animal's kidneys. However, the general approach to performing sonography includes the following steps. Preparation of the patient when the animal is subjected to general anaesthesia and placed on the operating table. Preparation of the device head and sonography (the ultrasound head is sterilised and applied to the animal's skin in the kidney area). Communication with the device is provided by means of a gel. The operating team receives an image of the kidneys on the device screen. Monitoring the operation with sonography: during the operation, the veterinary surgeon determines the exact location of the cysts and the degree of their interaction with the kidneys, noted M. Bertolotto *et al.* (2018). It also monitors the condition of the kidneys and other internal organs to detect possible complications during surgery. After surgery, a veterinary surgeon may perform additional sonography to assess the condition of the kidneys and other internal organs.

Based on the resulting images, as noted by I.C.K. Cruz *et al.* (2021), the doctor evaluates the condition of the kidneys, their shape, size, number, and size of cysts. If the kidneys appear substantially enlarged, with a large number of cysts, complications may occur during surgery, such as bleeding, impaired kidney function, and damage to surrounding organs. Therefore, P.G.S. Cardoso *et al*. (2019) indicate that it is important to accurately determine the size and number of cysts and the location of the kidneys before starting surgery. For this purpose, special sonography techniques can be used, such as dynamic sonography, which allows visualising changes in the size and shape of the kidneys during the animal's movements and various phases of breathing. 3D and 4D sonography can also be used, which allows getting a more detailed image of the structure of the kidneys and cysts.

When performing sonography during surgery, it is important, according to D.J.X. Liu *et*

*al.* (2019), to ensure the safety of the animal. Therefore, it is fixed in a soft fabric that prevents movement. In addition, special gels can be used to improve the connection between the sonograph sensor and the animal's skin, which improves image quality.

Although sonography is a useful tool for diagnosing and determining the location of cysts on the surface of the kidneys during abdominal surgery for polycystic kidney disease in animals, it has some disadvantages. F. Rossi *et al.* (2023) stated in their paper that sonography might be less effective if the kidneys are far from the surface of the skin, if there are thick layers of muscle and adipose tissue between them and the sensor that interfere with visualisation, or when the presence of air structures in the stomach or intestines reduces the effectiveness of sonography. This method can also provide only limited information about the internal structure of cysts, such as the presence of internal partitions or stones. As noted by S. Griffin (2020), sonography may be less effective in diagnosing smaller cysts because they tend to be less visible in the image. There may be cases when this method does not provide sufficient information to decide on the type of surgery and may require additional studies, such as computed tomography or magnetic resonance imaging. However, given these shortcomings, sonography is still a valuable tool for surgical intervention in polycystic kidney disease in animals. B. Vázquez & J. Daniel (2021) argues that in the case of non-standard situations, veterinarians combine sonography with other diagnostic methods, such as radiography, computed tomography, magnetic resonance imaging, etc., to obtain maximum information about the condition of the animal's kidneys and determine the optimal treatment tactics. The author's assessment of the use of sonography at different stages of abdominal surgery for polycystic kidney disease in animals indicates its effectiveness, informative value, and prospects. Thus, it was found that sonography allows getting a detailed three-dimensional image of the kidneys and other internal organs during surgery. This improves the ability to accurately locate and evaluate kidney cysts and provides real-time visualisation of blood circulation and blood flow (International renal..., n.d.).

In the early stages of surgery, when the question of the possibility of resection or extraction of cysts is decided, sonography provides the possibility of accurate diagnosis and assessment of the size of cysts, which contributes to more precise planning of surgical actions. In addition, during the operation itself, intraoperative sonography provides an opportunity to monitor the process of cyst removal, drainage, and other manipulations in real time. The data obtained confirm that the use of sonography allows the veterinary surgeon to perform accurate and safe operations, minimising the risk of possible complications. An important aspect is also to reduce the invasiveness of surgery due to the possibility of intraperitoneal sonography, which allows obtaining diagnostic information without interfering with the renal parenchyma (Frazier & Huppmann, 2020).

Thus, the results of the study confirm that sonography is an integral and effective component of abdominal surgery for polycystic kidney disease in animals, providing accurate diagnosis, greater controllability of surgical procedures, and improved treatment outcomes.

### Special sonography techniques: Dopplerography, colour dopplerography, 3D and 4D sonography, elastography, intraperitoneal sonography, duplex scanning of renal arteries and veins

In cases where the animal's kidneys have a complex structure or in the presence of complications during the development of polycystic

kidney disease, M.M. Larson *et al.* (2021) indicate that special sonography techniques may be involved:

♦ dopplerography – used to assess blood circulation in the kidneys, which allows identifying possible problems with blood flow, such as narrowing of the arteries, which can cause complications during surgery;

♦ colour dopplerography – used to determine the direction and speed of blood flow in the kidneys, which allows the veterinary surgeon to assess the condition of the kidneys in more detail and identify possible circulatory problems;

♦ 3D and 4D sonography – necessary for obtaining a three-dimensional image of the kidneys and other internal organs, allowing the veterinary surgeon to determine the size and shape of the kidneys in more detail and can be helpful when planning surgery;

♦ elastography – used to determine the density and stiffness of tissues, which can be useful in determining possible complications during surgery and helps the veterinary surgeon more accurately determine the condition of the kidneys;

♦ intraperitoneal sonography – allows getting images of organs in real time during surgery, which requires the use of a special probe that is connected to the sonograph and inserted into the animal's body through a small injection;

♦ intraperitoneal sonography – provides an image of the kidneys and parotid space without interfering with the parenchyma of the organ;

♦ duplex scanning of renal arteries and veins – allows assessing the degree of nodular damage and determine the possibility of preserving the renal parenchyma during surgery.

M. Vagias *et al.* (2022) noted in their paper that the following steps are used to perform intraoperative sonography during abdominal surgery for polycystic kidney disease in animals. An incision of the muscles and anterior abdominal wall is performed to access the abdominal cavity. A miniature probe is used, which is inserted into the abdominal cavity to do this. The next step is to perform a sonography of cysts to determine the location of the kidneys. The number of cysts that affect the size of the kidneys is assessed, and the number of polyps and their size located in the kidney cavity is estimated. Then, the size and condition of the kidneys are evaluated using a high-frequency probe, and colour Doppler sonography is used to examine the blood supply to the kidneys. In the next step, the renal arteries and veins are examined to determine their size and diameter. At the end, the cysts are punctured to examine their contents.

Based on the result of Y. Huang *et al.* (2022), the method of dopplerography for abdominal surgery for polycystic kidney disease in animals consists of the following steps. Preparation of the animal: the animal is subjected to general anaesthesia and fixed in the optimal position for the examination. Coat of the animal in the examination area should be trimmed and cleaned of various types of dirt. It is important to ensure the thermal comfort of the animal during the operation. Installation of the dopplerographic sensor on the surface of the animal's skin: before starting the examination, the sensor is lubricated with gel to ensure better contact with the surface of the animal's skin and better transmission of sound waves. Determination of test parameters: the doctor determines the test parameters, such as the signal penetration depth and sensor frequency. This allows obtaining the most accurate results. Image acquisition and analysis of results: a dopplerographic sensor is moved along the vessel to obtain an image of blood flow. After finding the appropriate blood vessels, dopplerography is performed to measure the blood flow rate. The image is transmitted to the monitor, where the doctor analyses the results. F. Freccero *et*

*al.* (2020) emphasise that the data obtained on the rate of blood flow in the arteries and veins help to assess the condition of the kidneys, diagnose the presence of bleeding, and determine the level of blood flow in the organs.

For example, the rate of blood flow in the renal artery can help assess the perfusion (patency) of the kidneys, that is, how fully the blood flow supplies the kidneys with oxygen and nutrients. If perfusion is impaired, problems with kidney function may occur. The blood flow rate in the renal vein can also indicate the degree of blood drainage (outflow) from the kidneys. A decrease in the rate of blood flow in the renal vein indicates impaired drainage and blood retention in the kidneys. The rate of blood flow in the renal artery is usually in the range of 80 to 180 cm/s. Therewith, a decrease in blood flow rate to 70 cm/s or less may be a consequence of impaired renal perfusion. S.M. McLeland *et al.* (2015) note that the rate of blood flow in the renal vein is usually in the range of 15 to 40 cm/s. A decrease in the blood flow rate to 10 cm/s or less may indicate a violation of blood drainage from the kidneys. Assessment of blood flow rate in arteries and veins using dopplerography is an important component of the diagnosis and treatment of animals with kidney pathology. These data help doctors more accurately assess the condition of the kidneys and plan further treatment tactics.

N.J. Patel *et al.* (2020) noted in their paper that colour dopplerography is a modern method of visualising and examining blood circulation in animal organs. The main method of colour dopplerography for abdominal surgery for polycystic kidney disease in animals is as follows. After opening the abdominal cavity, the researcher finds the animal's kidneys and discovers the blood vessels that supply these organs. Next, the device is configured for colour dopplerography, and optimal scanning parameters are determined. After that, the researcher begins scanning the arteries and veins that feed the kidneys using colour dopplerography. During the scan, the doctor receives an image of blood vessels with a colour-coded blood flow rate. This allows estimating the speed of blood flow in the arteries and veins and determining the direction of blood flow. The data on the speed of blood flow in the arteries and veins allowed assessing the condition of the kidneys and deciding on further actions during surgery. After completing the examination, the data obtained are analysed and the results are recorded. Colour dopplerography allows analysing not only the speed of blood flow in arteries and veins but also determining the size and structure of blood vessels, which is vital in the diagnosis and treatment of polycystic kidney disease in animals (Fig. 3).

J. Park *et al.* (2019) note that the optimal scanning parameters for colour dopplerography may vary depending on the specific situation and the scanning technique used by the doctor. However, in general, the following parameters are recommended: the frequency corresponds to the range between 2 and 5 Mhz, depending on the depth at which the examination is to be conducted; the flow rate is usually adjusted to the maximum value that can be measured during the study; angular correction: the angle adjustment between the direction of blood flow and the direction of the ultrasound wave, which affects the accuracy of measuring the flow rate; acoustic power should be adjusted to a safe level. The scan settings may change during the study, depending on what is displayed on the screen. Optimal scanning parameters should provide a high-quality and accurate image to assess the condition of the kidneys and blood circulation.

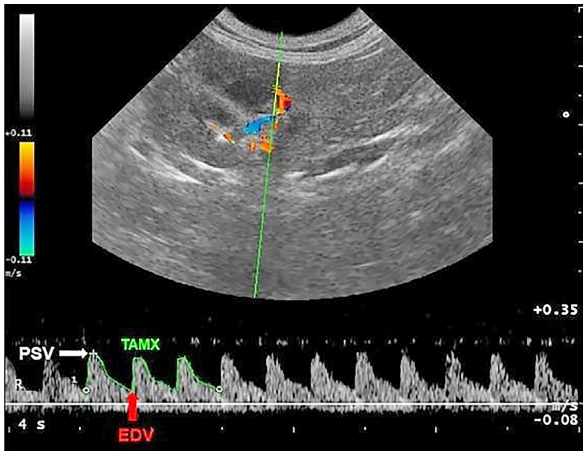


**Figure 3.** Colour dopplerography of the kidneys
***Notes**: measurement of resistivity index (RI) and pulsation index (PI) in the intrathecal artery. Doppler wave showing the difference for peak systolic velocity (PSV), end-diastolic velocity (EDV), and time averaged maximum velocity (TAMX)*
***Sourse**: study by G.C. Evangelista et al. (2023)*

During dopplerography, based on the results of the studies by K.R. Waller *et al.* (2007), it was established that the following parameters should be used to estimate the blood flow rate in arteries and veins. Peak Systolic Velocity (PSV) is the maximum blood flow rate in an artery during the systolic phase of the cardiac cycle. End Diastolic Velocity (EDV) – the rate of blood flow in an artery during the diastolic phase of the cardiac cycle. Resistive Index (RI) – the ratio of the difference between the maximum and minimum blood flow rate in the artery to the maximum blood flow rate. Pulsatility Index (PI) – the ratio of the difference between the maximum and minimum blood flow rate in an artery to the average blood flow rate. The direction of blood flow is determined using a coloured doppler, which reflects the direction of blood movement in the corresponding vessels (Parolini *et al.*, 2009). Optimal scanning parameters may vary depending on the specific technique and type of doppler equipment. As noted by B. Singh (2018), to accurately assess the blood flow rate and determine the direction of blood flow, it is crucial to follow the recommendations of the manufacturer of doppler equipment and conduct scans under optimal conditions.

3D and 4D sonography are methods that allow obtaining a three-dimensional image of an object. In the case of polycystic kidney disease, 3D and 4D sonography can help determine the size and shape of kidney cysts and the position and location of polyps and cysts. Y. Huang *et al.* (2022) indicate that the methods of 3D and 4D sonography for abdominal surgery for polycystic kidney disease in animals are the same as for conventional sonography. However, to get three-dimensional images, it is necessary to increase the number of cross-sections and change the position of the probe. In the case of 3D sonography, the resulting image can be viewed in different planes, which provides detailed information

about the internal structure of the kidneys. In the case of 4D sonography, the image can be viewed in real-time, which allows assessing the movement and dynamics of the object. Elastography is a method that allows assessing the elasticity of tissues. In abdominal surgery for polycystic kidney disease in animals, elastography can be used to assess the stiffness of renal tissue and detect signs of fibrosis.

M. Vagias *et al.* (2022) argue that the method of performing elastography in abdominal surgery for polycystic kidney disease in animals includes the following steps:

♦ *animal training:* the animal must be under anaesthesia, which will ensure maximum stability during elastography. At the moment, there is no special dietary preparation for elastography;

♦ *conducting elastography*: after standard sonography, elastography is performed, for which an elastography sensor is placed on the surface of the kidney to measure tissue stiffness. When using a device with integrated elastography, sonography usually proceeds immediately to measuring stiffness. The screen displays an image of fabrics, in which the stiffness is shown on a colourful scale. The darker the colour, the softer the fabrics, the lighter – the stiffer;

♦ *data analysis:* after performing elastography, the obtained data can be processed and analysed using special software.

For intraperitoneal sonography, based on the results of C.A. McAloney *et al.* (2018), a special catheter must first be inserted through the terminal intestine into the coccyx area, allowing access to the intraperitoneal cavity. Next, a special elastic hydrogel balloon is placed on top of the catheter, which ensures a tight seal at the entrance to the cavity and prevents fluid from escaping during the study. After that, a sonographic sensor is connected to the catheter, which allows getting an image of the kidneys and parotid space and assessing the size and number of cysts in the kidneys. These techniques reduce the risk of kidney damage during surgery and maximise its effectiveness.

**Disadvantages of using sonography for polycystic kidney disease in animals and evaluating the effectiveness of using sonography**

Although sonography is a useful tool for diagnosing and determining the location of cysts on the surface of the kidneys during abdominal surgery for polycystic kidney disease in animals, it has some disadvantages. This method can also provide only limited information about the internal structure of cysts, such as the presence of internal partitions or stones. As noted by S. Griffin (2020), sonography may be less effective in diagnosing smaller cysts because they tend to be less visible in the image. There may be cases when this method does not provide sufficient information to decide on the type of surgery and may require additional studies, such as computed tomography or magnetic resonance imaging. However, given these shortcomings, sonography is still a valuable tool for surgical intervention in polycystic kidney disease in animals. B. Vázquez & J. Daniel (2021) argues that in the case of non-standard situations, veterinarians combine sonography with other diagnostic methods, such as radiography, computed tomography, magnetic resonance imaging, etc., to obtain maximum information about the condition of the animal's kidneys and determine the optimal treatment tactics. The author's assessment of the use of sonography at different stages of abdominal surgery for polycystic kidney disease in animals indicates its effectiveness, informative value, and prospects. Thus, it was found that sonography allows getting a detailed three-dimensional image of the kidneys and other internal organs during surgery. This improves the ability to accurately locate and evaluate kidney cysts

and provides real-time visualisation of blood circulation and blood flow.

In the early stages of surgery, when the question of the possibility of resection or extraction of cysts is decided, sonography provides the possibility of accurate diagnosis and assessment of the size of cysts, which contributes to more precise planning of surgical actions. In addition, during the operation itself, intraoperative sonography provides an opportunity to monitor the process of cyst removal, drainage, and other manipulations in real time. The data obtained confirm that the use of sonography allows the veterinary surgeon to perform accurate and safe operations, minimising the risk of possible complications. An important aspect is also to reduce the invasiveness of surgery due to the possibility of intraperitoneal sonography, which allows obtaining diagnostic information without interfering with the renal parenchyma.

Thus, the results of the study confirm that sonography is an integral and effective component of abdominal surgery for polycystic kidney disease in animals, providing accurate diagnosis, greater controllability of surgical procedures, and improved treatment outcomes.

## Conclusions

The use of sonography during abdominal surgery for polycystic kidney disease in animals is a valuable tool that reduces the risk of complications and improves the accuracy of the operation. This method reduces the duration of surgery for polycystic kidney disease in animals, as it helps the surgeon to more accurately and quickly determine the location of cysts on the surface of the kidneys and not damage the surrounding tissues during their removal. In addition, using sonography reduces the risk of complications after surgery and increases its effectiveness. However, as indicated in studies, there are some disadvantages to the use of sonography in the case of abdominal surgery. This is primarily due to insufficient wave penetration depth and inefficiency in studying high-density structures, such as cysts. In addition, during the operation, it is not always possible to provide optimal conditions for sonography. Thus, the use of sonography in abdominal surgery for polycystic kidney disease in animals has both advantages and disadvantages. Given these shortcomings, sonography is still an effective tool for surgical intervention in polycystic kidney disease in animals. In general, the examination of the problem of polycystic kidney disease in animals is an urgent and vital task for veterinarians and researchers, which requires further scientific research and improvement of diagnostic and treatment methods.

Prospects for further research include determining the optimal parameters of sonography during abdominal surgery in the case of polycystic kidney disease, such as the size, number, and location of cysts, which is extremely important for such surgical interventions. Further investigation of the relationship between polycystic kidney disease and other animal diseases is necessary to identify possible common causes and develop effective methods of prevention and treatment. In addition, determining the possibility of developing new surgical methods that can reduce the risk of complications and improve the results of surgery, is important. Lastly, exploring the possibility of using new technologies, such as robotic systems, to improve the accuracy and efficiency of surgical intervention for polycystic kidney disease is also required.

## Acknowledgements

None.

## Conflict of Interest

None.

## References


[1] Agut, A., Soler, M., & Fernández-Del Palacio, M.J. (2020). Changes in renal resistive index values in healthy puppies during the first months of life. *Animals*, 10(8), article number 1338. doi: 10.3390/ani10081338.

[2] Bayazit, Y., Soyupak, B., Özdemir, S., Arıdoğan, I.A., Doran, S. (2003). Comparison of laparoscopy and open surgery in the treatment of symptomatic simple renal cysts. *Urology Research & Practice*, 29(3), 324-329.

[3] Bertolotto, M., Bucci, S., Valentino, M., Currò, F., Sachs, C., & Cova, M.A. (2018). Contrast-enhanced ultrasound for characterizing renal masses. *European Journal of Radiology*, 105, 41-48. doi: 10.1016/j.ejrad.2018.05.015.

[4] Bilgen, N., Bişkin Türkmen, M., Çınar Kul, B., Isparta, S., Şen, Y., Akkurt, M.Y., Çıldır, Ö.Ş., & Bars, Z. (2020). Prevalence of PKD1 gene mutation in cats in turkey and pathogenesis of feline polycystic kidney disease. *Journal of Veterinary Diagnostic Investigation*, 32, 549-555. doi: 10.1177/1040638720935433.

[5] Cardoso, P.G.S., Pinto, M.P.R., Moroz, L.R., Fontes, T.N., Santos, R.S., Freitas, J.L., Nogueira, V.A., & Peixoto, T.C. (2019). Dystrophic mineralization in uremic dogs: An update. *Pesquisa Veterinária Brasileira,* 39(11), 889-899. doi: 10.1590/1678-5150-PVB-6250.

[6] da Cruz, I.C.K., Gasser, B., Maronezi, M.C., Uscategui, R.A.R., Feliciano, M.A.R., Padilha-Nakaghi, L.C., Aires, L.P.N., & da Silva, P.D.A. (2021). Applicability of B-mode ultrasonography, ARFI elastography and contrast-enhanced ultrasound in the evaluation of chronic kidney disease in dogs. *Pesquisa Veterinária Brasileira*, 411, 67-85. doi: 10.1590/1678-5150-PVB-6785.

[7] Debruyn, K., Haers, H., Combes, A., Paepe, D., Peremans, K., Vanderperren, K., & Saunders, J.H. (2012). Ultrasonography of the feline kidney: Technique, anatomy and changes associated with disease. *Journal of Feline Medicine and Surgery*, 14(11), 794-803. doi: 10.1177/1098612x12464461.

[8] Dekerle, B., Fabres, V., Benchekroun, G., & Manassero M. (2022). Long-term follow-up of a juvenile simple renal cyst in a dog treated by laparoscopic deroofing, fulguration, and omentalization. *Journal of the American Veterinary Medical Association,* 260(11), 1-5. doi: 10.2460/javma.21.06.0272.

[9] Dekhnych, I.S., Zaviliy, M.F., & Rakitina, A.I. (2021). Diagnostic sonography during abdominal surgery in animals. *Bulletin of the PSAU*, 1, 241-249. doi: 10.31210/visnyk2021.01.30.

[10] Evangelista, G.C., Dornelas, L.R., Cintra, C.C., Valente, F.L., Fajardo, E.S., da Fonseca, L.A., & Reid, E.C.C. (2023). Evaluating feline lower urinary tract disease: Doppler ultrasound of the kidneys. *Journal of Feline Medicine and Surgery,* 25(1), article number 1098612X221145477. doi: 10.1177/1098612X221145477.

[11] Frazier, R.L., & Huppmann, A.R. (2020). Educational case: Autosomal dominant polycystic kidney disease. *Academic Pathology*, 7, article number 2374289520939257. doi: 10.1177%2F2374289520939257.

[12] Freccero, F., Petrucelli, M., Cipone, M., Nocera, I., & Sgorbini, M. (2020). Doppler evaluation of renal resistivity index in healthy conscious horses and donkeys. *PLoS ONE*, 15(2), article number e0228741. doi: 10.1371/journal.pone.0228741.

[13] Griffin, S. (2020). Feline abdominal ultrasonography: What's normal? What's abnormal? The kidneys and perinephric space. *Journal of Feline Medicine and Surgery,* 22(5), 409-427. doi: 10.1177/1098612X20917598.

[14] Huang, Y., Wu, X., Tao, Y. Wu, J., Yang Y., Ma, M., Yuan, X., Li, F., & Hou, X. (2021). *Diagnostic value of doppler ultrasound in early chronic kidney disease*. Retrieved from https://assets.researchsquare.com/files/rs-667000/v1/0dde79c4-eab6-4597-ad00-505cfd7e7b9c.pdf?c=1631885905.

[15] Hwang, Y., Noh, D., Choi, S., Choi, H., Lee, Y., & Lee, K. (2020). Changes of ultrasonographic pattern of the spleen examined with a high-frequency linear transducer during growth in puppies. *Veterinary Radiology & Ultrasound*, 61(5), 577-582. doi: 10.1111/vru.12873.

[16] International renal interest society. IRIS staging of CKD. (n.d.). Retrieved from http://www.iris-kidney.com/guidelines/staging.html.

[17] Kumar, G. (2012). Bilateral superior accessory renal arteries – its embryological basis and surgical importance – a case report. *Journal of Clinical Case Reports*, 2, article number e105. doi: 10.4172/2165-7920.1000e105.

[18] Larson, M.M., Mattoon, J.S., Lawrence, Y., & Sellon, R.K. (2021). Liver. In *Small animal diagnostic ultrasound* (pp. 355-421). Amsterdam: Elsevier. doi: 10.1016/B978-0-323-53337-9.00018-6.

[19] Liu, D.J.X., Hesta, M., Stock, E., Bogaerts, E., Broeckx, B.J.G., Saunders, J.H., & Vanderperren, K. (2019). Renal perfusion parameters measured by contrast-enhanced ultrasound in healthy dogs demonstrate a wide range of variability in the long-term. *Veterinary Radiology and Ultrasound,* 60(2), 201-209. doi: 10.1111/vru.12690.

[20] Matos, I., Azevedo, P., & Carreira, L.M. (2018). Pilot study to evaluate the potential use of the renal resistive index as a preliminary diagnostic tool for chronic kidney disease in cats. *Journal of Feline Medicine and Surgery,* 20(10), 940-947. doi: 10.1177/1098612X17736656.

[21] McAloney, C.A., Sharkey, L.C., Feeney, D.A., Seelig, D.M., Avery, A.C., & Jessen, C.R. (2018). Evaluation of the diagnostic utility of cytologic examination of renal fine-needle aspirates from dogs and the use of ultrasonographic features to inform cytologic diagnosis. *Journal of the American Veterinary Medical Association*, 252(10), 1247-1256. doi: 10.2460/javma.252.10.1247.

[22] McLeland, S.M., Cianciolo, R.E., Duncan, C.G. & Quimby, J.M. (2015). A comparasion of biochemical and histopathologic staging in cats with chronic kidney disease. *Veterinary Pathology*, 52(3), 524-534. doi: 10.1177/0300985814561095.

[23] Meomartino, L., Greco, A., Di Giancamillo, M., Brunetti, A., & Gnudi, G. (2021). Imaging techniques in veterinary medicine. part I: Radiography and ultrasonography. *European Journal of Radiology Open,* 18(8), article number 100382. doi: 10.1016/j.ejro.2021.100382.

[24] Park, J., Moon, C., Lee, M., Lee, H.B., & Jeong S.M. (2019). Surgical fenestration combined with omentalization for the treatment of renal cysts in 2 dogs. *Canadian Veterinary Journal*, 60(10), 1104-1110.

[25] Parolini, C., Noce, A., Staffolani, E., Giarrizzo, G.F., Costanzi, S., & Splendiani, G. (2009). Renal resistive index and long-term outcome in chronic nephropathies. *Radiology*, 252, 888-896. doi: 10.1148/radiol.2523080351.

[26] Patel, N.J., Brady, R., & Scarf, V.F. (2020). Single-incision laparoscopic deroofing and omentalization of a cystic renal adenoma in a dog. *Journal of the American Animal Hospital Association*, 56(6), article number 331. doi: 10.5326/jaaha-ms-6991.

[27] Perondi, F., Lippi, I., Marchetti, V., Bruno, B., Borrelli, A., & Citi, S. (2020). How ultrasound can be useful for staging chronic kidney disease in dogs: Ultrasound findings in 855 cases. *Veterinary Science*, 7(4), article number 147. doi: 10.3390/vetsci7040147.

[28] Rossi, F., Gianni, B., Marconato, L., Sabattini, S., Caleri, E., Mattolini, M., Camosci, V., & Carozzi, G. (2023). Comparison of sonographic and CT findings for the identification of renal nodules in dogs and cats. *Veterinary Radiology and Ultrasound*, 64(3), 439-447. doi: 10.1111/vru.13219.

[29] Schirrer, L., Marin-Garcia, P.J., & Llobat, L. (2021). Feline polycystic kidney disease: An update. *Veterinary Sciences*, 8(11), article number 269. doi: 10.3390%2Fvetsci8110269.

[30] Singh, B. (2018). Dyce, sack, and wensing's textbook of veterinary anatomy. Amsterdam: Elsevier.

[31] Stock, E., Paepe, D., Daminet, S., Vandermeulen, E., Duchateau, L., Saunders, J.H., & Vanderperren, K. (2018). Contrast-enhanced ultrasound examination for the assessment of renal perfusion in cats with chronic kidney disease. *Journal of Veterinary Internal Medicine,* 32(1), 260-266. doi: 10.1111/jvim.14869.

[32] Thaiss, W.M., Bedke, J., Kruck, S., Spira, D., Stenzl, A., Nikolaou, K., Horger, M., & Kaufmann, S. (2019). Can contrast-enhanced ultrasound and acoustic radiation force impulse imaging characterize CT-indeterminate renal masses? A prospective evaluation with histological confirmation. *World Journal of Urology*, 37(7), 1339-1346. doi: 10.1007/s00345-018-2520-3.

[33] Thanaboonnipat, C., Sutayatram, S., Buranakarl, C., & Choisunirachon, N. (2020). Renal ultrasonographic strain elastography and symmetric dimethylarginine (SDMA) in canine and feline chronic kidney disease. *Journal of Veterinary Science*, 82(8), 1104-1112. doi: 10.1292/jvms.19-0496.

[34] Vagias, M., Chanoit, G., Bubenik-Angapen, L., Gibson, E. A., Rooster, H., Singh, A., Scharf, V., Grimes, J., Wallace, M., Kummeling, A., Flanders, J., Evangelou, G., & Mullins, R. (2022). Perioperative characteristics, histologic diagnosis, complications, and outcomes of dogs undergoing percutaneous drainage, sclerotherapy or surgical management of intrarenal cystic lesions: 18 dogs (2004-2021). *BMC Veterinary Research*, 18, article number 233. doi: 10.1186/s12917-022-03327-z.

[35] Vázquez, B., & Daniel, J. (2021). Avances na ecografía transabdominal en vacas de produción láctea. Lugo: Internacional da Universidade de Santiago de Compostela.

[36] Waller, K.R., O'Brien, R.T., & Zagzebski, J.A. (2007). Quantitative contrast ultrasound analysis of renal perfusion in normal dogs. *Veterinary Radiology & Ultrasound*, 48(4), 373-377. doi: 10.1111/j.1740-8261.2007.00259.x.

[37] Widmer, W.R., Biller, D.S., & Adams, L.G. (2004). Ultrasonography of the urinary tract in small animals. *Journal of the American Veterinary Medical Association*, 225(1), 46-54. doi: 10.2460/javma.2004.225.46.

[38] Zhang, X., Cao, D., Han, P., Ren, Zh., Wang, J., & Wei, Q. (2020). Aspiration-sclerotherapy versus laparoscopic de-roofing in the treatment of renal cysts: Which is better? *BMC Nephrology*, 21, article number 193.

# Характер абдомінальної операції при полікістозі нирок у тварин та роль показників сонографічного дослідження на різних етапах хірургічного втручання: огляд літератури

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**Анотація.** Аналіз методики використання сонографії під час хірургічного втручання у тварин із полікістозом нирок є актуальним завданням, оскільки, по-перше, сонографія є безпечним та неінвазивним методом обстеження, що дозволяє визначити структурні особливості нирок перед, під час та після операції. По-друге, з точки зору покращення результатів операції, використання сонографії допомогає уточнити локалізацію кіст та визначити їхні розміри. По-третє, важливим фактором у використанні сонографії є зниження болю та ризику післяопераційних ускладнень. Крім того, завдяки цьому методу дослідження нирок можливо точніше визначати оптимальний шлях доступу до кіст, що сприяє зменшенню травматизації тканин та забезпечує швидке відновлення тварини після операції. Мета дослідження полягала в детальному аналізі та описі методики використання сонографії під час хірургічного втручання у тварин з полікістозом нирок. Дослідження орієнтоване на необхідності визначення, як сонографія впливає на зменшення тривалості операції, поліпшення якості видалення кіст та зниження ризику ускладнень під час проведення абдомінальних операцій. Дослідницький підхід базується на аналізі наукових праць за цією тематикою, зокрема на досвіді ветеринарних лікарів, які вже використовували сонографію під час абдомінальних операцій у тварин із полікістозом нирок. Так, спеціальні техніки сонографії та хірургічного лікування кіст у нирках включають доплерографію, кольорову доплерографію, 3D та 4D сонографію, еластографію, інтроперативну сонографію, інтраперитонеальну сонографію, а також дуплексне сканування ниркових артерій та вен. У хірургічних методах лікування відзначаються екстракція окремих кіст, дренування кіст, резекція, а також нефректомія. Використання сонографії на різних етапах хірургічного втручання допомогає оптимізувати процес операції та знизити ризик виникнення ускладнень, а також сприятиме впровадженню нових підходів у лікуванні тварин з полікістозом нирок, що істотно поліпшить якість їхнього життя

**Ключові слова:** ветеринарна медицина; переродження органа; епітеліальні клітини; діагностика; черевна порожнина; малоінвазивна хірургія

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# Features of exotoxin production of vaccine strains of anthrax pathogen for use in the veterinary industry

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**Abstract.** Exotoxins that produce vaccine strains of the anthrax pathogen are the main source of immunogenicity of anti-selective vaccines used in veterinary medicine. The relevance of the study is due to the search for the most suitable vaccine strains of the anthrax pathogen to obtain

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high production of exotoxin as a factor of the effectiveness of drugs for the implementation of preventive and safety measures in the field of veterinary medicine. In this regard, the purpose of the study was to examine the productive properties of microbes of the *Bacillus* genus regarding the production of exotoxin under changes in cultivation conditions during incubation. Microbiological and biotechnological methods and comparative statistical analysis are used to examine vaccine strains of the anthrax pathogen. Strains are selected according to the intensity of growth on nutrient media. A biotechnological approach to obtaining a specific anthrax protein is used to analyse the production of exotoxin by vaccine strains of the anthrax pathogen. When cultured on identical nutrient media, the vaccine strains produce different amounts of exotoxin. Virulent (*B. anthracis* IBM-92 Z), vaccine *(B. anthracis* K-79 Z*, B. anthracis Sterne* 34F 2*, B. anthracis* 55*, B. anthracis* SB. *anthracis Tsenkovsky* II) strains, and anthrax cultures (*B. cereus* 8035*, B. anthracoides* 67*, B. subtilis* BKM 17) are examined. In the course of experimental work, it is determined that the production of exotoxin of various anthrax pathogen strains depends on the medium's pH. It is established that with identical pH values of the medium and cultivation conditions, the highest production of exotoxin was shown by the vaccine strain *B. anthracis* K-79 Z. The titer of a specific anthrax protein was 1:64. Changes in the pH of the medium during the cultivation of strains affect the amount of exotoxin formation – the main factor in the formation of specific immunity against the anthrax pathogen. The results of the study can be applied by specialists of the veterinary service to select antigen producers in the development of new drugs against anthrax in animals based on exotoxins

**Keywords:** anthrax; toxin formation; cultivation; medium pH; virulent strains; reference cultures

## Introduction

Anthrax is an infectious disease caused by a spore-forming gram-positive, rod-shaped bacterium, *Bacillus anthracis*. In the large Bacillus genus, *B. anthracis* is the only obligate pathogen. As noted by M. Omodo *et al.* (2023), anthrax is a zoonotic disease transmitted through the soil by a pathogen found on all continents of the globe. Anthrax is potentially contagious to most mammals. However, first of all, it affects ruminants. This bacterium survives for decades as spores in meadows contaminated with abandoned or buried corpses of animals that died from anthrax. R. Liddington (2021) draws attention to the fact that people become infected with anthrax through contact with infected animals or by eating contaminated animal products. In 95% of cases, when a person is infected with anthrax, the skin form is most common. E. Kisaakye *et al.* (2020) indicate that WHO estimates that between 2,000 and 20,000 human cases of anthrax are reported annually in the world.

Infection with *B. anthracis* is possible through the skin, gastrointestinal, or respiratory tract. Anthrax exists in two forms: vegetative cells (inside the host) and endospores in the soil or environment, where, as noted by S. Topluoglu *et al.* (2021), it can remain viable for decades to come. The global distribution of anthrax, in most cases, is determined by the presence of soils that support the survival of spores, namely those that are rich in organic substances and calcium and have a pH>6.4. W. Ashenefe *et al.* (2022) proved that a warming climate will increase the risk of anthrax in some regions of the world. The preservation of spores and unpretentiousness to the conditions of existence created prerequisites for the potential

use of bacteria as a weapon for bioterrorism. As stated by E.K. Dumas *et al.* (2020), anthrax outbreaks and isolated cases of animal and human infection have been reported in many countries. High mortality in the invasive form of anthrax and the development of shock during the disease are quite common consequences of this disease compared to the effect of other bacteria on the body. Therefore, developed countries pay considerable attention to this deadly infection.

*Bacillus anthracis*, the etiological agent of anthrax, becomes particularly virulent due to the capsule and two AB-type toxins: lethal factor LF and edematous factor EF. These toxins primarily disable immunocompetent cells. Both toxins are transferred to the host cell via the adhesin-Internalin subunit, a protective PA antigen. T.G. Sumithra *et al.* (2021) report that PA allows LF to reach intraluminal vesicles, where it remains active for a long time.

The main criterion for the development of modern anti-anthrax agents in veterinary medicine is the use of anthrax vaccine strains. According to W. Liu & E.M. Nestorovich (2021), it follows that the feature of *B. anthracis* is a property to produce exotoxin outside its cell. This product of metabolism of the vegetative form of bacilli consists of three fractions: protective, edematous, and lethal. The ability of anthrax microbes to produce exotoxin varies. All anthrax vaccines form antitoxic immunity in the vaccinated animal. The intensity and duration of immunity depends on this property, so the examination of toxigenicity of anthrax strains is relevant.

The toxin contains various combinations of protective antigen (PA), lethal factor (LF), and edema factor (EF). These three proteins combine to form two toxins: a lethal toxin (LT, a combination of PA and LF) and an edematous toxin (ET, a combination of PA and EF). Toxins alter the signalling pathways of host cells to interfere with the innate immune response in the early stages of infection and cause a vascular collapse in the later stages of the disease. According to R. Liddington (2021), toxins not only affect the innate and adaptive immune system but also determine the subsequent consequences of the disease. Due to the key role of the protective antigen (PA) in the action of anthrax toxin, efforts are being made to develop new vaccines consisting of a purified recombinant form of PA (rPA) adsorbed on an aluminium adjuvant that can cause a persistent neutralising effect. PA is immunogenic and causes the formation of toxin-neutralising antibodies that correlate with anthrax protection. Vaccines against toxin-mediated bacterial diseases such as diphtheria, tetanus, and anthrax protect by triggering sustained antibody responses that neutralise the toxin. However, attempts to develop such vaccines are difficult due to the lack of stability of the vaccine during storage.

When cultivating field virulent strains of *B. anthracis* in the laboratory, a slight production of the toxin is recorded, but it has a high aggressiveness. In the composition of the toxin of the field strain, the lethal and edematous fractions occupy more than 70%. Thus, as proved by W. Liu & E.M. Nestorovich (2021), the protective fraction accounts for less than 30%. Anthrax vaccine strains produce exotoxin, which is dominated by the protective fraction. When grown under identical conditions, they produce different amounts of exotoxin. Saprophytic spore-forming aerobic microbes from the *Bacillus* genus produce their own metabolites in the culture fluid but do not form a specific anthrax toxin. The ability of a vaccine strain to produce exotoxin *in vitro* depends on many factors: genetic makings, composition and type of nutrient medium, pH values, duration of cultivation, temperature, and number of cultivation passages. M. Manish *et al.* (2020) note that an important role in the creation of drugs using anthrax is played by the selection of anthrax vaccine strains.

From the above, the purpose of the study is to investigate the ability of various microbes of the *Bacillus* genus to produce exotoxins outside the cell wall when culture conditions change during incubation in a veterinary laboratory. Therewith, special attention was paid to the effect of the pH of the medium on the production of exotoxin, which is an important stage in the toxin formation and further use of anthrax pathogen strains in the case of creating new veterinary drugs. The main development of drugs based on anthrax exotoxins was carried out during the lifetime of Academician A.I. Zaviryukha, under his leadership a group of scientists (DNU State Center for Innovative Biotechnologies, Ukraine, Kyiv). This study is a continuation of scientific work on the development of modern technologies for obtaining exotoxins of pathogenic microorganisms.

## Literature Review

K.A. Simonsen & K. Chatterjee (2019) indicated that anthrax actors, once in a favourable environment, during reproduction in a living organism (*in vivo*) and on liquid nutrient media (*in vitro*), germinate quickly and secrete exotoxin outside the cell wall. Anthrax toxin consists of threeproteins: protective antigen (PA, 83 kDa), lethal factor (LF, 90 kDa), and edema factor (EF, 89 kDa). These three polypeptides are encoded by the pXO1 plasmid and form dicotyledonous combinations. The combination of LF and PA forms a lethal toxin (LT), whereas EF, in combination with PA, creates an edematous toxin (ET). Two protein components of anthrax toxin (EF and PA) increase the host's susceptibility to infection by inhibiting PMN function and increasing the body's resistance. The researchers proved that the combination of LT and ET is a disease factor. Instead, separately, the three toxin components (PA, LF, and EF) are non-toxic, which follows from the mortality of mice with LT and ET.

According to the results of the study, today, there is a wide possibility of using attenuated toxins as drug delivery systems. J.L. Dale *et al.* (2018) noted that experimental preparations containing exotoxins had high preventive properties. PA was shown to be a harmless toxin subunit that triggers a protective immune response and is the basis for all preventive measures against anthrax. According to J. Frydrych *et al.* (2018), M. Manish *et al.* (2020) and J. Tournier & C. Rougeaux (2020), anthrax protein vaccines licensed for human use include partially purified PA enhanced with various excipients.

In recent years, anthrax toxin has been modernised, and it acts as a targeted anti-angiogenic antitumour agent that kills tumours and has excellent therapeutic performance in experiments on animal models. Two conceptually different functions were added to PA to achieve high specificity when targeting the tumour. Anthrax toxin is a powerful tool for delivering proteins and drugs to cells. The ability of PA to various modifications and the growing number of new LF drug conjugates, as proved by C.J. Young *et al.* (2018) and E.S. Fischer *et al.* (2019), make this system a fairly versatile tool not only for antitumour therapy but also for use in other areas of veterinary medicine and biomedicine. J.M. Fonseca *et al.* (2020) indicate that anthrax toxin has proven to be a universal system for delivering drugs to cells from multiple enzyme fragments. This highly efficient delivery system was further modified by introducing ubiquitin as a cytosolic cleavage site into lethal fusion proteins. Such developments are conducted for the purpose of targeting tumours and delivering drugs to them.

Anthrax toxin receptors: tumour endothelial marker 8 (TEM8) and capillary morphogenesis gene 2 (CMG2) are endothelial receptors involved in extracellular matrix homeostasis and angiogenesis that are selectively activated in many tumours. One of the methods of

influencing these receptors is the protective antigen (PA), a protein produced by *B. anthracis*. T. Crawford *et al.* (2019) specified that toxins targeting PA selectively inhibit tumour growth and angiogenesis, but the selectivity of PA for the tumour is still being investigated. Application of *B. anthracis* strains with reduced protease was identified to be appropriate in combination with drugs to prevent the trigger of an immune response at the stage of cancer treatment. M.A. Walker *et al.* (2020) used protective antigen (PA) for specific targeting of different types of tumours.

D. Diaz-Arévalo *et al.* (2021) proved that the involvement of anthrax exotoxin in needle-free mucosal vaccines has positive results. Therewith, the immunogenic properties of anthrax exotoxin, which was used to develop combined drugs, were examined. It is noted that during the development of an influenza vaccine, its composition should be reviewed annually due to antigenic changes in circulating strains of the influenza virus. Due to seasonal drift and changes in circulating strains, the flu vaccine does not always correspond to circulating strains, and the adjuvants included in it are insufficient to induce a protective effect on long-lived memory cells. Adjuvants play an important role in the immune response to the vaccine and *Bacillus anthracis* the detoxified anthrax edema toxin, which consists of the protective antigen PA and the N-fragment of edema factor (EFn), showed an improved effect on humoral and cellular immune responses. As a result, a universal influenza vaccine design was developed, consisting of three tandem repeats of influenza antigen M2E plus HA2 and detoxified toxin EFn, which is associated with the PA component, and methods used to confirm protection. This will help develop a vaccination strategy using detoxified anthrax toxin for intranasal delivery of influenza antigen.

Despite the effectiveness of modern vaccines, scientists are searching for the development of the most areactogenic drugs, namely, improving anti-selective vaccines to reduce irritation and inflammation at the injection site. Similar reactions, as noted by S. Alameh *et al.* (2020) and T.G. Sumithra *et al.* (2021), occur when exposed to edematous and fatal factors. M.A. Smiley *et al.* (2019), D. Żakowska *et al.* (2019), and D.R. Weilhammer *et al.* (2020) proved that anthrax vaccines based on mutant variants of the exotoxin PA have less toxicity and greater immunogenicity. PA is a central component of anthrax toxin, which plays a leading role in protecting against encapsulated and non-encapsulated anthrax strains. Vaccines based on subunit rPA have a good safety and protection profile. However, there are problems of PA instability that are substantially aggravated by the use of aluminium adjuvants. New adjuvant compositions, dry preparations, and mutant forms of PA that are resistant to proteolysis and deamidation can solve this problem. Therefore, as stated by O.A. Kondakova *et al.* (2019), the development of a modern anthrax vaccine requires further research.

The protective antigen is the main protective immunogen in anthrax vaccines licensed in the United Kingdom and the United States. Repeated administration of vaccines is required to protect the body from this disease. However, depending on how they are developed, vaccines are relatively coarse products containing trace amounts of LF, EF, and other bacterial antigens that contribute to reactogenicity. LF and its individual domains have been confirmed to stimulate a protective antibody response in animals and humans. Combining protective regions into a single fusion protein is a more efficient, cost-effective, and practical approach. A fusion protein containing the N-terminal PA-binding domain LF (LFn) and the C-terminal PA-binding domain of the host cell can protect mice

from lethal anthrax infection. T.B. Gallagher *et al.* (2019) proved that the production of a single vaccine containing protective regions against LF and PA is cost-effective and can provide a wider range of protection than a vaccine containing only PA.

P. Pilo & J. Frey (2018) and V. Savransky *et al.* (2019) conducted a number of studies on the development of anthrax vaccine with recombinant protective antigen (rPA) (rPA7909), which is intended for post-exposure prevention of diseases caused by suspicious or confirmed *Bacillus anthracis*. The reaction at the injection site in animals treated with the control adjuvant is similar to that in animals treated with rPA7909 for inflammation. The inflammatory response at the injection site and drained lymph nodes was consistent with the expected immune stimulation and indicated a favourable safety profile for rPA7909. Although anthrax is rare, this microorganism can potentially be used in bioterrorism because it produces endospores that can withstand harsh environmental conditions. Therefore, as noted by Ch.K. Kang *et al.* (2019), in the future, the demand for an effective and safe anthrax vaccine will grow.

Thus, according to recent scientific studies, the use of extracellular toxins *B. anthracis* when creating new drugs is promising, and there is a need for further scientific developments on the prevention and treatment of anthrax.

## Materials and Methods

The research was conducted in 2021 based on the Veterinary Laboratory of the State Scientific Institution "Centre for Innovative Biotechnologies" (Kyiv) under the programme No. 0119U100900 "Exotoxin of the causative agent of anthrax. Characteristics, identity between strains, alternative to antibiotic therapy".

The following nutrient media were used in the study: nutrient broth (HIMEDIA) and nutrient agar (HIMEDIA), nutrient broth (GRM) – Kesler medium based on fish meal hydrolysate, meat-peptone Agar (MPA), and meat-peptone broth (MPB). Vaccine strains of anthrax and anthrax microorganisms: *B. anthracis* K-79 Z, *B. anthracis* IBM-92Z (Institute of Veterinary Medicine, Kyiv), *B. anthracis Sterne* 34F 2, *B. anthracis* 55, *B. anthracis* SB, *B. anthracis Tsenkovsky* II, *B. cereus* 8035, *B. anthracoides* 67, *B. subtilis* BKM 17 (museum collection of microorganisms).

Microbiological, biotechnological, and statistical research methods were used in the the process. The selection of strains was applied depending on the intensity of growth on nutrient media. A biotechnological method of cultivation and filtration was used to obtain a specific anthrax protein and examine the production of exotoxin by vaccine strains of the anthrax pathogen. The effect of pH on exotoxin production by experimental strains was determined by changes in the pH of the medium and the ability of different cultures to exhibit toxigenic properties. The HANNA H198128 pH meter was used. Experiments began with the production of pure working cultures of strains (Fig. 1-9), which were introduced to the HIMEDIA Agar (India) on Petri dishes and incubated at a temperature of 37°C for 48 hours.

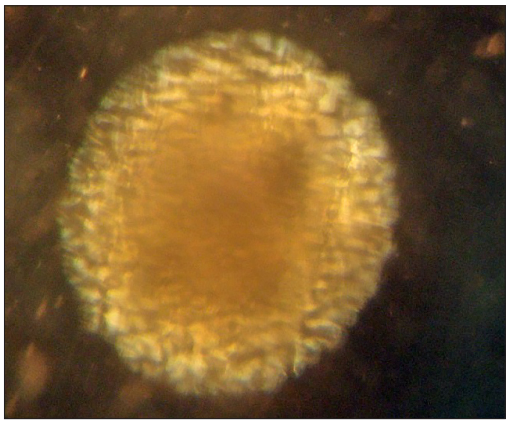

**Figure 1.** *B. anthracis* K-79 Z culture growth on nutrient agar (HIMEDIA)

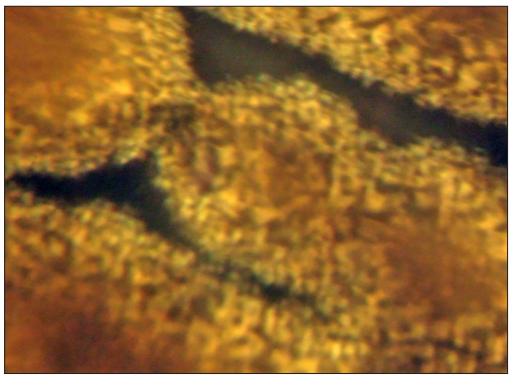

**Figure 2.** *B. anthracis Tsenkovsky* II (IBM92 Z) culture growth on nutrient agar (HIMEDIA)

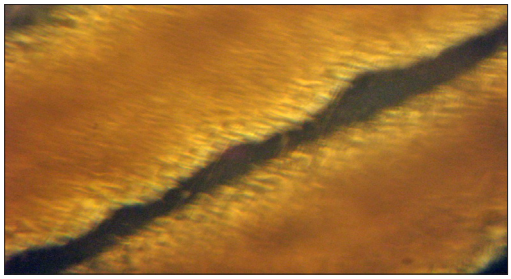

**Figure 3.** *B. anthracis Sterne* 34f2 culture growth on nutrient agar (HIMEDIA)

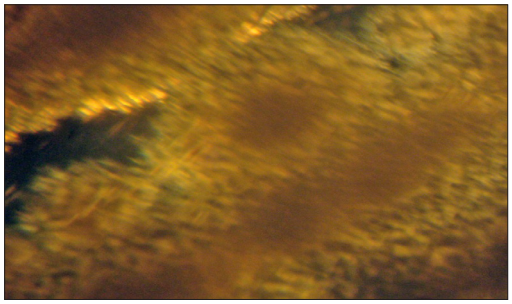

**Figure 4.** *B. anthracis* 55 culture growth on nutrient agar (HIMEDIA)

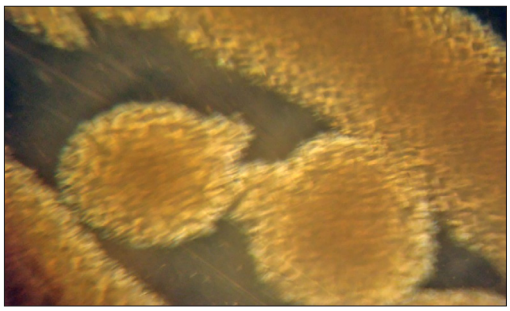

**Figure 5.** *B. anthracis* culture growth on nutrient agar (HIMEDIA)

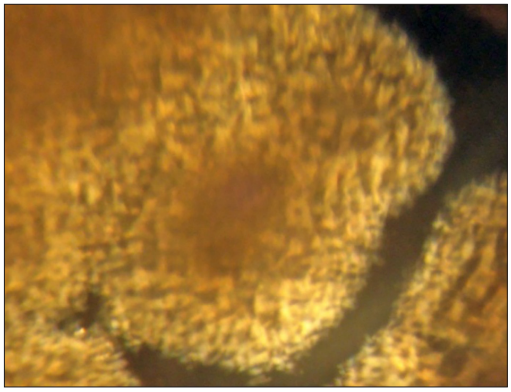

**Figure 6.** *B. anthracis Tsenkovsky* I culture growth on nutrient agar (HIMEDIA)

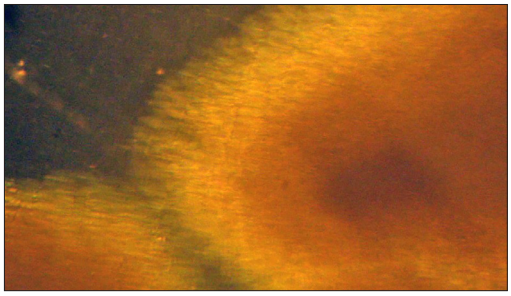

**Figure 7.** *B. cereus* 8035 culture growth on nutrient agar (HIMEDIA)

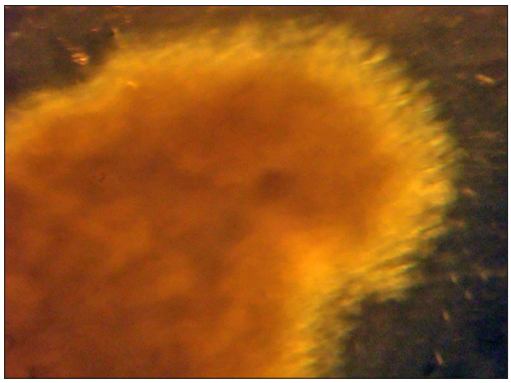

**Figure 8.** *B. anthracoides* 67 culture growth on nutrient agar (HIMEDIA)

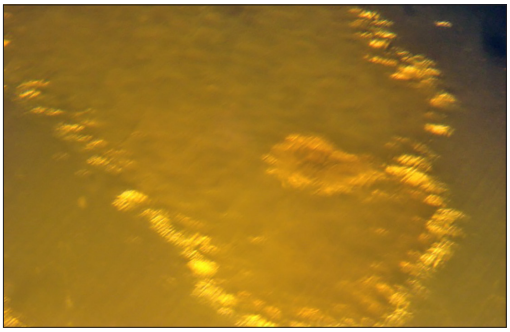

**Figure 9.** *B. subtilis* BKM 17 culture growth on nutrient agar (HIMEDIA)

***Sourse**: compiled by the author*

After the first 24 hours of incubation, the cultures were checked under a microscope for the presence of alien microflora. After 48 hours, at the end of incubation on a solid medium, microscopy of the colonies was performed. The number of microbial cells was determined using the generally accepted method of counting the colony forming units (CFU) in veterinary medicine. At the end of incubation, it was washed off with a spatula on the nutrient broth (HIMEDIA).

Incubation was conducted for 48 hours in nutrient broth (HIMEDIA) at a temperature of 37.0±1°C. In the first 24 hours, the purity of crop growth was monitored. Grown colonies were selected using a bacteriological loop, and a "crushed drop" smear was applied and viewed under a microscope. pH control was conducted using a HANNA H198128 pH meter (Germany), the pH of the medium was 7.4. After testing, incubation was continued for up to 48 hours. At the end of incubation, exotoxins were obtained. Further, filtration through a bacteriological filter of the F5 brand (China) was conducted according to the author's certificate No. 1022362. The resulting filtrates were checked for contamination according to DSTU 4483:2005 (2006). The titer of anthrax protein was determined in the obtained exotoxins.

The precipitation disk reaction (RDP) was used to determine the titer of exotoxins. The disk-precipitation reaction was staged. The formulation of the disk-precipitation reaction is aimed at detecting in the culture liquid a specific for *B. anthracis* metabolite – exotoxin by immunodiffusion reaction in Agar gel using a specific anthrax serum. According to the Ascoli reaction formulation method, 0.5-1.0 $cm^3$ opaque precipitation of anthrax serum was added to the bottom of a clean bacteriological test tube. A 1% agar gel, melted and cooled to 41-45°C, was layered on the serum using a Pasteur pipette along the wall of the test tube. After layering the Agar with a column 7-10 mm high, the test tube was left in an upright position for 15-20 minutes until it completely cooled down. The number of test tubes with such a system should correspond to the number of test tubes with sediment and

sediment dilutions. 1.0-2.0 cm $^3$ of appropriate dilution of sediment and sediment was layered on the agar surface. The prepared test tubes were placed in a thermostat at a temperature of 37°C for 16-24 hours. The test tubes were visible in the penetrating light against a black background. The presence of a white precipitation line (disk) in the agar column was determined. The presence of a disk in the test tube indicated the presence of spencific anthrax protein. Precipitating anthrax serum (Kherson State Enterprise – Biological Dactory) was used in the reaction.

## Results and Discussion

During experimental studies, the influence of changes in cultivation conditions on experimental strains of microbes of the *Bacillus* genus was determined. The results of the determination of exotoxin titers are presented in Table 1.

**Table 1**. Results of determination of anthrax protein titers in filtrates of anthrax pathogen strains during cultivation in nutrient broth (HIMEDIA)

| Strain name | Colony-forming units (CFU) | Nutrient medium | Incubation temperature, °C | Incubation period, h | Titer of the resulting filtrate (genus) |
|---|---|---|---|---|---|
| *B. anthracis K-79 Z* | $30\times10^6$ | HIMEDIA broth | 37.0±1.0 | 48 | 1:64 |
| *B. anthracis IBM-92Z* | $31\times10^6$ | | | | 1:16 |
| *B. anthracis Sterne 34F$_2$* | $32\times10^6$ | | | | 1:32 |
| *B. anthracis 55* | $30\times10^6$ | | | | 1:32 |
| *B. anthracis SB* | $29\times10^6$ | | | | 1:32 |
| *B. anthracis Tsenkovsky II.* | $31\times10^6$ | | | | 1:16 |
| *B. cereus 8035* | $30.2\times10^6$ | | | | - |
| *B. anthracoides 67* | $31\times10^6$ | | | | - |
| *B. subtilis BKM 17* | $29\times10^6$ | | | | - |

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

Based on the results (Table 1), it can be seen that the strain *B. anthracis* K-79 Z, under identical growing conditions in a nutritious broth (HIMEDIA), produces an exotoxin with a titer of 1:64. Strains *B. anthracis Sterne* 34F$_2$, *B. anthracis* 55, and *B. anthracis* SB produced an exotoxin with a titer of 1:32. Therewith, it was established that the strains *B. anthracis* IBM-92Z and *B. anthracis Tsenkovsky* II produced an exotoxin with a specific anthrax protein titer of 1:16. Anthrax strains *B. cereus* 8035, *B. anthracoides* 67, and *B. subtilis* BKM 17 produced no anthrax exotoxin. The reaction for the genus was negative. Cultivation of virulent and avirulent strains of anthrax pathogen on nutritious GRM broth showed that the cultivation of vaccine strains of anthrax pathogen *B. anthracis Sterne* 34F$_2$, *B. anthracis* 55, and *B. anthracis* SB is less productive than the strain *B. anthracis* K-79 Z under the same conditions (Table 2).

**Table 2**. Results of determination of anthrax protein titers in filtrates of anthrax pathogen strains during cultivation on nutritious GRM broth

| Strain name | Colony-forming units (CFU) | Nutrient medium | Incubation temperature, °C | Incubation period, h | Titer of the resulting filtrate |
|---|---|---|---|---|---|
| *B. anthracis K-79 Z* | $28\times10^6$ | GRM broth | 37.0±1.0 | 48 | 1:64 |
| *B. anthracis IBM-92Z* | $29\times10^6$ | | | | 1:16 |
| *B. anthracis Sterne 34F$_2$* | $27\times10^6$ | | | | 1:32 |

Table 2. Continued

| Strain name | Colony-forming units (CFU) | Nutrient medium | Incubation temperature, °C | Incubation period, h | Titer of the resulting filtrate |
|---|---|---|---|---|---|
| *B. anthracis 55* | $28\times10^6$ | GRM broth | 37.0±1.0 | 48 | 1:32 |
| *B. anthracis SB* | $30\times10^6$ | | | | 1:32 |
| *B. anthracis Tsenkovsky II.* | $27\times10^6$ | | | | 1:16 |
| *B. cereus 8035* | $28\times10^6$ | | | | - |
| *B. anthracoides 67* | $31\times10^6$ | | | | - |
| *B. subtilis BKM 17* | $29\times10^6$ | | | | - |

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

As can be seen from the results (Table 2), the lowest titer of exotoxin when incubated in GRM broth corresponded to *B. anthracis* IBM-92 Z and *B. anthracis Tsenkovsky* II, the titer of exotoxin in the RDP reaction was 1:16. The results of growing crops on HIMEDIA broth with the addition of 40% glucose solution in the amount of 5% are presented in Table 3.

**Table 3**. Results of determination of anthrax protein titers in filtrates of anthrax pathogen strains when cultured in nutrient broth (HIMEDIA) + glucose 5%

| Strain name | Colony-forming units (CFU) | Nutrient medium | Incubation temperature, °C | Incubation period, h | Filtrate titer |
|---|---|---|---|---|---|
| *B. anthracis K-79 Z* | $31\times10^6$ | HIMEDIA broth+glucose 5% | 37.0±1.0 | 48 | 1:128 |
| *Bac. anthracis IBM-92Z* | $29\times10^6$ | | | | 1:16 |
| *B.anthracis Sterne 34F$_2$* | $30\times10^6$ | | | | 1:32 |
| *B.anthracis 55* | $30\times10^6$ | | | | 1:32 |
| *B.anthracis SB* | $30\times10^6$ | | | | 1:32 |
| *B.anthracis Tsenkovsky II.* | $29\times10^6$ | | | | 1:16 |
| *B. cereus 8035* | $28\times10^6$ | | | | - |
| *B. anthracoides 67* | $31\times10^6$ | | | | - |
| *B. subtilis BKM 17* | $31\times10^6$ | | | | - |

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

The research shown in Table 3 indicate that the titer of the exotoxin strain *B. anthracis K-79 Z* when grown in HIMEDIA + 5% glucose broth was 1:128. This indicator is the highest among vaccine strains of the anthrax pathogen. Changes in cultivation conditions did not affect the production of exotoxin by vaccine strains *B. anthracis Sterne* 34F$_2$, *B. anthracis* 55, *B. anthracis* SB, and the exotoxin titer was 1:32. Exotoxins of strains *B. anthracis* IBM-92Z and *B. anthracis Tsenkovsky* II had a specific protein titer of 1:16. Saprophytic spore-forming bacilli *B. anthracoides* 67, *B. subtilis* 17, and *B. cereus* 8035 do not produce a specific toxin, and therefore, their filtrates of culture fluids did not react by precipitation (Ascoli) with antibodies of precipitating anthrax serum.

The examination of the effect of the pH of the medium on the production of exotoxin of anthrax strains began with the production of a bacterial mass with an identical number of microbial cells – 30.2 - 30.8 × $10^6$ CFU. Strains were introduced in the amount of: *B. anthracis* K-79 Z - 30.5 × $10^6$, *B. anthracis* IBM-92Z – 30.7 × $10^6$, *B. anthracis Sterne* 34F2 – 30.2 × $10^6$, *B. anthracis* 55 – 30.8 × $10^6$, *B. anthracis* SB – 30.5 × $10^6$

and B. *anthracis Tsenkovsky* II – $30.2 \times 10^6$. Colonies grown on MPA were introduced in a liquid nutrient medium – MPB with different pH values – 6.5; 8.5; 7.5. Incubation was conducted in a thermostat for 48 hours at a temperature of 37°C. The bacterial mass was filtered through F5 grade bacterial filters. The obtained filtrates were checked according to DSTU 4483:2005 (2006). With filtrates of the culture liquid, RDP was applied to determine the titer of the anthrax protein. According to the results of the study, it was determined that vaccine strains produce different amounts of exotoxin when the pH of the medium changes.

According to the results of the experiment, it was found that *B. anthracis* K-79 Z when cultured at pH 6.5, showed an exotoxin titer according to RDP – 8, when cultured at pH 7.5, the exotoxin titer was 64, and when cultured at pH 8.5-32. The average titer of the resulting exotoxin corresponded to a value of 1:34.66±16.22. Culture *B. anthracis* IBM-92 Z, when cultured at pH 6.5, showed an exotoxin titer of 4, and when incubated at pH 7.5-8.0. If the pH of the medium changed to 8.5, the exotoxin titer corresponded to 4. The average titer of exotoxin strain *B. anthracis* IBM-92 Z was equal to 1:5.33±1.33. When the pH value of the medium changes to 6.5, the vaccine strain *B. anthracis Sterne* 34F2 showed the title 8. When cultured on a medium at pH 7.5, the exotoxin titer corresponded to 32. When cultured at pH 8.5, a titer of 1:16 was obtained. The average exotoxin score was 1: 18.66±7.05.

Exotoxin production indicators also changed in *B. anthracis* 55. When cultured at pH 6.5, exotoxin production was 1:8 in titer. If the pH value of the medium changes to 7.5, an exotoxin titer of 1:32 is obtained. For changes in the pH of the medium to 8.5, an exotoxin titer of 1:16 was noted. Average titer of exotoxin *B. anthracis* 55 in the experiment was 1:18.66±7.05. Vaccine strain *B. anthracis* SB, when cultured on a nutrient medium, a pH value of 6.5 showed a titer of anthrax protein of 1:8. When cultured at a medium pH of 7.5, an exotoxin with a titer of 1:32 was obtained. When the pH of the medium changed to 8.5, an exotoxin was noted in the titer of 1:16. The average exotoxin production of the strain *B. anthracis* SB was 1: 18.66±7.05.

Culture *B. anthracis Tsenkovsky* II, when cultured at pH 6.5, showed an exotoxin titer of 1:8. When cultured at pH 7.5, the exotoxin titer was 1:16. When cultured at a medium pH of 8.5, an exotoxin titer of 1:8 was obtained. Average exotoxin production of the strain *B. anthracis* Tsenkovsky II corresponded to 1:10.66±2.66. The results of experimental studies show that during the cultivation of vaccine strains of anthrax at different pH values of the medium, the content of a specific protein in exotoxins varied. Despite changes in the pH value of the medium, the highest titer of exotoxin was obtained from the strain *B. anthracis* K-79Z, the average amount of specific anthrax protein in the bacterial mass filtrate was 1:34.66±16.22. Notably, at pH values of 8.5 of the medium, *B. anthracis* K-79Z produced exotoxin in a titer of 1:32. This is the highest rate of formation of a specific anthrax protein among the experimental strains at a pH of 8.5 of the medium.

According to the results, it was established that the production of exotoxin can change with fluctuations in the pH values of the medium, which has practical application in the development of a biotechnological process for manufacturing a vaccine from a strain or creating diagnosticums as, for example, a standard anthrax antigen. Changing the pH value of the medium allows correcting the biotechnological process of spore formation, the intensity of spore germination, the formation and accumulation of exotoxin in the cultural fluid. In accordance with the literature data, the research on the exotoxin production of anthrax pathogen strains is relevant. Typically, studies use one or two strains to produce vaccines or

diagnosticums. This study compares the vaccine strains used for the production of vaccines for veterinary medicine in Ukraine and abroad. Therewith, over the past five years, comparative studies of such vaccine strains regarding the production of exotoxin and the influence of external factors on toxin formation have not been conducted by other researchers.

M.H. Norris *et al.* (2020) analysed the spore formation of field and vaccine strains of the anthrax pathogen in the laboratory using nutrient media. The number of viable spores was taken into account according to the CFU. The authors confirmed that laboratory strains grown on media without animal protein quickly lose their ability to spore. Laboratory strains *B. anthracis* produce fewer proteins associated with growth and spore formation compared to wild strains isolated as a result of zoonotic diseases. According to the results of the paper, the rate of their growth on nutrient media is more slowed down than in field strains. It was also confirmed that wild strains grow faster than laboratory strains, showing greater sensitivity to the availability of nutrients. Spore formation in wild strains was substantially faster compared to laboratory strains, which indicates the ability of wild strains to accumulate spores faster due to nutrient restrictions. Meanwhile, in laboratory strains, there is a decrease in the rate at which they use nutrients and an increase in the time of spore formation. In addition, the authors noted that the mechanisms of growth retardation in laboratory strains require further investigation. Therefore, studies on the influence of the medium and cultivation conditions of vaccine strains are relevant and have a practical need.

D. Galante *et al.* (2022) noted that PA plays a crucial role in the immune inactivation of anthrax toxins, as it induces the production of neutralising antibodies that are necessary to provide protection against anthrax. The researchers proved a clear correlation between neutralising antibodies and protective immunity and highlighted the dependence of the effectiveness of anthrax vaccines on the magnitude of the humoral anti-PA response induced in animals or humans. In addition to toxin proteins, in media containing bicarbonate and providing 5% $CO_2$, which happens when mammalian tissues are infected, *B. anthracis* secretes many other proteins that play an important role in the virulence process. Therefore, proteases and degrading enzymes were identified that can inactivate proteins, cleave host antimicrobial factors, and modify secreted bacterial virulence factors, thereby performing additional functions for the complete virulence of *B. anthracis*.

As noted by A. Verma & D.L. Burns (2018) and Ch.K. Kang *et al.* (2019), the demand for an effective and safe anthrax vaccine is growing as humanity prepares for possible bioterrorism in the future. However, the currently adsorbed anthrax vaccine (AVA, BioThrax TM, Lansing, Michigan) remains the only U.S. FDA-approved vaccine that has been researched for decades. The authors noted that the optimal scheme and route of administration of AVA is still unknown and expressed several reservations about its pharmaceutical quality and consistency from batch to batch, which requires the creation of a new anthrax vaccine. Therewith, a randomised, blind, placebo-controlled phase II clinical trial was conducted on healthy adult volunteers to assess the immunogenicity and safety of a new anthrax vaccine with recombinant protective antigen (rPA), GC1109. The new anthrax vaccine rPA GC1109 was identified to have immunogenic activity after three doses of intramuscular administration and was well tolerated.

J.B. Felix *et al.* (2020) proved that the Sterne vaccine cannot elicit an immune response after oral vaccination. This vaccine contains a suspension of live attenuated spores of *B. anthracis* strain Sterne 34f2 (Sterne spores) in saponin

and is a modern injectable vaccine for livestock. When administered orally, it is ineffective, and individual subcutaneous injections are not a practical method of vaccination of wild animals. Based on the conducted studies, the authors established a direct dependence of the effectiveness of the drug on the pH of the medium and the effect on the vaccine strain when creating various cultivation conditions, which confirms the results described in this paper. The stability of Sterne spores at different pH values was evaluated *in vitro*, and it was determined that the medium at pH 2 leads to the death of spores of the vaccine strain. The authors confirmed that protection from the stomach environment is a major problem in the production of oral vaccines. Therefore, there is a need to create an effective oral anthrax vaccine for wild animals. An oral anthrax vaccine is urgently needed to prevent annual anthrax outbreaks that lead to catastrophic losses of livestock and wildlife around the world.

V. Savransky *et al.* (2019) noted that existing licensed preventive vaccines are heterogeneous products and may include inactivated bacterial and viral vaccines, live attenuated vaccines, recombinant proteins, polysaccharide and conjugated vaccines, and DNA vaccines. They often contain immunostimulating components represented by various adjuvants and require developing new anthrax vaccines designed to improve the currently licensed one. These improvements include fewer vaccinations and faster protection Times. V. Savransky *et al.* (2019) indicated that rPA7909 is an anthrax vaccine with recombinant protective antigen (rPA) supplemented with the immunostimulating oligonucleotide CpG 7909 and was developed for post-exposure anthrax prevention. The vaccine is a sterile lyophilised form, which, after dilution, has the appearance of a suspension from white to almost white in color. The final reconstituted drug contains 75 mcg of rPA, 750 mcg of aluminium (as an adjuvant of algidrogel), and 250 mcg of CpG 7909 in 0.5 mL (full dose) with other excipients. The rPA7909 vaccine was well tolerated by the animal body and caused local and systemic effects associated with the inflammatory process provoked by the injected material. The expected antigen-specific antibody response was observed in animals that received the rPA7909 vaccine.

M.A. Smiley *et al.* (2019), D. Żakowska *et al.* (2019), and D.R. Weilhammer *et al.* (2020) presented a study on mutant variants of the PA exotoxin used to improve vaccines. T.B. Gallagher *et al.* (2019) proved that PA is the main protective immunogen in anthrax vaccines licensed in the UK and US. The use of recombinant protective antigen (rPA) (rPA7909) as a post-exposure anthrax prevention was proven by V. Savransky *et al.* (2019). Anthrax vaccine production in the UK (AVP) is focused on reproducing protective antigen (PA) from the *Bacillus anthracis* Sterne strain. However, some fundamental properties of AVP still need to be examined. Therefore, it is necessary to investigate the differences in degrees of protection in different animal species, even though AVP has been used for decades, as noted by T. Modi *et al.* (2021).

S. Jauro *et al.* (2020) noted that the vaccine from the *Bacillus anthracis* Sterne strain with live spores (SLSV, strain *Bacillus anthracis* 34F2) is an effective veterinary anthrax vaccine, although its use with antimicrobials is contraindicated. However, the use of a non-live anthrax vaccine (NLAV) may overcome the limitations of SLSV. The authors presented the results of a study on cattle vaccinated with either NLAV (purified recombinant PA (PrPA) or rPA (CrPA) and formaldehyde-inactivated spores (strain FIS *B. anthracis* 34f2) and emulsigen-D®/Alhydrogel® adjuvant. The potential of NLAV (PrPA+FIS or CrPA+FIS) applied together with combined adjuvants (Emulsigen-D®/Alhydrogel®) to induce an immune response against toxins and spores *B. anthracis* in vaccinated cattle

was established. Immune response with Pr-PA+FIS+Emulsigen-D®/Alhydrogel® demonstrated a substantial level of protection in the passive protection analysis on mice, which is comparable to SLSV. Due to the inanimate property of the vaccine, it is compatible with antibiotic treatment in the event of an outbreak. It can be used for vaccination of wild animals and on grazing grounds. It lacks residual virulence in vaccinated animals. Field clinical trials of the vaccine are currently underway. The use of antigens as components of new drugs against anthrax is a promising direction in improving methods of control and Prevention of this disease in animals.

H. Mirhaj *et al.* (2019) noted that the standard approach to anthrax therapy is to destroy germinating bacilli by injecting aggressive antibiotics. However, antibiotic therapy is ineffective when systemic symptoms of anthrax appear since, by this time, deadly concentrations of anthrax toxin accumulate in the body of sensitive animal species, and as a result of natural evolution, antibiotic-resistant strains appear. Deliberate modification through genetic engineering is also a new challenge for traditional antibiotic treatment. Therefore, the development of an antitoxin for combined use with antibiotic therapy is a top priority. Modern vaccines are produced in England and the United States on the basis of precipitation of cell extracts. The production of next-generation anthrax vaccines focuses on various recombinant expression systems. As a result of this study, it was determined that antibodies generated against four PA regions (PAD4) can neutralise anthrax toxin and PA mixed with LF, which increases the specific antibody response to PA. Thus, from the available literature sources, it is known about the research on toxin formation of a vaccine strain *B. anthracis Sterne* $34F_2$ used for the production of anthrax vaccines in the United States and England.

In Ukraine, biofactories produce veterinary preventive drugs from three strains: *B. anthracis Sterne* 34F2, *B. anthracis* K-79Z, and *B. anthracis* SB. The creation of vaccines is based on the spore mass of the anthrax pathogen. Foreign developments are based on the products of *B. anthracis* strain metabolism and are mostly aimed at preventing infection in humans. Academician A.I. Zaviriukha and a team of scientists from the relevant laboratory of the Institute of Molecular Biology and Genetics of the National Academy of Sciences of Ukraine (Kyiv) commenced the examination and practical application of exotoxins of infectious diseases pathogens in animals in the composition of biological preparations. The selection and deposition of strains producing toxins was conducted: anthrax (strains B. anthracis K-79 Z and IBM 92 Z), colibacteriosis (*E. coli* IBM-1), pasteurellosis (*P. multocida* 84z), salmonellosis. The possibility of using anthrax exotoxins in the manufacture of specific diagnosticums and inactivated immunogenic vaccines were experimentally proven. The results presented in this paper are a continuation of research on the development of modern technologies for obtaining exotoxins of pathogenic microorganisms and their determination, presence, and quantity in the culture fluid and elucidation of their effect on the immune system of animals.

The authors of this paper consider all the above aspects regarding preventive drugs from the vaccine strain *B. anthracis Sterne* 34F2, especially its properties related to the formation of exotoxin. Due to the museum of microorganisms, in particular, the presence of the aforementioned strains of anthrax and anthrax bacilli, researchers have the opportunity to compare the parameters of cultures of the *Bacillus genus.* Long-term work with various reference strains indicates substantial advantages of the vaccine strain *B. anthracis* K-79 Z regarding the production of exotoxin in comparison

with *B. anthracis Sterne* 34F$_{2,}$ *B. anthracis* 55, and *B. anthracis* SB, which is the main source of immunogenicity of the vaccine, namely the effectiveness of its use for animals and humans. At the present stage of creating vaccines for effective anthrax prevention, it is vital to use anthrax pathogens with a lower allergenic effect and the ability to preserve the stability of exotoxin as a source of immunogenicity. Therefore, examining vaccine strains at different stages of the creation of anti-selective drugs is a relevant direction of scientific and practical search.

## Conclusions

On the basis of the Veterinary Laboratory of the State Scientific Institution "Centre for Innovative Biotechnologies", a study of the ability of various microbes of the *Bacillus* genus to produce exotoxins outside the cell wall when culture conditions change during incubation was conducted. It was established that when the cultivation conditions and pH values of the medium change, vaccine strains of the anthrax pathogen produce different amounts of exotoxin. The *B. anthracis* K-79 Z vaccine strain was the most resistant to changes in the pH of the medium, the average amount of specific anthrax protein in the bacterial mass filtrate was 1: 34.66±16.22. Cultivation in a medium with a pH value of 7.5 was the most optimal for strains *B. anthracis Sterne* 34F$_{2,}$ *B. anthracis* 55, and *B. anthracis* SB. The titer of the resulting anthrax protein was 1:32. Under the same cultivation conditions with the experimental vaccine strains and a certain favourable pH 7.5 of the medium, the highest titer of a specific anthrax protein was 1:64, obtained from the bacilli of the vaccine strain *B. anthracis* K-79 Z. It was determined that a change in the pH of the medium to 8.5 provoked experimental strains of the anthrax pathogen to produce exotoxin in the titer of a specific anthrax protein – 1:16, which is much lower than that under optimal cultivation conditions. Change in the incubation pH values of the nutrient medium to 6.5 affected the growth properties of strains and the process of toxin formation, which manifested itself in a decrease in the production of specific anthrax protein to 1:8 in filtrates of bacterial masses of vaccine strains *B. anthracis Sterne* 34F$_{2,}$ *B. anthracis* 55, and *B. anthracis* SB. When the cultivation conditions changed – an increase in the pH of the medium to 8.5, the experimental strains showed a similar decrease in the production of a specific anthrax protein, which was 1:32. This indicates similar characteristics of these vaccine strains and an identical effect of the pH value of the medium on exotoxin production. Under the same cultivation conditions with the experimental vaccine strains and a certain favourable pH 7.5 of the medium, the highest titer of a specific anthrax protein was 1:64, obtained from the bacilli of the vaccine strain *B. anthracis* K-79 Z.

The presence of high rates of exotoxin production in vaccine strains of anthrax pathogen is an important indicator in the development of new drugs against anthrax pathogen for use in veterinary medicine. In the future, studies of toxigenic properties of vaccine strains of the anthrax pathogen will be conducted and their further use in veterinary medicine during the development of new preventive drugs for anthrax, which will contribute to the epizootic security of the state.

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## Conflict of Interest

None.

## References


[1] Alameh, S., Bartolo, G., O'Brien, S., Henderson, E.A., Gonzalez, L.O., Hartmann, S., Klimko, C.P., Shoe, J.L., Cote, C.K., Grill, L.K., Levitin, A., & Martchenko, Sh.M. (2020). Anthrax toxin component, Protective Antigen, protects insects from bacterial infections. *PLoS ONE,* 16(8), article number 1008836. doi: 10.1371/journal.ppat.1008836.

[2] Ashenefe, W., Fantaw, S., Mekonene, Y., Teshale, A., Yitagesu, Y., Tsige, E., Getahun, D., Geremew, T., Abichu, G., Moges, B., Abate, E., Abayneh, T., Zeru, T., Belay, Z., & Mor, S. (2022). First PCR Confirmed anthrax outbreaks in Ethiopia-Amhara region, 2018-2019. *PLOS Neglected Tropical Diseases,* 16(2), article number e0010181. doi: 10.1371/journal.pntd.0010181.

[3] Crawford, T., Fletcher, N., Veitch, M., Gonzalez, J.L., Pett, N., Brereton, I., Wells, J.W., Mobli, M., & Tesiram, Y. (2019). *Bacillus anthracis* protective antigen shows high specificity for a UV induced mouse model of cutaneous squamous cell carcinoma frontiers in medicine (Lausanne). *Frontiers in Medicine*, 6, article number 22. doi: 10.3389/fmed.2019.00022.

[4] Dale, J.L., Raynor, M.J., Ty, M.C., Hadjifrangiskou, M., & Koehler, T.M. (2018). A dual role for the bacillus anthracis master virulence regulator AtxA: Control of sporulation and anthrax toxin production. *Frontiers in Microbiology,* 9, article number 482. doi: 10.3389/fmicb.2018.00482.

[5] Diaz-Arévalo, D., Chen, Y., & Zeng, M. (2021). Vaccine delivery with a detoxified bacterial toxin, methods in molecular biology, 2183, 423-435. doi: 10.1007/978-1-0716-0795-4_22.

[6] DSTU 4483:2005. (2006). *Veterinary immunobiological preparations. Methods of determining bacterial and fungal contamination*. Retrieved from http://surl.li/qqvrj.

[7] Dumas, E.K., Demiraslan, H., & Ingram, R.J., Sparks, R.M., Muns, E., Zamora, A., Larabee, J., Garman, L., Ballard, J.D., Boons, G.J., James, J.A., Kayabas, U., Doganay, M., & Darise Faris, A. (2020). Toxin neutralizing antibodies elicited by naturally acquired cutaneous anthrax are elevated following severe disease and appear to target conformational epitopes. *PLoS ONE,* 15(4), article number e0230782. doi: 10.1371/journal.pone.0230782.

[8] Felix, J.B., Chaki, S.P., Xu, Y., Ficht, T.A., Rice-Ficht, A.C., & Cook, W.E. (2020). Protective antibody response following oral vaccination with microencapsulated *Bacillus Anthracis* Sterne strain 34F2 spores. *NPJ Vaccines*, 5, article number 59. doi: 10.1038/s41541-020-0208-3.

[9] Fischer, E.S., Campbell, W.A., Liu, Sh., Ghirlando, R., Fattah, R.J., Bugge, T.H., & Leppla, S.H. (2019). Bismaleimide cross-linked anthrax toxin forms functional octamers with high specificity in tumor targeting. *Protein Science*, 28(6), 1059-1070. doi: 10.1002/pro.3613.

[10] Frydrych, J., Skácel, J., Šmídková, M., Mertlíková-Kaiserová, H., Dračínský, M., Gnanasekaran R., Lepšík, M., Soto-Velasquez, M., Watts, V.J., & Janeba, Z. (2018). Synthesis of α-branched acyclic nucleoside phosphonates as potential inhibitors of bacterial adenylate cyclases. *ChemMedChem,* 13(2), 199-206. doi: 10.1002/cmdc.201700715.

[11] Fonseca, J.M., Mackowiak da Fonseca, I.I., Nagamine, M.K., Oliveira Massoco, C., Nishiya, A.T., Ward, J.M., Liu, Sh., Leppla, S.H., Bugge, T.H., & Dagli, M.L. (2020). Inhibitory effects of a reengineered anthrax toxin on canine and human osteosarcoma cells. *Toxins (Basel),* 12(10), article number 614. doi: 10.3390/toxins12100614.

[12] Gallagher, T.B., Mellado-Sanchez, G., Jorgensen, A.L., Moore, S., Nataro, J.P., Pasetti, M.F., & Baillie, L.W. (2019). Development of a multiple-antigen protein fusion vaccine candidate that confers protection against *Bacillus anthracis* and *Yersinia pestis*. *PLOS Neglected Tropical Diseases,* 13(8), article number e0007644. doi: 10.1371%2Fjournal.pntd.0007644.

[13] Galante, D., Manzulli, V., Donatiello, A., Fasanella, A., Chirullo, B., Francia, M., Rondinone, V., Serrecchia, L., Pace, L., Iatarola, M., Tarantino, M., & Adone, R. (2022). Production of a *Bacillus anthracis* secretome with suitable characteristics as antigen in a complement fixation test. *Life*, 12(2), article number 312. doi: 10.3390/life12020312.

[14] Jauro, S., Ndumnego, O.C., Ellis, Ch., Buys, A., Beyer, W., & Heerden, H. (2020). Immunogenicity of non-living anthrax vaccine candidates in cattle and protective efficacy of immune sera in A/j mouse model compared to the sterne live spore vaccine. *Pathogens*, 9(7), article number 557. doi: 10.3390/pathogens9070557.

[15] Kang, Ch.K., Kim, N.H., Kim, Ch.J., Rhie, Gi.E., Jo, S.K., Ahn, M., Kang, J., Choe, P.G., Park, W.B., Kim, N.J., & Oh, M.D. (2019). Immunogenicity and safety of a novel recombinant protective antigen anthrax vaccine (GC1109), a randomized, single-blind, placebo controlled phase II clinical study. *Vaccine,* 37(29), 3820-3824. doi: 10.1016/j.vaccine.2019.05.057.

[16] Kisaakye, E., Riolexus Ario, A., Bainomugisha, K., Cossaboom, C., Lowe, D., Bulage, L., Kadobera, D., Sekamatte, M., Lubwama, B., Tumusiime, D., Tusiime, P., Downing, R., Buule, J., Lutwama, J., Salzer, J.S., Matkovic, E., Ritter, J., Gary, J., & Zhu, B. (2020). Outbreak of anthrax associated with handling and eating meat from a cow, Uganda, 2018. *Emerging Infectious Diseases*, 26(12), 2799-2806. doi: 10.3201/eid2612.191373.

[17] Kondakova, O.A, Nikitin, N.A., Evtushenko, E., Ryabchevskaya, E.M., Atabekov, J.G., & Karpova, O.V. (2019). Vaccines against anthrax based on recombinant protective antigen: Problems and solutions. *Expert Review of Vaccines*, 18(8), 813-828. doi: 10.1080/14760584.2019.1643242.

[18] Liddington, R.C. (2021). Assembly and Function of the anthrax toxin protein translocation complex. *Subcellular Biochemistry*, 96, 563-577. doi: 10.1007/978-3-030-58971-4_18.

[19] Liu, W., & Nestorovich, E.M. (2021). Anthrax toxin channel: What we know based on over 30 years of research. *Biochimica et Biophysica Acta*, 1863(11), article number 183715. doi: 10.1016/j.bbamem.2021.183715.

[20] Manish, M., Verma, Sh., Kandari, D., Kulshreshtha, P., Singh, S., & Bhatnagar, R. (2020). Anthrax prevention through vaccine and post-exposure therapy. *Expert Opinion on Biological Therapy,* 20(12), 1405-1425. doi: 10.1080/14712598.2020.1801626.

[21] Mirhaj, H., Honari, H., & Zamani, E. (2019). Evaluation of immune response to recombinant Bacillus anthracis LFD1-PA4 chimeric protein. *Iranian Journal of Veterinary,* 20(2), 112-119.

[22] Modi, T., Gervais, D., Smith, S., Miller, J., Subramaniam, Sh., Thalassinos, K., & Shepherd, A. (2021). Characterization of the UK anthrax vaccine and human immunogenicity. *Human Vaccines & Immunotherapeutics*, 17(3), 747-758. doi: 10.1080/21645515.2020.1799668.

[23] Norris, M.H., Zincke, D., Leiser, O.P., Kreuzer, H., Hadfied, T.L., & Blackburn, J.K. (2020). *Laboratory strains of Bacillus anthracis lose their ability to rapidly grow and sporulate compared to wildlife outbreak strains*. Retrieved from https://pubmed.ncbi.nlm.nih.gov/31978128/.

[24] Omodo, M., Gardela, J., Namatovu, A., Okurut, A., Esau, M., Acham, M., Nakanjako, M., Israel, M., Isingoma, E., Moses, M., Paul, L., Ssenkeera, B., Atim, S., Gonahasa, D., Sekamatte, M., Gouilh, M., & Gonzalez, J. (2023). Anthrax bio-surveillance of livestock in Arua District, Uganda, 2017-2018. *Acta Tropica,* 240, article number 106841. doi: 10.1016/j.actatropica.2023.106841.

[25] Pilo, P., & Frey, J. (2018). Pathogenicity, population genetics and dissemination of *Bacillus anthracis*. *Infection, Genetics and Evolution*, 64, 115-125. doi: 10.1016/j.meegid.2018.06.024.

[26] Savransky, V., Lacy, M., Ionin, B., Skiadopoulos, M.H., & Shearer, J. (2019). Toxicity study of a lyophilized recombinant protective antigen-based anthrax vaccine adjuvanted. *International Journal of Toxicology*, 38(3), 163-172. doi: 10.1177/1091581819848722.

[27] Simonsen, K.A., & Chatterjee, K. (2019). *Anthrax*. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK507773/.

[28] Smiley, M.A., Sanford, D.C., Triplett, Ch.A., Callahan, D., Frolov, V., Look, J., Ruiz, Ch., Reece, J.J., Miles, A., Ruiz, E., Ionin, B., Shearer, J.D., & Savransky, V. (2019). Comparative immunogenicity and efficacy of thermostable (lyophilized) and liquid formulation of anthrax vaccine candidate AV7909. *Vaccine,* 37(43), 6356-6361. doi: 10.1016/j.vaccine.2019.09.015.

[29] Sumithra, T.G., Chaturvedi, V.K., Gupta, P.K., Bincy, J., Siju, S.J., Sunita, S.C., Reshma, K.J., Patel, C.L., & Rai, A.K. (2021). A novel bicistronic DNA vaccine with enhanced protective immune response against *Bacillus anthracis* through DNA prime-protein boost vaccination approach. *Microbial Pathogenesis*, 158, article number 105104. doi: 10.1016/j.micpath.2021.105104.

[30] Topluoglu, S., Aktas, D., Celebi, B., Kara, F., Doganay, M., & Alp, E. (2021). Human anthrax in Turkey: A ten years' experience (2009-2018). *Tropical Doctor*, 51(1), 80-83. doi: 10.1177/0049475520969542.

[31] Tournier, J., & Rougeaux, C. (2020). Anthrax toxin detection: From *in vivo* studies to diagnostic applications. *Microorganisms*, 8(8), article number 1103. doi: 10.3390/microorganisms8081103.

[32] Verma, A., & Burns, D.L. (2018). *Improving the stability of recombinant anthrax protective antigen vaccine*. Retrieved from https://pubmed.ncbi.nlm.nih.gov/30228030/.

[33] Walker, M.A., Uribasterra, M., Asher, V., Getz, W.M., Ryan, S.J., Ponciano, J.M., & Blackburn, J.K. (2021). Anthrax surveillance and the limited overlap between obligate scavengers and endemic anthrax zones in the United States. *Vector-Borne and Zoonotic Diseases,* 21(9), 675-684. doi: 10.1089/vbz.2020.2747.

[34] Weilhammer, D.R., Dunkle, A.D., Boone, T., Gilmore, S.F., Khemmani, M., Peters, S.K.G., Hoeprich, P.D., Fischer, N.O., Blanchette, C.D., Driks, A., & Rasley, A. (2020). Characterization of *Bacillus anthracis* spore proteins using a nanoscaffold vaccine platform. *Frontiers in Immunology*, 11, article number 1264. doi: 10.3389/fimmu.2020.01264.

[35] Young, C.J., Richard, K., Beruar, A., Lo, S.Y., & Siemann, S. (2018). An investigation of the pH dependence of copper-substituted anthrax lethal factor and its mechanistic implications. *Journal of Inorganic Biochemistry,* 182, 1-8. doi: 10.1016/j.jinorgbio.2018.01.015.

[36] Żakowska, D., Graniak, G., Rutyna, P., Naylor, K., Głowacka, P., & Niemcewicz, M. (2019). Protective antigen domain 4 of *Bacillus anthracis* as a candidate for use as vaccine for anthrax. *Annals of Agricultural and Environmental Medicine,* 26(3), 392-395. doi: 10.26444/aaem/99669.

# Особливості продукції екзотоксину вакцинних штамів збудника сибірки для застосування у ветеринарній галузі

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**Анотація.** Основним джерелом імуногенності протисибіркових вакцин, які застосовуються у ветеринарній медицині є екзотоксини, що продукують вакцинні штами збудника сибірки. Актуальність дослідження зумовлена пошуком найпридатніших до використання вакцинних штамів збудника сибірки, з метою отримання високої продукції екзотоксину, як фактора ефективності препаратів для здійснення профілактичних і безпекових заходів у галузі ветеринарії. У зв'язку з цим, мета роботи полягала у дослідженні продуктивних властивостей мікробів роду *Bacillus* щодо продукції екзотоксину за зміни умов культивування під час інкубації. При дослідженні вакцинних штамів збудника сибірки використовували мікробіологічні та біотехнологічні методи, а також порівняльний статистичний аналіз. Проводили відбір штамів за інтенсивністю росту на поживних середовищах. Для вивчення продукції екзотоксину вакцинними штамами збудника антракса застосовували біотехнологічний підхід отримання специфічного сибіркового білка. При культивуванні на ідентичних поживних середовищах вакцинні штами продукували різну кількість екзотоксину. Досліджено вірулентні (B. anthracis IBM-92 Z), вакцинні (*B.* anthracis K-79 Z*, B. anthracis Sterne*

34F 2, *B. anthracis* 55, *B. anthracis* СБ, *B. anthracis* Ценковського II) штами, а також сибіркоподібні культури (B. cereus 8035, *B. anthracoides* 67, *B. subtilis* ВКМ 17). У процесі експериментальної роботи визначено, що продукція екзотоксину різних штамів збудника сибірки залежить від pH середовища. Встановлено, що при ідентичних показниках pH середовища та умов культивування найвищу продукцію екзотоксину показав вакцинний штам B. anthracis K-79 Z. Титр специфічного сибіркового білка становив 1:64. Зміна pH середовища при культивуванні штамів впливає на кількість утворення екзотоксину – основного фактору формування специфічного імунітету проти збудника антракса. Результати досліджень можуть бути застосовані фахівцями ветеринарної служби для відбору продуцентів антигену при розробці нових препаратів проти сибірки в тварин на основі екзотоксинів

**Ключові слова:** антракс; токсиноутворення; культивування; pH середовища; вірулентні штами; референтні культури

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# Prevalence and diagnostic methods of surgical pathology in the digestive system of animals

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**Abstract.** The relevance of the study is driven by the rapid increase in the number of small domestic animals with surgical pathology of the digestive system, requiring effective diagnosis and surgical intervention. In this regard, the purpose of this study is to investigate the prevalence of various diseases of the digestive organs in small domestic animals and to determine the most informative methods of their diagnosis. The primary approach in the study is to compare the informativeness of results obtained from examining sick animals, including general (history collection, examination, palpation) and special (ultrasound diagnostics of abdominal organs, endoscopy of the digestive

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tract, radiography) diagnostic methods. The advantages and disadvantages of different special diagnostic methods were identified and analysed, providing a comprehensive assessment of indications and contraindications for their application in clinical veterinary practice. Out of 1863 animals that presented to the veterinary clinic, pathologies of the digestive tract of various origins were diagnosed in 957 animals. Conservative care was provided to 79.7% of the affected animals (763 individuals). Complicated pathologies requiring surgical treatment were diagnosed in 194 (20.3%) animals. Among these, 170 (17.7%) had foreign bodies in the stomach, 14 (1.5%) had gastric erosions, 4 (0.4%) had neoplasms, 3 (0.3%) had perforations, and 3 (0.3%) had inflammatory diseases. The use of ultrasound diagnostics allowed for the detection of linear foreign bodies in the stomach and the identification of associated complications such as stomach or intestinal perforations caused by foreign bodies. Radiographic examination proved effective when radiopaque structures (metals, stones, bones) were present as foreign bodies. The materials of the study provide practical value for both researchers and practising veterinarians, offering the opportunity to utilise new instrumental diagnostic methods to assess the feasibility of surgical manipulations on the stomach and intestines in dogs and cats

**Keywords:** esophagogastroduodenoscopy; ultrasound diagnostics; radiographic examination; plateau level; diverticulum; hernia; inflammatory bowel disease

## Introduction

In recent years, there has been a rapid increase in the number of animals with pathologies in the digestive tract. S.G. Lishchuk *et al.* (2023) indicate that among internal pathologies, gastrointestinal organ disorders were most frequently diagnosed, accounting for 44.6% of the total cases. The process of breaking down complex nutrients in the feed into simple ones, accessible for absorption by the animal's body, is referred to as digestion. A.M. Power *et al.* (2021) note that the digestive tract of small domestic animals, specifically dogs and cats, includes all organs involved in the intake and processing of food. The digestive system can be conventionally divided into three sections. The anterior part includes the oral cavity with auxiliary organs, the pharynx, and the oesophagus. The middle section comprises the stomach and the small intestine with digestive glands. The posterior section includes the large intestine. According to N.R. Barash *et al.* (2022), the anterior section is responsible for capturing, chewing, and swallowing food, the middle section for digestion and absorption of nutrients, and the posterior section for the formation of faecal masses from undigested feed residues.

Studies by R.A. Didier *et al.* (2021) emphasise that the process of digestion in animals begins as soon as the feed enters the oral cavity. It involves chewing, processing food with saliva, the catalytic transformation of its components with the participation of enzymes, and further movement along the digestive tract. However, as mentioned by M. Murakami *et al.* (2022), the movement of feed mass from the stomach can be limited or completely stopped due to partial or complete obstruction of the intestinal lumen by tumours, foreign objects, polyps, excessive mucosal growth, or the presence of ulcers. M.G. Derré *et al.* (2022) clarified that intestinal obstruction can be partial or complete, caused by foreign objects, invagination, swelling, strangulation (e.g., hernia strangulation), certain infections, and neoplasms.

Symptoms indicating disruptions in the functioning of the digestive organs are diverse. A.B. Bongard *et al.* (2019) noted that clinical manifestations of digestive system pathologies are primarily characterised by excessive salivation and diarrhoea. R.A. Didier *et al.* (2021) mention that the onset of abdominal pain, abdominal distension, loss of appetite, vomiting or regurgitation, defecation disorders, constipation, dehydration, and shock can also indicate pathology of the digestive system. According to C. Demars *et al.* (2023), based on the clinical manifestation of symptoms in affected animals, it is possible not only to determine the nature but also to establish the localisation of the pathological process. For diseases of the oral cavity – teeth, jaws, or oesophagus in animals, worsening of biting, chewing, and swallowing processes is observed. Gastritis most commonly leads to vomiting due to irritation or infectious processes. Nevertheless, as asserted by V.I. Woolhead *et al.* (2020), the act of vomiting is also a characteristic symptom of kidney pathology.

C. Demars *et al.* (2023) demonstrated that attention should be paid to faeces, as any deviations from the norm (changes in colour, consistency, frequency of discharge) are also symptoms of intestinal diseases. In the case of gastric bleeding or in the area of the small intestine, faeces may turn black. R.A. Didier *et al.* (2021) described that straining during defecation occurs in cases of inflammation of the mucous membrane in the rectal area or obstruction of its lumen by a foreign body. Abdominal distension in animals can result from the accumulation of gases, fluids, or ingested feed, usually due to a decrease in the activity of muscles responsible for moving feed along the digestive tract. Intestinal stretching is possible, caused by physical obstruction due to the presence of a foreign object, invagination, or overeating.

Furthermore, E. Taillieu *et al.* (2021) note that abdominal pain arises from stretching or inflammation of the peritoneal membranes and can vary in severity. With the development of a painful reaction from the abdominal organs, animals often adopt unnatural body positions (stretching front limbs, lowering the chest to the floor, raising the pelvic limbs). Given the diversity of symptoms that develop in pathological conditions of the digestive system organs, the question of choosing the most informative method for diagnosing digestive tract diseases remains relevant. Unfortunately, there are only isolated studies by Ukrainian scientists on this issue in the literature. For example, I.S. Dekhnych *et al.* (2021) noted in their study that ultrasonography is an effective diagnostic method for diseases of most abdominal organs and is used in veterinary abdominal surgery. Nevertheless, a drawback of this method is the inability to perform the procedure in animals with a high content of adipose tissue around the examined organ.

Considering all of the above, the purpose of this study is to investigate the prevalence and types of pathologies most commonly encountered in the digestive system of small domestic animals and to analyse the informativeness of different diagnostic methods, the results of which may indicate the need for surgical intervention on the stomach or intestines.

## Literature Review

In the study by F. Davoodi *et al.* (2021), attention was drawn to the fact that digestion is crucial not only for providing the body with nutrients but also for maintaining proper fluid and electrolyte (salt) balance in the body. I. Hennink *et al.* (2021), through their investigation of digestive system functions, suggested that they can be categorised into four main categories: digestion, absorption of nutrients, motility (movement along the digestive tract), and faecal elimination.

Diarrhoea is often a sign of digestive system disorders, and R.A. Didier *et al.* (2021) noted in their study that it can have various causes. Watery diarrhoea of large volume is usually associated with hypersecretion, a condition where excess fluid is secreted into the intestine. This can be caused by bacterial infection. Diarrhoea can also occur due to disruptions in absorption, affecting the assimilation of nutrients from the feed. Impaired absorption of hydrolytic breakdown products of feed components can occur due to destructive changes in intestinal cells responsible for absorption. This condition can be caused by various viruses (e.g., canine parvovirus, coronavirus, rotavirus).

C.I. Pratt *et al.* (2014) asserted that the treatment of animals with digestive system pathology should begin with determining the localisation of the pathology in the digestive tract, followed by identifying its cause and prescribing a treatment regimen, selecting the most optimal method for each specific case according to R. Terragni *et al.* (2014). D.A. Elsemore *et al.* (2017) indicated that the visualisation of tissue regeneration in damaged tissues due to digestive system pathologies will expand existing concepts of the course of the healing process and contribute to the search for innovative technologies to activate their full regeneration.

Refusal of food and vomiting in dogs and cats – all of these, according to B.A. Brisson *et al.* (2018), are vivid manifestations of diseases of the digestive system. Conventionally, for targeted diagnosis of this group of diseases in veterinary practice, both general methods (palpation, auscultation) and instrumental methods (radiography, contrast radiography) of examination are used, according to D.A. Elsemore *et al.* (2017). As noted by R. Terragni *et al.* (2014), using these methods, it is not always possible to objectively diagnose the presence of pathology. V.I. Woolhead *et al.* (2020) and F. Davoodi *et al.* (2021) demonstrated that achieving a definitive diagnosis using these methods without detailed visualisation of digestive system structures, such as the oesophagus, stomach, and duodenum, is impossible.

K. Allenspach *et al.* (2015), Q. Cabon *et al.* (2017), and M. Binvel *et al.* (2020) emphasised that decisions regarding the necessity and advisability of surgical or conservative treatment should be based solely on research results. B.J. Rivers *et al.* (2017) noted that during surgical interventions on the stomach or intestines, attention should be focused on optimal tissue regeneration and the formation of a full-fledged scar. Studies on wound repair in the stomach, as indicated by M. Binvel *et al.* (2020), have allowed expanding existing concepts of the healing process and continuing the search for technologies to activate complete tissue regeneration. Various methods for activating regenerative processes, such as cellular technologies, fibrin gel, and platelet-rich plasma, were used for this purpose, possessing anti-inflammatory effects and containing adhesion molecules, cytokines, and platelet growth factors that stimulate reparative and anabolic processes in damaged tissues.

Scientific developments by R.K. Sellon *et al.* (2003) and D.A. Elsemore *et al.* (2017) related to veterinary medicine in Europe, the United States, and Ukraine have immensely enriched information on the diagnosis and methods of surgical interventions in small animals in recent years. R.A. Didier *et al.* (2021) improved diagnostic methods and treatment schemes, performed minimally traumatic surgical interventions, and developed methods to enhance healing and restoration of organ functions after surgical interventions. This is particularly important considering statistical data on the necessity of surgical interventions of varying complexity. As highlighted by B.J. Rivers *et al.* (2017), substantial progress has been made in recent years in surgical interventions on the

stomach and intestines, associated with the widespread adoption of endoscopic research methods. Using these methods, researchers refine the diagnosis with subsequent implementation of conservative or surgical treatment. A.M. Power *et al.* (2021) demonstrated that surgical interventions on the stomach (gastrotomy) in dogs and cats are typically performed in the presence of neoplasms, foreign bodies, or ulcers, allowing the preservation of the animal's life.

According to E. Gibson *et al.* (2020), special diagnostic methods for diseases of the digestive system include ultrasound examination, radiography, and endoscopy. E. Taillieu *et al.* (2021) noted that, when necessary, the most informative method of investigation was chosen, or several types of additional instrumental studies were conducted, as they effectively complement each other. Based on research results, decisions were made regarding the need for further surgical or conservative treatment. According to M. Mikiewicz *et al.* (2019), during surgical interventions on the stomach and intestines, special attention was paid to optimal tissue regeneration and the formation of a complete scar. Investigating the wound repair process in the stomach will allow expanding existing concepts of the healing process and contribute to continuing the search for technologies to activate full tissue regeneration. V.I. Woolhead *et al.* (2020) employed various methods to activate regenerative processes (cellular technologies, fibrin gel, platelet-rich plasma) with anti-inflammatory effects, containing adhesion molecules, cytokines, and platelet growth factors that stimulate reparative and anabolic processes in damaged tissues.

In connection with this, it is relevant to conduct a study on the use of various methods for diagnosing pathologies of the digestive system in dogs and cats to determine their informativeness and subsequently assess the advisability of choosing conservative or surgical treatment methods in each clinical case.

## Materials and Methods

The study was conducted from 2022 to 2023 at the veterinary clinics "Zoolux" in Kyiv and at the Department of Surgery and Pathophysiology named after Academician I.O. Povazhenko, National University of Life and Environmental Sciences of Ukraine. Experiments on animals were conducted following the requirements of the "General Ethical Principles of Conducting Experiments on Animals" approved by the I National Congress on Bioethics (Law of Ukraine No. 3447-IV, 2006), and the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (1986).

The object of the study was 957 animals (dogs – n=586; cats – n=371), aged from 3 months to 12 years. Upon admission to the veterinary clinic, anamnesis collection and clinical examination of sick patients were conducted. Animals with clinical symptoms characterising digestive disorders were selected for further investigation. During the preliminary acquaintance with the animal, the following clinical symptoms were identified: nausea, vomiting, refusal of food, diarrhoea (in some patients with blood and mucus), abdominal pain, abdominal wall tension, and absence of defecation.

To determine the type and exact location of the pathological process, animals underwent ultrasound examination (USG) using the Esaote 70 ultrasound machine (Italy) and a linear probe UST-5512U (Japan) with a frequency of 5-7.5 MHz. Instrumental diagnostics were performed to examine the abdominal organs. Before manipulation, the animal's fur was shaved in the abdominal area because it could degrade the quality of the study, and the animals were fixed in a ventro-dorsal position. Before the ultrasound examination, the skin was treated with an alcohol solution, and gel was applied on a starch basis. During the ultrasound examination, the condition of all organs of the

abdominal cavity was assessed to establish the cause of the deterioration of the patient's general condition. The complex of studies included the following organs: abdominal aorta, pancreas, stomach, liver, gallbladder and its ducts, small and large intestine, urinary bladder, urethra, ureters, spleen, kidneys, and adrenal glands, uterus and ovaries in females, and testicles and prostate in males.

During the diagnosis of diseases of the digestive system organs, survey radiographs were taken. When studying animals, a device for digital image reading, IMAX 1010 (China), was used, and animals were positioned laterally on the right and ventro-dorsally. Patients mainly did not require specific preparation or sedation. In cases where proper positioning for the examination was impossible, sedative drugs such as butorphanol, dexmedetomidine, gabapentin, or trazodone hydrochloride were used according to the instructions, considering the body weight of each patient. Prior to the use of medications, a thorough history was collected, and the overall condition of the patient was assessed. In breeds prone to cardiovascular pathologies, additional echocardiography and blood sampling were conducted to clarify not only the structural pathologies of organs but also the presence of functional disorders.

Endoscopic examinations of animals were performed using the Olympus 2T10 endoscope (Japan), under anaesthesia, with intravenous access using catheterisation of the subcutaneous vein of the forearm. Patient intubation was done with an individualised endotracheal tube, considering the thickness of the animal's nasal septum. Propofol (Lipuro, Germany) was used for anaesthesia as an ultra-short-acting hypnotic. Patient monitoring was conducted using a pulse oximeter (EDAN VE-H100B, China) and physical monitoring of the depth of anaesthesia, with the aid of a stethoscope (Littmann classic III, USA), manual pulse palpation on the femoral and dorsal arteries, pressure in the tail artery region, femoral artery, or on the main brachial artery, counting the respiratory rate per minute, eye position, and the level of masticatory muscle (jaw muscles) relaxation. In specific cases, if the duration of manipulation exceeded 30-45 minutes, inhalation anaesthesia was used for animal anaesthesia. This provided better control of inhalation anaesthesia and reduced the percentage of statistical complications during prolonged anaesthetic support for various types of surgical interventions. The inhalation anaesthetic level was monitored using a capnograph and an air gas analyser, Capnomac ULTIMA (Germany).

Propofol was used for short-term manipulations and for the induction of patient anaesthesia, followed by a transition to inhalation anaesthesia (for patient intubation). The dosage was as follows: for continuous infusion at a rate of 6 mg/kg per hour and for bolus induction, 2-4 mg/kg. When using sedative or tranquillising drugs for premedication, the dosage for induction was kept at a minimum of 2 mg/kg. For sedation of animals, in the absence of contraindications, medetomidine or dexmedetomidine was used at a dose of 0.01 mL/kg for dogs, and butomidor (relaxant) at 0.1-0.4 mg/kg.

## Results and Discussion

During 2022-2023, about two thousand animals were examined to identify the spread of pathologies of the digestive tract in dogs and cats. Based on the results of collecting anamnesis and clinical and instrumental studies, the types of pathologies of the digestive canal organs most often identified in small pets were established. The total number of receptions of animals with surgical pathology and pathology of the digestive system organs is presented in Table 1.

**Table 1**. The total number of animals in which pathologies of the digestive system are detected (on the basis of the veterinary clinic "Zoolux" in Kyiv)

| Total number of animals with surgical pathology, heads | Total number of pathologies of the digestive system, heads | Number of cases of conservative care | Complicated pathologies of the digestive canal (number of operations). | | | | |
|---|---|---|---|---|---|---|---|
| | | | Foreign bodies in the stomach | Neoplasms in the stomach area | Gastric perforations | Gastric erosions | Inflammatory bowel diseases |
| 1863 | 957 | 763 | 170 | 4 | 3 | 14 | 3 |
| 100% | 51.4% | 79.7% | 17.7% | 0.4% | 0.3% | 1.5% | 0.3% |

***Source***: *compiled by the author of this study*

The total number of animals diagnosed with the pathology of the digestive system was 957 animals. Conservative care was provided to seven hundred sixty-three animals, accounting for 79.7%. Gastroscopy was performed on 122 patients with stomach pathology, accounting for 12.7% of the total number of visits. 194 surgical interventions were performed for stomach and intestinal pathology, representing 20.3% of the total number of visits. During gastric surgery, 170 animals (87.6%) had foreign bodies removed, and 4 (2.1%) operations were performed to remove neoplasms. Surgical intervention for gastric perforation was performed in 3 animals (1.5%), for gastric erosion – in 4 animals (7.4%), surgical care for Inflammatory bowel diseases was provided to 3 animals (1.5%). Of the 194 operated animals, four underwent repeated gastric surgeries associated with repeated eating of foreign objects.

During the collection of anamnesis, it was established that most often, the clinics were visited by owners of animals with functional disorders of the digestive system, which occurred due to unbalanced feeding and violations of the conditions of keep. For the most part, in the animals under study, pathologies occurred during injury to the tissues of the gastrointestinal tract due to eating foreign bodies (feeding meat with bones), during games (swallowing rubber, plastic toys, and other foreign bodies), or a distorted appetite (eating inedible objects). Complicated pathologies (194 cases) of the digestive canal organs caused perforation of the stomach wall, erosion, and inflammatory bowel diseases. In a separate group of animals (4 cases), malignant neoplasms in the stomach and intestines were diagnosed, which, like foreign bodies, completely or partially obturated the intestinal lumen.

Some of the examined animals had digestive disorders caused by bacterial or viral factors and irrational use of antibiotics (dysbiosis) applied by animal owners without consulting a doctor. During the analysis of statistical indicators, it was noted that the development of pathology of the digestive system can occur as a concomitant pathology or symptom of other diseases, such as chronic renal pathology and, especially in the terminal stage, with erosive lesions of the digestive system, due to a substantial and prolonged increase in the

concentration of urea in the blood. Results of the study by M. Binvel *et al.* (2017) highlighted that the absorption process can be disrupted due to any induced defect that restricts the intestinal ability to absorb fluid or disorders in the secretion of pancreatic juice necessary for efficient digestion. In rare cases, newborn pups may have diarrhoea, as noted by R.A. Didier *et al.* (2021), when they are fed milk because they are unable to digest lactose. During diarrhoea, shock can occur due to dehydration and an electrolyte (salt) imbalance.

According to the results of the analysis of the obtained statistical data, it was determined that among all surgical pathologies, 52% are diseases that occur in the organs of the digestive system. Among these diseases, in 25% of cases, animals needed surgical treatment. The following instrumental diagnostic methods were used to clarify the diagnosis of surgical pathology of the digestive system in animals: X-ray examination (Figs. 1-11), ultrasound diagnostics (Fig. 12), and esophagogastroduodenoscopy (Fig. 13).

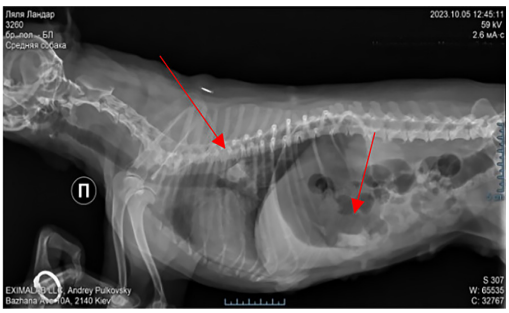

**Figure 1.** Overview image of the dog's thoracic and abdominal organs, lateral projection
***Note:*** *arrows indicate multiple foreign bodies in the thoracic oesophagus and stomach of the dog. The examination was conducted without the use of contrast agents*
***Sourse****: developed by the author*

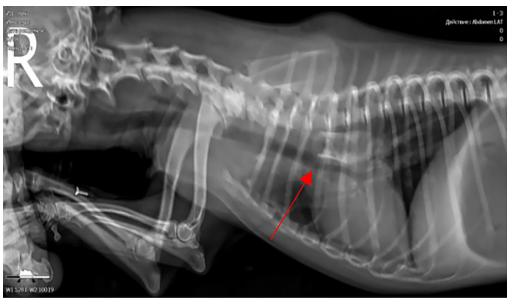

**Figure 2.** Overview image of the chest organs, lateral projection
***Note:*** *the arrow indicates a foreign body in the thoracic oesophagus*
***Sourse****: developed by the author*

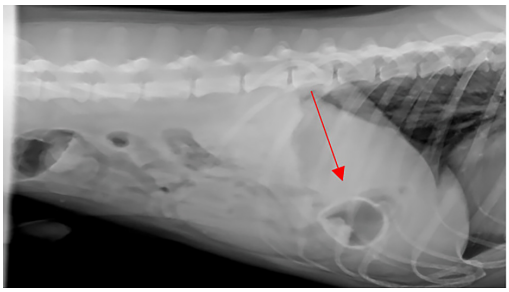

**Figure 3.** Overview image of the abdominal organs, lateral projection
***Note:*** *the arrow marks a hollow foreign object in the patient's stomach*
***Sourse****: compiled by the author*

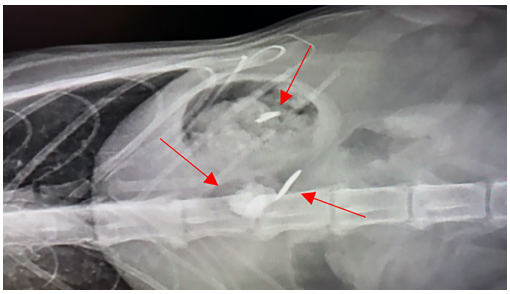

**Figure 4.** Overview image of the abdominal organs, front projection
***Note:*** *arrows indicate multiple foreign bodies and their fragments in the dog's stomach*
***Sourse****: compiled by the author*

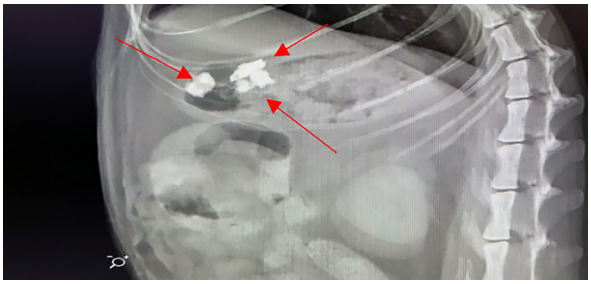

**Figure 5.** Overview image of the abdominal organs, lateral projection
***Note:*** *arrows indicate multiple X-ray contrast foreign bodies in the animal's stomach cavity*
***Sourse****: compiled by the author*

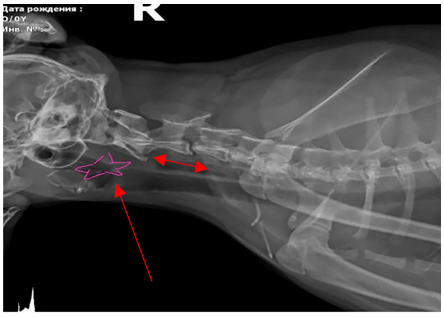

**Figure 6.** Overview image of the cervical oesophagus, lateral projection
***Note:*** *the red arrow indicates a foreign body, the contours of which are circled in pink to improve visualisation, and the air-expanded lumen of the oesophagus is also visible (marked with a red two-sided arrow)*
***Sourse****: compiled by the author*

**Figure 7.** Patient after the removal of the alien body
***Note:*** *cat after removal of the side body (star) in the area of the oesophagus, which was previously visualised in Fig. 6*
***Sourse****: developed by the author*

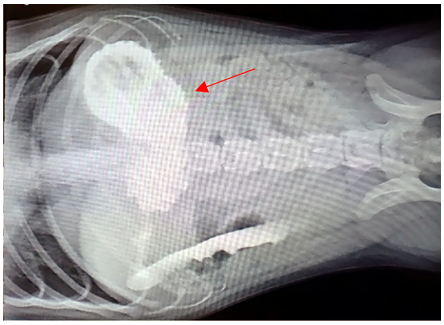

**Figure 8.** Overview image of the abdominal cavity, ventro-dorsal projection
***Note:*** *X-ray examination with a contrast agent (barium sulfate) identified a foreign body (marked with an arrow) in the stomach without signs of mechanical obstruction*
***Sourse****: compiled by the author*

**Figure 9.** Foreign body after removal from the animal's stomach
***Note:*** *removed foreign body visualised (marked with a red arrow)*
***Sourse****: compiled by the author*

According to the results of the examination, it was established that X-ray examination is especially effective in the presence of X-ray contrast structures (foreign objects) in the digestive organs, such as metals, stones, bones (and other objects that are more noticeable in their density when using this diagnostic method). In particular, C.I. Pratt *et al.* (2014) and A.M. Power *et al.* (2021) emphasised that using this method is not 100% effective because not all foreign bodies are X-ray contrast or denser than body tissues. In the example of clinical cases, they showed the informative value of various diagnostic methods for surgical pathology of the gastrointestinal tract of animals.

Example 1. Cat Arnold, Abyssinian breed, age 3 years. From the history data, it was determined that the animal had swallowed a toy. Based on the diagnostic investigation results, it was found that the removal of the foreign body through endoscopy was not possible due to the risk of perforation during manipulation and the presence of feed masses in the stomach. Therefore, the decision was made to perform upper midline laparotomy along the white line and gastrotomy, followed by the removal of feed masses and the foreign body. Lembert-Schmieden sutures were applied to the stomach wall using monofast 4.0 suture material with a cutting needle. The abdominal cavity was flushed with sterile physiological saline at a volume of 200 mL/kg. The abdominal wall was sutured with a nodular suture using PDS 4.0 thread, the subcutaneous tissue with a continuous suture, and the skin with an intradermal suture.

In cases where the presence of foreign bodies in the gastrointestinal tract was confirmed, esophagogastroscope or esophagogastroduodenoscopy was not always performed on animals. According to the research results, linear foreign bodies that passed into the small intestine and caused its corrugation were not subjected to endoscopic removal due to the high risks of perforation, which could lead to the development of peritonitis.

Example 2. Luna dog, Labrador breed, 7 months old. Owners presented to the clinic with complaints of the animal's attempts to vomit and refusal to eat. There was suspicion of ingestion of a foreign body (they allowed chewing on ribs). To refine the diagnosis, a radiographic examination was performed, revealing the foreign body. A radiographic examination was conducted in two projections (lateral on the right side and ventro-dorsally) (Fig. 10, 11).

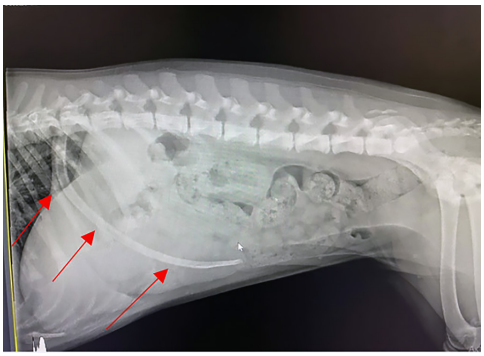

**Figure 10.** Overview image of the dog's abdominal organs (lateral projection on the right side)
***Note:*** *the red arrows indicate the extent of the foreign body (pig rib) in the dog's stomach*
***Sourse****: developed by the author*

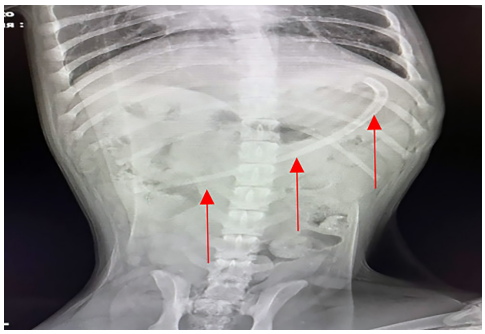

**Figure 11.** Overview radiograph of the abdominal organs (ventro-dorsal projection)
***Note:*** *the red arrows on the image indicate the extent of the foreign body in the dog's stomach*
***Sourse****: developed by the author*

In many cases, radiographic examination is highly informative and allows choosing the most effective surgical treatment method for animals with foreign bodies in the digestive system. In addition to radiographic examination, ultrasound diagnostics (US) were used to detect linear foreign bodies in the stomach and accompanying complications, such as thickening of the stomach wall due to inflammation. They diagnosed perforations in the stomach and intestine caused by a foreign body, the presence of fluid in the abdominal cavity, and neoplasms in the stomach, protruding into the pyloric region and causing obstruction of gastric outflow. This type of study provided information on the shape, size, echogenicity, and location of the foreign body, identified signs of thickening of the stomach and intestinal walls, and determined the sizes of regional lymph nodes. It is worth noting that ultrasound also has a drawback.

With a large amount of feed masses in the stomach, the informative value of this research method decreases. The results described are consistent with those of other researchers. For example, E. Gibson *et al.* (2020) indicated that the presence of feed masses or gases in the stomach makes ultrasound examination of the digestive tract less informative. Therewith, J.S. Mattoon *et al.* (2020) demonstrated the usefulness of ultrasound examination, especially in the diagnosis of neoplasia in the stomach of dogs. The results of the ultrasound corresponded to the localisation of the tumour obtained by other diagnostic methods. The authors stated that the clinical picture of ultrasound is difficult to interpret because benign gastric polyps are rarely found in dogs, and most of them are discovered accidentally. Despite the fact that ultrasound examination allowed assuming the presence of a polyp in the gastric mucosa, the final diagnosis can be established based on histopathological examination.

V. Barberet *et al.* (2008) used advanced ultrasound diagnostic methods in their studies. Using transgastric ultrasound, they examined organs located centrally, such as the pancreas. These organs can be well examined transgastrically using endoscopic ultrasound, without interfering with the upper intestinal segments, as is usually the case with transabdominal ultrasound. It is necessary to consider factors that can affect the quality of the study. Parameters that negatively affect the visibility of organs are air or feed in the digestive organs. According to A. Otomo *et al.* (2020), these parameters positively influenced the visibility of the pancreas and duodenum, likely related to the different body positions used for the study. E. Gibson *et al.* (2020) expressed that a retrospective analysis of cases involving simultaneous abdominal US and endoscopy to assess the presence of anomalies in the stomach walls, foreign bodies, tumour localisation, and changes between the two examinations is more informative. Sonography and endoscopy are useful for diagnosing gastric neoplasia, with endoscopy being a more precise method for detecting gastric neoplasia. However, sonography can raise clinical suspicion of gastric neoplasia and provide a less invasive way to gather information before endoscopy. The presence of gas or fluid in the stomach lumen limited the diagnostic capabilities of sonographic evaluation.

Example 3. Dog Nicole, Labrador breed. During the history collection, it was established that at 9:00 in the morning, the owners observed the dog playing with a ball, and within 5 minutes, the toy was not found. Therefore, the decision was promptly made to seek veterinary care. The animal underwent an ultrasound examination of the abdominal cavity to determine the presence of a foreign body in the stomach (Fig. 12). After confirming the presence of the foreign body, endoscopic removal was performed (Fig. 13).

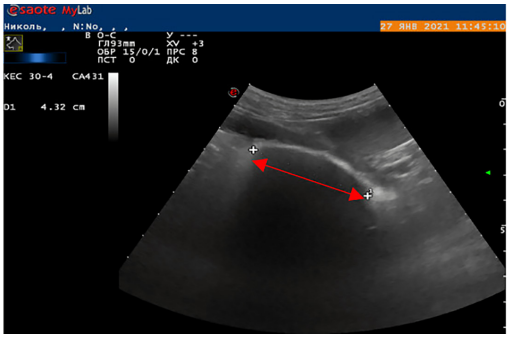


**Figure 12.** Ultrasonographic diagnosis of the foreign body in the stomach of the dog Nicole
***Note:*** *the red arrow indicates the extent of the foreign body in the patient's stomach*
***Sourse****: developed by the author*

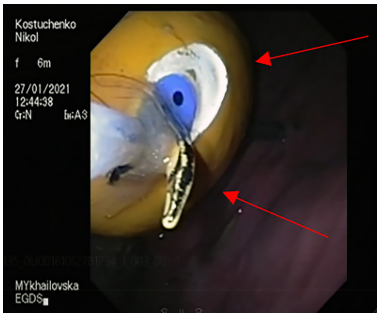


**Figure 13.** Endoscopic removal of the foreign body from the stomach of the dog Nicole
***Note:*** *the red arrows indicate the foreign body in the stomach, which was removed using endoscopy (esophagogastroduodenoscopy)*
***Sourse****: developed by the author*

Esophagogastroduodenoscopy (EGD) is one of the most effective diagnostic methods for detecting foreign bodies in the oesophagus, stomach, and duodenum. Through EGD (with the availability of special equipment), the removal of a foreign body from the stomach and biopsy for oesophagal strictures or mucosal trauma and neoplasms in the gut were conducted. This diagnostic method allows for the final diagnosis to be established, as supported by the findings of E. Gibson *et al.* (2020) and helps determine the need for surgical intervention on the stomach and intestines.

Endoscopic examination was also used to diagnose and perform therapeutic balloon dilation of oesophageal strictures. According to the results of the study by R.K. Sellon *et al.* (2003), who conducted endoscopic balloon dilation of benign oesophageal structures in animals, regurgitation is the most common clinical symptom observed on average 4 weeks before the dilation of the oesophageal segment to the stenotic zone. Oesophageal perforation was the only serious complication and occurred in one patient. I. Hennink *et al.* (2021) indicated that this manipulation should be performed under anaesthesia, preceded by necessary diagnostics, to determine the presence of systemic disorders and reduce the patient's anaesthetic risk. D.A. Elsemore *et al.* (2017) emphasised the importance of examining the stomach wall throughout its entire area to select the treatment strategy based on the patient's overall condition and considering all aspects of the history and results of previous investigations. However, M. Pinheiro Souto *et al.* (2023) demonstrated that a drawback of this diagnostic method is the need for anaesthesia, with contraindications being the occurrence of perforation in the stomach or intestines. The risk of perforation may arise during the examination or attempted removal of a foreign body and in the presence of free fluid or diaphragmatic or hiatal hernias.

R.K. Sellon *et al.* (2003) noted that the diagnosis and treatment of diseases of the digestive system organs in dogs and cats increasingly rely on the collection and interpretation of biopsy samples from the mucous membrane obtained endoscopically from one or more areas of the digestive system. The physician's goal is to distinguish between normal and affected tissues, characterise the nature and severity of their changes, and make an accurate diagnosis based on morphological or etiological features,

facilitating prognosis formation and appropriate therapy selection. The application of histopathological methods allows for the rapid diagnosis of adenocarcinomas or alimentary lymphomas. Conversely, the interpretation of inflammatory changes in the mucous membrane proved much more complex.

A. Zamprakou *et al.* (2016) indicated that veterinary clinics often create surgical intervention protocols based on generally accepted rules and modern trends in veterinary medicine development. The authors compared various suture materials with Monocryl, which were selected according to surgical recommendations and research results. Nevertheless, the use of Monofilament Maxon contributes to obtaining much more reliable knots. Sliding knots with Monocryl (poliglecaprone 25) were similar, square knots were three times less reliable than those tied with PDS-2 monofilament, and Braided Dexon-Plus suture material was stronger than Monocryl for sliding knots. Therewith, braided Vicryl did not significantly differ from Monocryl.

I. Hennink *et al.* (2021) demonstrated that the mechanical properties of the Monocryl knot are identical to those of Vicryl. Still, they are less reliable than those in other synthetic absorbable monofilament threads (Maxon) and respective alternatives (PDS). These conclusions and other characteristics, such as degradation rate, should be used to determine the application of Monocryl. The positive aspect of using Monocryl is the lower trauma volume during surgery and the use of appropriate biological preparations that stimulate the activation of regenerative activity in the damaged area of the organ or tissue under investigation.

Therefore, the use of modern diagnostic methods in most cases allows for a clear diagnosis and resolution of the problem without surgical intervention or the most effective implementation of surgery on the stomach and intestines with minimal possible complications. The choice of the method depends on the situation and the reason for the surgical intervention.

## Conclusions

Pathologies of the digestive system are quite often recorded in dogs and cats. The sharp increase in their incidence is attributed to the irresponsible attitude of owners who violate the conditions and rules of keeping and feeding their pets. It was established that among animals with surgical pathology, 51.4% had diseases of the gastrointestinal tract, of which 25% of animals required surgical treatment. The most common surgical intervention was performed for the removal of foreign bodies (87.5%), erosion of the stomach (7.4%), diagnosis of neoplasms (2.1%), and stomach perforation (1.5%). Instrumental, radiographic, and ultrasound methods were used for the diagnosis of gastrointestinal tract pathologies, and their informativeness was determined. The results of these methods were considered when choosing the surgical treatment method for sick animals.

High efficiency of radiographic examination was observed in the presence of radiopaque structures in the digestive organs, which are more visible in this diagnostic method due to their density. The drawback of this method is that not all foreign bodies are radiopaque or denser than the body's tissues. Ultrasound diagnostics allows the detection of linear foreign bodies in the stomach and complications, such as thickening of its wall due to inflammation. This method is also informative for perforations in the stomach and intestine caused by a foreign body, the presence of fluid in the abdominal cavity, and tumours in the stomach protruding into the pyloric part, causing obstruction of gastric outflow.

The application of ultrasound allowed obtaining information about the shape, size,

echogenicity, and location of foreign bodies and identifying signs of thickening of the stomach and intestinal walls. The drawback of this method is a decrease in informativeness due to the large volume of feed masses and gases in the stomach of the examined animal. Clinical cases demonstrated the algorithm for examining animals with digestive system pathologies and the use of various diagnostic methods, the results of which allowed choosing the most optimal method of surgical treatment. It should be noted that in some cases, it is possible to avoid invasive treatment methods for pathological processes in the digestive system. These include minimally invasive surgical intervention using non-invasive methods such as gastroscopy, which shortened the patient's recovery process after eliminating the cause of the pathological process. The correct choice of the instrumental diagnostic method in each specific case, considering the data of the history and clinical examination of the animal, allowed selecting the most effective method of surgical intervention, reducing tissue trauma, and shortening the recovery period for patients.

In the future, a study on the use of cell-regenerative therapy to activate the process of reparative regeneration and the formation of a complete scar on the stomach to reduce postoperative complications is planned.

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## Conflict of Interest

None.

## References

[1] Allenspach, K. (2015). Diagnosis of small intestinal disorders in dogs and cats. *Clinics in Laboratory Medicine*, 35(3), 521-534. doi: 10.1016/j.cll.2015.05.003.

[2] Barash, N.R., Lashnits, E., Kern, Z.T., Tolbert, M.K., & Lunn, K.F. (2022). Outcomes of esophageal and gastric bone foreign bodies in dogs. *Journal of Veterinary Internal Medicine*, 36(2), 500-507. doi: 10.1111/jvim.16383.

[3] Barberet, V., Schreurs, E., Rademacher, N., Nitzl, D., Taeymans, O., Duchateau, L., & Saunders, J.H. (2008). Quantification of the effect of various patient and image factors on ultrasonographic detection of select canine abdominal organs. *Veterinary Radiology and Ultrasound*, 49(3), 273-276. doi: 10.1111/j.1740-8261.2008.00365.x.

[4] Binvel, M., Poujol, L., Peyron, C., Dunie-Merigot, A., & Bernardin, F. (2017). Endoscopic and surgical removal of oesophageal and gastric fishhook foreign bodies in 33 animals. *Journal of Small Animal Practice,* 59(1), 45-49. doi: 10.1111/jsap.12794.

[5] Bongard, A.B., Furrow, E., & Granick, J.L. (2019). Retrospective evaluation of factors associated with degree of esophagitis, treatment, and outcomes in dogs presenting with esophageal foreign bodies (2004-2014): 114 cases. *Journal of Veterinary Emergency and Critical Care,* 29(5), 528-534. doi: 10.1111/vec.12875.

[6] Brisson, B.A., Wainberg, S.H., Malek, S., Reabel, S., Defarges, A., & Sears, W.C. (2018). Risk factors and prognostic indicators for surgical outcome of dogs with esophageal foreign body obstructions. *Journal of the American Veterinary Medical Association*, 252(3), 301-308. doi: 10.2460/javma.252.3.301.

[7] Cabon, Q., Carmel, E.N., & Cabassu, J. (2017). Cholecystopexy and pericardial pseudocyst removal in a dog with a congenital peritoneopericardial diaphragmatic hernia. *Journal of the American Animal Hospital Association*, 53(5), 270-276. doi: 10.5326/JAAHA-MS-6457.

[8] Davoodi, F., Valizadeh, Y., Raisi, A., Mozaffari, N., & Gohardehi, K. (2021). Esophageal foreign body removal through gastrotomy using a covault hook in a female dog: A case report. *Veterinary Research Forum: An International Quarterly Journal*, 12(3), 387-389. doi: 10.30466/vrf.2020.128301.2967.

[9] Dekhnych, I.S., Zavaliy, M.F., & Rakitina, A.I. (2021). Diagnostic sonography in animal abdominal surgery. *Bulletin of Poltava State Agrarian Academy*, 1, 241-249. doi: 10.31210/visnyk2021.01.30.

[10] Demars, C., Boland, L., & Minier, K. (2023). Surgical removal of intestinal foreign bodies using a laparotomy-assisted endoscopic approach in dogs and cats and comparison with enterotomy. *Journal of Small Animal Practice*, 64(1), 43-50. doi: 10.1111/jsap.13550.

[11] Derré, M.G., Findji, L., McLauchlan, G., & Guilherme, S. (2022). Surgical treatment of a circumferential oesophagogastric mass associated with a peritoneopericardial diaphragmatic hernia in a Maine Coon cat. *JFMS Open Reports*, 8(1), article number 20551169221090449. doi: 10.1177/20551169221090449.

[12] Didier, R.A., Oliver, E.R., Rungsiprakarn, P., Debari, S.E., Adams, S.E., Hedrick, H.L., Adzick, N.S., Khalek, N., Howell, L.J., & Coleman, B.G. (2021). Decreased neonatal morbidity in 'stomach-down' left congenital diaphragmatic hernia: Implications of prenatal ultrasound diagnosis for counseling and postnatal management. *Ultrasound in Obstetrics & Gynecology: The Official Journal of The International Society of Ultrasound in Obstetrics and Gynecology*, 58(5), 744-749. doi: 10.1002/uog.23630.

[13] Elsemore, D.A., Geng, J., Cote, J., Hanna, R., Lucio-Forster, A., & Bowman, D.D. (2017). Enzyme-linked immunosorbent assays for coproantigen detection of Ancylostoma caninum and Toxocara canis in dogs and Toxocara cati in cats. *Journal of Veterinary Diagnostic Investigation: Official Publication of the American Association of Veterinary Laboratory Diagnosticians,* 29(5), 645-653. doi: 10.1177/1040638717706098.

[14] European convention for the protection of vertebrate animals used for experimental and scientific purposes. (1986). Retrieved from http://zakon4.rada.gov.ua/laws/show/994_137.

[15] Gibson, E., Culp, W., Mayhew, P., Runge, J.J., Peterson, L.C., Balsa, I.M., & Kim, S.Y. (2020). Laparoscopic-assisted gastrotomy for foreign body retrieval in four dogs. *Veterinary Record Case Reports*, 8(2), article number e000966. doi: 10.1136/vetreccr-2019-000966.

[16] Hennink, I., Düver, P., Rytz, U., Meneses, F., Moioli, M., Adamik, K.N., & Kovačević, A. (2021). Case report: Unusual peritoneopericardial diaphragmatic hernia in an 8-month-old german shepherd dog, associated with a pericardial pseudocyst and coexisting severe pericardial effusion resulting in right-sided heart failure. *Frontiers in Veterinary Science*, 8, article number 673543. doi: 10.3389/fvets.2021.673543.

[17] Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty". (2006, February). Retrieved from https://zakon.rada.gov.ua/laws/show/3447-15#Text.

[18] Lishchuk, S.G., Dobrovolsky, V.A., & Smoljak, V.V. (2023). Analysis of the dynamics and establishment of trends regarding the frequency of disease due to pathology of various dogs organ systems. *Podilian Bulletin: Agriculture, Engineering, Economics: Veterinary Sciences,* 1(38), 179-184. doi: 10.37406/2706-9052-2023-1.27.

[19] Mattoon, J.S., Sellon, R.K., & Berry, C.R. (2020). *Small animal diagnostic ultrasound*. Chatswood: Elsevier Health Sciences.

[20] Mikiewicz, M., Paździor-Czapula, K., Gesek, M., Lemishevskyi, V., & Otrocka-Domagała, I. (2019). Canine and feline oral cavity tumours and tumour-like lesions: A retrospective study of 486 cases (2015-2017). *Journal of Comparative Pathology*, 172, 80-87. doi: 10.1016/j.jcpa.2019.09.007.

[21] Murakami, M., Heng, H.G., & Sola, M. (2022). CT features of confirmed and presumed gastric wall edema in dogs. *Veterinary Radiology & Ultrasound: The Official Journal of the American College of Veterinary Radiology and the International Veterinary Radiology Association*, 63(6), 711-718. doi: 10.1111/vru.13123.

[22] Otomo, A., Singh, A., Valverde, A., Beaufrere, H., Mrotz, V., Kilkenny, J., & Linden, A.Z. (2019). Comparison of outcome in dogs undergoing single-incision laparoscopic-assisted intestinal surgery and open laparotomy for simple small intestinal foreign body removal. *Veterinary Surgical*, 48(1), 83-90. doi: 10.1111/vsu.13131.

[23] Pinheiro Souto, M., dos Santos Ribas, P., Monteiro, F.D.O., Corrêa da Silva, A., Monteiro Barroso, J.P., Jales Gurgel, H., Bueno Borges, L.P., & Maia Teixeira, P.P. (2023). Gastroesophageal foreign bodies in dogs – endoscopy and surgical removal. *Acta Scientiae Veterinariae*, 51. doi: 10.22456/1679-9216.129590.

[24] Power, A.M., Diamond, D.W., & Puetthoff, C. (2021). Laparotomy-assisted transoral foreign body retrieval in small animals: 10 cases (2018-2020). *Topics in Companion Animal Medicine*, 42, article number 100504. doi: 10.1016/j.tcam.2020.100504.

[25] Pratt, C.L., Reineke, E.L., & Drobatz, K.J. (2014). Sewing needle foreign body ingestion in dogs and cats: 65 cases (2000-2012). *Journal of the American Veterinary Medical Association*, 245(3), 302-308. doi: 10.2460/javma.245.3.302.

[26] Rivers, B.J., Walter, P.A., Johnston, G.R., Feeney, D.A., & Hardy, R.M. (2017). Canine gastric neoplasia: Utility of ultrasonography in diagnosis. *Journal of the American Animal Hospital Association*, 33(2), 144-155. doi: 10.5326/15473317-33-2-144.

[27] Sellon, R.K., & Willard, M.D. (2003). Esophagitis and esophageal strictures. *The Veterinary Clinics of North America. Small Animal Practice*, 33(5), 945-967. doi: 10.1016/s0195-5616(03)00075-5.

[28] Taillieu, E., Chiers, K., Amorim, I., Gärtner, F., Maes, D., Van Steenkiste, C., & Haesebrouck, F. (2022). Gastric Helicobacter species associated with dogs, cats and pigs: Significance for public and animal health. *Veterinary Research*, 53(1), article number 42. doi: 10.1186/s13567-022-01059-4.

[29] Terragni, R., Vignoli, M., van Bree, H.J., Gaschen, L., & Saunders, J.H. (2014). Diagnostic imaging and endoscopic finding in dogs and cats with gastric tumors: A review. *Schweizer Archiv fur Tierheilkunde*, 156(12), 569-576. doi: 10.1024/0036-7281/a000652.

[30] Woolhead, V.L., Whittemore, J.C., & Stewart, S.A. (2020). Multicenter retrospective evaluation of ileocecocolic perforations associated with diagnostic lower gastrointestinal endoscopy in dogs and cats. *Journal of Veterinary Internal Medicine*, 34(2), 684-690. doi: 10.1111/jvim.15731.

[31] Zamprakou, A., Berg, C., Strizek, B., Müller, A., Heydweiller, A., Gembruch, U., & Geipel, A. (2016). Morgagni hernia presenting with massive pericardial effusion and ascites: Prenatal management by thoraco-amniotic shunting and fetal endoscopic tracheal occlusion (FETO) and review of the literature. *Archives of Gynecology and Obstetrics*, 294(5), 953-958. doi: 10.1007/s00404-016-4103-0.

# Поширеність та методи діагностики хірургічної патології апарату травлення тварин

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**Анотація.** Актуальність дослідження зумовлена стрімким зростанням чисельності дрібних домашніх тварин з хірургічною патологією органів апарату травлення, що потребує ефективної діагностики та хірургічного втручання. У зв'язку з цим, мета цієї роботи полягала у дослідженні поширеності різних видів хвороб органів травного каналу в дрібних домашніх тварин, а також у визначенні найінформативніших методів їх діагностики. Провідним підходом у роботі було порівняння інформативності результатів досліджень хворих тварин, а саме: загального (збір анамнезу, огляд, пальпація) та спеціальних (ультразвукова діагностика органів черевної порожнини, ендоскопія травного каналу, рентгенографія). Визначено та проаналізовано переваги та недоліки різних спеціальних діагностичних методів дослідження, що дозволяє комплексно оцінити показання та протипоказання до їх застосування у клінічній ветеринарній практиці. Встановлено, що з 1863 тварин, які звернулися до ветеринарної клініки у 957 тварин діагностовано патології травного тракту різного генезу. Хворим тваринам у 79,7 % випадків (763 голів) надано консервативну допомогу. У 194 (20,3 %) тварин діагностовано ускладнені патології, що потребували оперативного лікування. Серед останніх виявлено у 170 (17,7 %) тварин – сторонні тіла у шлунку, в 14 (1,5 %) – ерозії шлунку, у 4 (0,4 %) – новоутворення, у 3 (0,3 %) – перфорації і у 3 (0,3 %) – запальні захворювання. Використання ультразвукової діагностики дозволяє виявляти лінійні сторонні тіла в шлунку, наявність супутніх ускладнень (перфорації шлунка чи кишечнику стороннім тілом). Рентгенологічне

дослідження ефективне за наявності в якості сторонніх предметів рентген-контрастних структур (метали, каміння, кістки). Матеріали статті становлять практичну цінність як для науковців, так і для практикуючих лікарів, надаючи можливість використовувати нові інструментальні методи діагностики для з'ясування доцільності хірургічних маніпуляцій на шлунку та кишечнику в собак і котів

**Ключові слова:** езофагогастродуоденоскопія; ультразвукова діагностика; рентгенологічне дослідження; рівень плато; дивертикул; кила; запальне захворювання кишечнику

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# Determination of the time of death of a domestic cat by measuring the area of a wet spot

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**Abstract.** Animal cruelty is a criminal offence under the current legislation of Ukraine. When investigating criminal proceedings related to animal cruelty, including domestic cats, it is often necessary to establish the time of death. Therefore, the development of new methods for determining it and improving existing ones is extremely relevant. The purpose of the study was to establish the relationship between the obtained values of wet spot area indicators and the time of death. To achieve this goal, the authors propose a new method for determining the age of death of domestic cats based on the results of measuring the area of a wet spot obtained from skeletal muscle tissue samples of corpses (Shkundia method). To do this, samples of muscle tissue were taken from the corpses of cats, a wet spot preparation was obtained by pressing and its area was calculated to establish a correlation between the values of this indicator and the time that has passed since death. The study of the obtained indicators established that these values are constant, and the features of fluctuations in the values of this indicator with the time elapsed since the death of the animals were established and shown graphically. Using a number of statistical methods, the absence of dependence of wet spot area indicators on the muscles from which samples were taken for research and animal breeds was determined. The nature of changes in the values of the wet spot area indicator depending on time is established and the features of their fluctuations in different

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periods during 27 days from the moment of animal death were explained. Reference values of these indicators depending on the time elapsed since the death of animals are presented, which can be used by forensic veterinary experts in practical work to determine the time of the occurrence of death of domestic cats. Due to a number of advantages, this method can be widely implemented in the practice of forensic veterinary medicine

**Keywords:** forensic veterinary examination; postmortem interval; *Felis silvestris catus*; Shkundia method; muscle tissue

## Introduction

One of the problems that hinders the development of a modern legal society is the cruelty to animals. Cruelty to animals is defined as the actions of certain persons towards animals that are committed intentionally and lead to their injury or death, as defined in Article 299 of the Criminal Code of Ukraine (Criminal Code of Ukraine, 2001). W. Huang *et al.* (2020), I. Yatsenko & O. Parilovsky (2022) report that quite often domestic cats (*Felis silvestris catus*) appear as the object of abuse, which are among the most common pets. There are known cases of killing cats as a result of the use of weapons, various tools or items, as noted by D. Doukas (2022). C.M. Romano *et al.* (2020) report that crimes committed by throwing cats from a height, strangling, poisoning, torturing, and targeting dogs on cats are not uncommon, as a result of which the animals die. In the vast majority of cases related to animal cruelty, it is the cases of their death that are considered and forensic veterinary examinations of corpses are appointed. In the process of investigating such crimes, there are cases when a body is discovered and sent for examination after a certain period of time after death, which raises a number of questions for the investigation. J. Viciano *et al.* (2022) considered one of the most common issues – determination of the time of animal death. The need to determine the time of death of cats may be related to the need to obtain information necessary for inquiry and investigation about the date of death of the animal, with clarification of the correspondence in time of the testimony of persons involved in the investigation and trial. Such data can also refute or confirm the alibi of one of the participating parties. In addition, even in cases not related to criminal proceedings, the determination of the time of death is necessary to confirm or deny the reliability of anamnestic data provided for conducting a pathologic and anatomical autopsy of an animal. The determination of the time of death, therefore, is one of the main issues to be resolved by a forensic veterinary expert based on the results obtained after performing a forensic veterinary autopsy, and during the inspection of the scene or the discovery of a corpse, if the expert participates in these activities. I. Yatsenko & O. Parilovsky (2022) note that such questions are often raised before a forensic veterinary expert in decisions on the appointment of forensic veterinary examinations.

To solve the issue of establishing the time of death of animals, in particular, domestic cats, it is of great importance to choose the method that allows this to be done. Methods for determining the time and duration of death are based on recording the regularities of the dynamics of cadaveric phenomena, the time of residual vital activity of various body tissues in the first hours after death, the nature of biochemical changes that develop in the tissues of the corpse, in particular, according to E.K. Hryhorian (2019).

Some of these methods help to determine the duration of death directly, some – indirectly, for example, by determining the time of stay of the corpse in certain conditions, for example, in water, soil, etc. To more accurately determine the time of death of animals, it is also necessary to consider natural and other factors that affect the condition of the corpse. S.J. Jeong *et al.* (2020), in particular, note that such factors can be climatic components, that is, temperature, humidity and air velocity, the impact of natural phenomena (rain, snowfall, etc.), the presence of a corpse in the ground, reservoirs, premises, under the rubble of buildings, death in fires and other man-made accidents and catastrophes. Damage to corpses by representatives of fauna and flora also has a certain impact. All these conditions can accelerate or slow down the development of cadaveric phenomena, that is, the criteria by which the time of death is determined. Of great importance is also the time interval from the moment of death, during which each of the methods can provide information that can be correlated with the time of its occurrence. Most methods are suitable for use only for short periods of time after death.

The purpose of the study was to correlate the obtained values of the wet spot area indicator with the time of death of a domestic cat (*F. silvestris catus*). To achieve the goal, the following tasks were set: to take material from the corpses of cats whose time of death is known; to examine tissue samples of cat corpses using the wet spot method; to conduct statistical processing of the obtained data, with the help of which to prove their reliability; to find out the patterns of changes in the studied indicators relative to the time that has passed since the onset of death and the presence of correlation.

## Literature Review

To date, quite a few methods have been developed for determining the time of death. Yu.V. Sarkisova (2021), I.G. Savka *et al.* (2021), V.K. Sokol (2022) note that the vast majority of these methods were invented by scientists in the field of forensic medicine. These methods are primarily intended for the study of objects such as human corpses. E.K. Hryhorian (2019), Yu.V. Sarkisova & S.M. Malanchuk (2020), M. Merck & D. Miller (2013) note that only some of these methods are used by veterinary doctors in forensic veterinary practice in the case of examination of animal corpses. The most common methods in use are classical methods. These include, for example, the visual and palpation method, which involves assessing the state of development of cadaveric changes by examination and palpation. Thermometry is widely used, as noted by S.J. Jeong *et al.* (2020), the essence of which is to measure the temperature of the corpse at certain points. H. Muggenthaler *et al.* (2012) emphasise the need to correlate the obtained values with tabular data reflecting the dynamics of postmortem cooling for animals of a certain species, weight, etc. A variation of this method is to measure the temperature of the place of death of an animal or person. Of the biochemical methods used, as reported by G. Piegari *et al.* (2023), studies of protein concentrations in cadaveric tissues; a number of authors, such as M.T. Ave *et al.* (2021), J. Garland *et al.* (2020), and M. Nioi *et al.* (2021), propose to investigate changes in the vitreous body in the eyeballs; some other methods have also been developed. There are a number of methods that are based on image analysis. E. Watson & J.Kr. Baucom (2020) suggest analysing data obtained using computed tomography, and M. Zhang (2022) – magnetic resonance imaging. There is also an elastographic method. J. Byrd & L. Sutton (2021) recommend the widespread use of forensic entomology.

According to S. Matuszewski (2021), this method consists of estimating the time after death, which can often coincide with the time of

colonisation of cadaver tissue by entomofauna. Such a study is based on determining the activity and number of insects that contaminate the corpse, and determination of the age and stage of development of these insects. M. Heba El-Sayed *et al.* (2023) propose to apply DNA studies in the aspect of assessing the degree of its degradation over the time that has passed since the onset of death, and to identify the species affiliation of the remains of an animal's corpse. A number of methods consist in studying the microstructure of cadaver tissues using light, luminescent, and electron microscopy. A method for determining the time of death based on the results of microscopic examination, as reported by V.K. Sokol (2022), is based on visual and morphometric assessment of tissue-level changes caused by tertiary cadaveric changes, namely autolysis and putrefaction. The development of destructive changes directly depends on the time of death. In the field of forensic medical examination today, in addition to histological examination, some of its varieties are used. Thus, Yu.V. Sarkisova & S.M. Malanchuk (2020) suggest using the spectropolarimetric method, the essence of which is to establish the time of death in a long-term interval by analysing data obtained by spectral-selective laser-induced fluorescence microscopy of tissue sections, and O.Iu. Lytvynenko (2022) – reconstruction of the polycrystalline structure of tissue sections, which consists in determining the relationships between changes of values that characterise the distribution of values of the degree of crystallisation of tissue sections of various organs and the time of death.

D.Yu. Shkundia *et al*. (2023) proposed a method for determining the time of death by measuring the area of a wet spot obtained from cadaver tissue samples. In turn, the literature, both Ukrainian and foreign, devoted to the problem of determining the time of death, does not reflect the features of such studies of cadaveric material, especially in the aspects of studying corpses of different types and breeds of animals; their gender and age categories.

## Materials and Methods

The study was conducted in 2022-2023 at the Academician Volodymyr Kasyanenko Department of Animal Anatomy, Histology and Pathomorphology of the National University of Life and Environmental Sciences of Ukraine, Kyiv. The selection of cadaveric material from the animals used in the experiment was carried out in accordance with the ethical norms of international and Ukrainian legislation (Law of Ukraine No. 3447-IV, 2006; European convention…., 1986).

The material was taken from the corpses of 4 domestic cats (Felis silvestris catus), of which two animals belonged to the Burmese breed, the other two – to the Maine Coon breed. The animals involved in the experiment were male, aged 2.5-3.5 years, of average fatness. Their corpses were received by the department from various veterinary clinics in Kyiv after euthanasia on the recommendations of veterinarians, subject to the consent of the animal owners, and were used by the authors to establish the time of death. After that, pathologic and anatomical autopsy of cat corpses was performed according to generally accepted methods (without evisceration). In the future, samples of skeletal muscle tissue weighing 3 g were taken from animal corpses from the shoulder and thigh area, provided that there were no visible pathologic and anatomical changes. Samples were taken once a day, for 27 days from the moment of arrival of the corpse. Corpses were stored in sterile conditions at a stable temperature of 20°C during the material selection period. After that, the sampling was stopped.

The selected samples were examined using the Shkundia method (Shkundia *et al.*, 2023). To do this, each sample was placed in the middle of a separate sheet of 10×10 cm plastic film,

and then placed between two 10×10 cm glass plates. A sheet of decontaminated filter paper was previously placed on the bottom plate. The polyethylene film with the sample was placed so that the surface of the sample was in contact with the filter paper. After placing the sample between two glass plates in the described manner, the plates were placed under a 1 kg press for 15 minutes. Next, the top plate and plastic film were removed, the contours of the wet spot and the contours of the pressed sample were outlined with a simple pencil, the pressed sample was removed, and then the filter paper was dried.

The wet spot area was measured using a polar planimeter PP-2k and calculated using the equation:

$$S = \frac{x_1+x_2}{2} \text{ x } P - \frac{y_1+y_2}{2} \text{ x } P, \qquad (1)$$

where $S$ – area of the wet spot, cm$^2$; $x_1$, $x_2$ – indicators of planimeter divisions when measuring the external contour of a wet spot; $y_1$, $y_2$ – indicators of planimeter divisions when measuring the internal contour of a wet spot; $P$ – price of dividing the planimeter in units of measurement (cm$^2$).

Statistical processing of the obtained results was carried out in the following sequence. The normality of the distribution was determined by the Shapiro-Wilk test (González-Estrada *et al.*, 2022; Rodrigues de Souza *et al.*, 2023). The equality of variances was determined by the Levene test (Wang *et al.*, 2022). The T-test (for two independent samples) was used to compare samples between animals of the same breed (Novak, 2022). ANOVA (univariate analysis of variance) was used to compare samples between animals of different breeds (Emerson, 2022). The calculations were performed using the Real Statistics Resource Pack 2007, which is an add-on to Excel-2010 software suite.

## Results and Discussion

Based on the results of calculating the wet spot area obtained from samples of skeletal muscle tissue of cat corpses, their values for various muscle groups in the animals under study were obtained (Table 1).

**Table 1**. Value of the wet spot area obtained from the muscles of experimental animals at different time intervals from the moment of death, n=4

| Days after death | Wet spot area, shoulder muscles, Maine Coon, cm$^2$ | Wet spot area, thigh muscles, Maine Coon, cm$^2$ | Wet spot area, shoulder muscles, Burmese, cm$^2$ | Wet spot area, thigh muscles, Burmese, cm$^2$ |
|---|---|---|---|---|
| 0 | 3.42 | 4.01 | 2.36 | 3.39 |
| 1 | 4.36 | 2.34 | 3.67 | 2.55 |
| 2 | 2.07 | 5.62 | 2.88 | 4.92 |
| 3 | 9.36 | 6.53 | 7.00 | 6.31 |
| 4 | 5.08 | 4.45 | 5.28 | 7.66 |
| 5 | 3.78 | 10.67 | 4.70 | 9.09 |
| 6 | 8.73 | 8.05 | 7.03 | 7.18 |
| 7 | 6.88 | 5.22 | 6.00 | 5.89 |
| 8 | 9.00 | 8.10 | 8.50 | 8.24 |
| 9 | 7.10 | 6.58 | 7.36 | 6.60 |
| 10 | 6.70 | 7.89 | 6.83 | 7.05 |
| 11 | 10.02 | 7.24 | 9.10 | 8.19 |
| 12 | 5.49 | 5.04 | 5.66 | 5.44 |
| 13 | 7.29 | 4.45 | 7.00 | 5.05 |
| 14 | 6.75 | 3.06 | 5.24 | 3.39 |

Table 1. Continued

| Days after death | Wet spot area, shoulder muscles, Maine Coon, cm² | Wet spot area, thigh muscles, Maine Coon, cm² | Wet spot area, shoulder muscles, Burmese, cm² | Wet spot area, thigh muscles, Burmese, cm² |
|---|---|---|---|---|
| 15 | 3.11 | 4.20 | 3.72 | 3.48 |
| 16 | 5.00 | 4.54 | 4.49 | 4.01 |
| 17 | 2.25 | 5.49 | 3.42 | 3.65 |
| 18 | 6.34 | 2.16 | 5.93 | 3.92 |
| 19 | 4.05 | 4.46 | 4.71 | 4.09 |
| 20 | 6.35 | 2.62 | 6.19 | 3.62 |
| 21 | 4.05 | 3.75 | 5.88 | 3.83 |
| 22 | 6.17 | 3.91 | 5.24 | 4.00 |
| 23 | 4.23 | 5.68 | 5.94 | 5.86 |
| 24 | 10.58 | 6.84 | 8.09 | 6.51 |
| 25 | 8.73 | 7.12 | 9.12 | 7.00 |
| 26 | 10.35 | 8.03 | 9.86 | 8.29 |
| 27 | 10.83 | 9.52 | 10.59 | 9.72 |
| Average | 6.36 | 5.63 | 6.14 | 5.68 |
| Standard deviation | 2.59 | 2.17 | 2.10 | 2.01 |
| Sample variance | 6.70 | 4.70 | 4.40 | 4.02 |

***Source**: developed by the author*

Changes in the wet spot area index depending on the time elapsed since the animal's death in samples taken from the shoulder muscles of a Maine Coon cat are graphically shown in Figure 1.

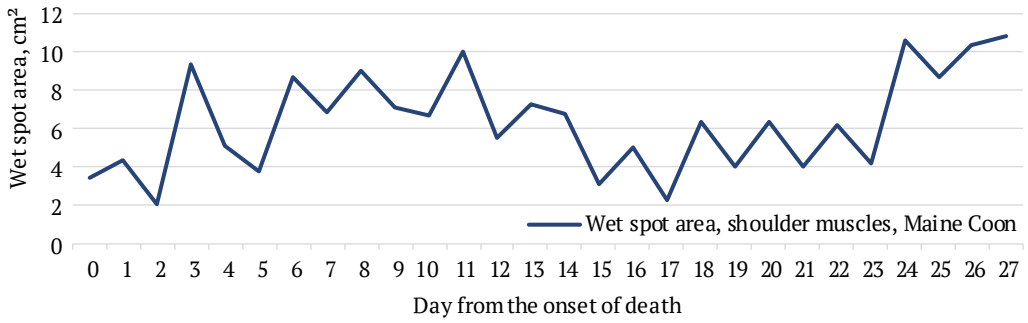


**Figure 1.** Diagram of changes in the wet spot area indicator depending on the time since death, Maine Coon, shoulder muscle

***Source**: developed by the authors*

From days 1 to 3 after the death of the animal, the indicator of the wet spot area first slightly decreased, and then sharply increased, to 9.36 cm² on day 3. From days 3 to 5, a decrease was again observed, and from days 6 to 12 – a stable increase to values in the range of 8-10 cm². From days 12 to 17, a gradual decrease in the wet spot area indicator to values in the range of 2-4 cm² was detected, from days 17 to 23, the indicator was consistently in the range of 4-6 cm², after which it grew abruptly to values of 10-11 cm² and remained at this level in the future.

Changes in the wet spot area depending on the time elapsed since the animal's death in samples taken from the thigh muscles of a Maine Coon are graphically shown in Figure 2.

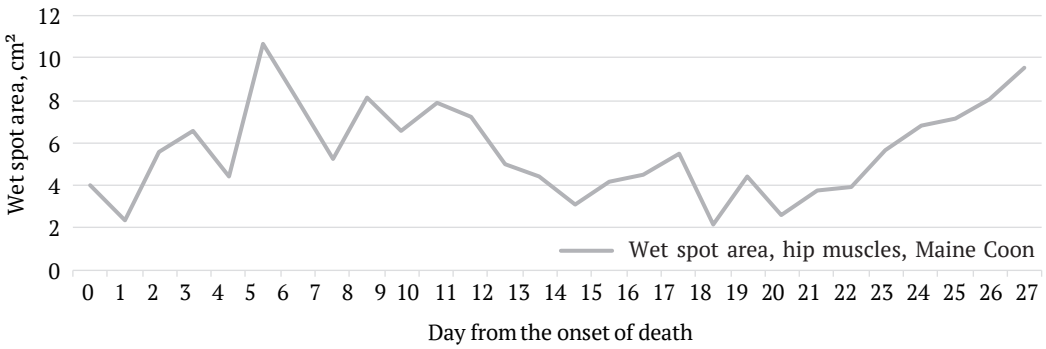


**Figure 2.** Graph of changes in the wet spot area indicator depending on the time since death, Maine Coon, thigh muscles

***Source**: developed by the authors*

From days 1 to 3 after the death of the animal, the indicator of the area of the wet spot first slightly decreased, and then increased, to 6.53 cm² on day 3. From days 4 to 5, a sharp increase to 10.67 cm² was observed, and from day 6 to 14 – a stable decrease to a value of 3.06 cm². From days 15 to 17, a gradual increase in the value of the wet spot area in the range of 4-6 cm² was detected, and from days 18 to 20, this indicator steadily decreased and was in the range of 2-4.5 cm², after which it gradually increased to a value of 9.52 cm². Changes in the size of the wet spot area depending on the time elapsed since the animal's death in samples taken from the shoulder muscles of a Burmese cat are graphically shown in Figure 3.

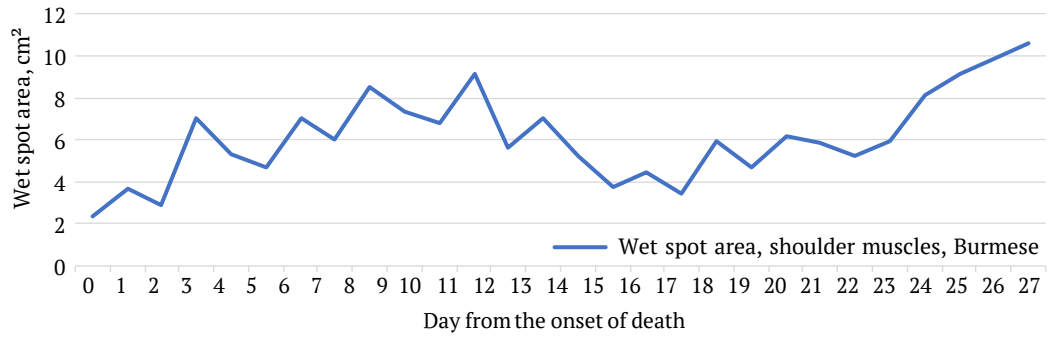


**Figure 3.** Diagram of changes in wet spot area indicator depending on the time since death, Burmese, shoulder muscles

***Source**: developed by the authors*

From days 1 to 2 after the death of the animal, the indicator of the wet spot area first slightly decreased, and then sharply increased, to 7 cm² on day 3. From days 4 to 5, a gradual decrease in its value to 4.7 cm² was observed, and from days 6 to 11, an abrupt increase in values in the range of 6-9 cm² was observed. From days 12 to 17, first a sharp and then gradual

decrease in the wet spot area to values in the range of 3-4 cm² were detected. From days 18 to 24, the parameters of this indicator increased and were in the range of 5-6 cm², after which they sharply increased to 10.59 cm².

Changes in the wet spot area indicator depending on the time elapsed since the animal's death in samples taken from the thigh muscles of a Burmese cat are graphically shown in Figure 4.

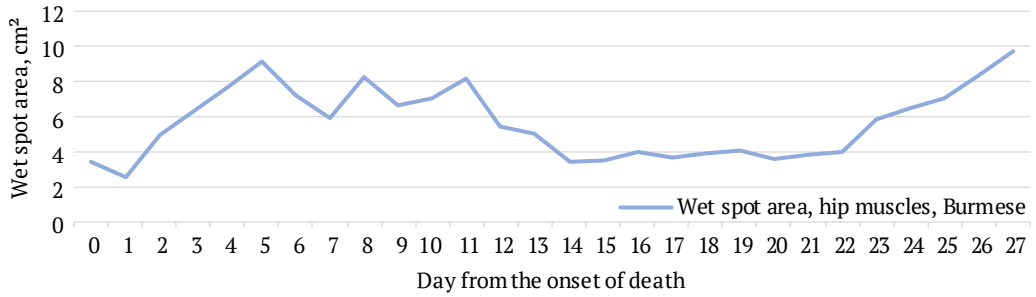


**Figure 4.** Graph of changes in the wet spot area indicator depending on the time since death, Burmese, thigh muscle

***Source**: developed by the authors*

From day 1 to 5 after the death of the animal, the indicator of the area of the wet spot initially increased very sharply to a value of 9.09 cm², and from days 6 to 11 this indicator remained in the range of values of 6-8 cm². From days 12 to 14, a sharp decrease in its value to 5.66 cm² was observed, and then from days 15 to 22 – the values almost did not change and were in the range of 3-4 cm². From days 23 to 27, the indicator increased sharply to a value of 9.72 cm². Changes in wet spot area indicators as a function of the time elapsed since animal death in samples taken from the shoulder and thigh muscles of Maine Coon and Burmese cats are graphically shown in Figure 5.

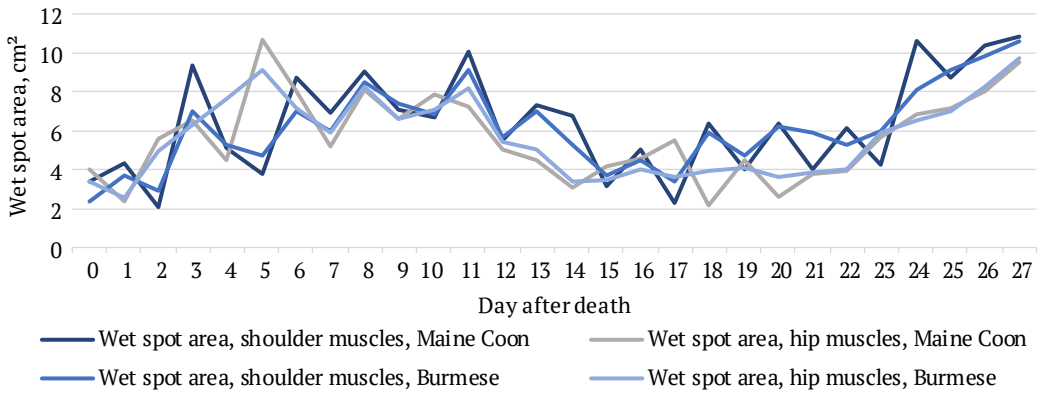


**Figure 5.** Diagram of changes in wet spot area indicators depending on the time since death of all animals under study

***Source**: developed by the authors*

When assessing the results obtained from the animals corpses, it is possible to identify general patterns of changes in the wet spot area obtained from samples of their skeletal muscles depending on the time elapsed since their death. From the onset of death to days 1-2, the indicator was in the range of 2-4 $cm^2$, sometimes even having a tendency to decrease. Starting from days 1-2, an abrupt increase in the values of this indicator was observed, which reached peak values at days 3-5, in the range of 8-11 $cm^2$. In the future, their values with small fluctuations corresponded to the range of 6-10 $cm^2$, and starting from day 11, a sharp decrease in the value of these indicators was observed, which corresponded to the range of 2-4 $cm^2$. The values were in the range of 2-6 $cm^2$ up to days 22-23. Next, there was a sharp abrupt increase in the size of the wet spot area obtained from skeletal muscle samples of cats, depending on the time that has passed since the death of animals, with a subsequent tendency to increase.

For mathematical processing of data as a null hypothesis, the statement was taken as a basis that according to the results of measuring the area of a wet spot obtained from samples of muscle tissue of cat corpses, there should be no significant difference between the indicators taken from animals of different breeds, and the difference between the indicators obtained during the study of samples taken from different skeletal muscles. Thus, the values obtained at certain time intervals, regardless of the breed of cats and the muscle group from which the samples were taken, should correlate with the time that has passed since the onset of death, and these indicators can be used to calculate the time of death of animals, using the data obtained by the authors as a reference. Using the Shapiro-Wilk test, the distribution normality was established in all the data samples under study (Table 2).

**Table 2**. Normality of the distribution of samples of wet spot area values obtained from the muscles of experimental animals at different time intervals from the moment of death, n=4

| **Shapiro-Wilk test** | **Wet spot area, shoulder muscles, Maine Coon** | **Wet spot area, thigh muscles, Maine Coon** | **Wet spot area, shoulder muscles, Burmese** | **Wet spot area, thigh muscles, Burmese** |
|---|---|---|---|---|
| Distribution normality indicator (W-stat) | 0.957271813 | 0.969241318 | 0.979122624 | 0.940969325 |
| Significance level (p-value) | 0.299880488 | 0.560395402 | 0.828890163 | 0.11699154 |
| Threshold significance level (alpha) | 0.05 | 0.05 | 0.05 | 0.05 |
| Normality | Yes | Yes | Yes | Yes |

***Source**: developed by the author*

The Shapiro-Wilk test (Table 2) shows that the distribution is normal in all the data samples under study, i.e., no sample has values that differ significantly from all the others. Using the Levene test, equality of variances was established in all the data samples under study (Table 3).

**Table 3**. Equality of sample dispersions of wet spot area values obtained from the muscles of experimental animals at different time intervals from the moment of death, n=4

| Levene test | Variance between wet spot area samples of shoulder and thigh muscles, Maine Coon | Variance between wet spot area samples of shoulder and thigh muscles, Burmese | Variance between wet spot area samples of Maine Coon and Burmese cats |
|---|---|---|---|
| Average values (means) | 0.325441 | 0.798616 | 0.492355 |
| Threshold significance level (alpha) | 0.05 | 0.05 | 0.05 |

***Source***: *developed by the author*

The Levene test (Table 3) determined the equality of variances between the values of the wet spot area of the shoulder and thigh muscles in animals of the same breed and between the animals of different breeds. The average values of variances exceeded the threshold level of significance. Since the difference in variances in the cases was not statistically significant, the variances can be considered homoscedastic. Thus, with the normality of the distribution and equality of variances, the null hypothesis is confirmed. Using the T-test (for two independent samples with equal variances in the Student's distribution), the absence of differences between the samples of these values of the wet spot area of the shoulder and thigh muscles in animals of each breed was established (Table 4).

**Table 4**. Value of the t-criterion for samples of wet spot area indicators obtained from the muscles of experimental animals at different time intervals from the moment of death, n=4

| Cat breed | Resulting value of the Student's criterion (t-stat) | Critical value of the Student's criterion (t-crit) | Significance level (p-value) |
|---|---|---|---|
| Maine Coon | 1.1474 | 2.0048 | 0.2562 |
| Burmese | 0.8375 | 2.0048 | 0.4060 |

***Source***: *developed by the author*

Using the T-test, it was found that in animals of each studied breed there was no difference between the samples of data on the area of the wet spot of the shoulder and thigh muscles, since the obtained values of the Student's criterion for the data samples of each breed compared with each other were less than the corresponding critical values of this variable. Therefore, the values of the wet spot area indicator do not significantly depend on which group of skeletal muscles (shoulder or thigh) to take samples for research using this method.

As a result of using univariate analysis of variance (ANOVA) with the calculation of the Fischer criterion, there was no difference between the samples of these values of the wet spot area of the shoulder and thigh muscles in cats of different breeds (Table 5).

**Table 5**. Value of the F-criterion for samples of wet spot area indicators obtained from the muscles of experimental animals at different time intervals from the moment of death, n=4

| Source of variation | Resulting value of the Fischer criterion (F) | Critical value of the Fischer criterion (F-crit) | Significance level (p-value) |
|---|---|---|---|
| Between groups, Maine Coon and Burmese breeds | 0.7179 | 2.6887 | 0.5433 |

***Source**: developed by the author*

Based on the results of univariate variance analysis, it was found that there is no difference between the value of the wet spot area of the shoulder and thigh muscles in cats of different breeds, since the obtained values of the Fischer criterion for comparable data samples obtained from cats of different breeds were less than the corresponding critical values of this variable. Thus, the breed of cats whose corpses were used for research by this method did not significantly affect the value of the wet spot area indicator. Thus, the null hypothesis, according to the calculations performed on the Shapiro-Wilk, Levene, t-test, and univariate analysis of variance (Wang *et al*; 2022, Emerson, 2022; Rodrigues de Souza *et al.*, 2023), it is confirmed that the values obtained at certain time intervals, regardless of the breed of cats and the muscle group from which the samples were taken, correlate with the time elapsed since the onset of death.

The idea of this study was based on the assumption that changes in wet spot area indicators over time would represent uniform growth in the form of a linear relationship. However, the data obtained had a slightly different trend. The wet spot area increased on day 3-5 and remained relatively stable for days 5-11, slightly decreased from day 11 to day 15, stabilised again between days 15 and 22-23, and began to increase sharply thereafter. The increase in the area of the wet spot on days 3-5 can be explained by the release of water due to the collapse of the structures of intermuscular connective tissue, and the result of the diffusion of the liquid part of the blood after death from the vessels that supply blood to the muscles into the intermuscular space. At this time, water remained in the muscle fibres, and their structure was preserved in this period of time from the moment of death. The relative stability of the indicators during days 5-11 after the death of the animals can be explained by the limited amount of water in the intermuscular space, as muscle fibres are not yet destroyed during this period. The decrease in the area of the wet spot during days 11-15 from the moment of death is explained by the loss of a certain amount of water through diffusion to other parts of the corpse and through its surface. The stability of indicators from days 15 to 22-23 may be associated with the beginning of the breakdown of muscle fibres, which compensates for the diffusion of water to other parts of the corpse and through its surface. An abrupt increase in the area of a wet spot, starting from days 22-23, can be the result of the breakdown of a large number of muscle fibres, and the destruction of macromolecules of proteins and lipids, as a result of which liquid is released. However, in all data samples under study, the described trend was clearly observed, so the data obtained can serve as a reference against which to compare the data from the corpses of cats obtained by the Shkundia method (Shkundia *et al.*, 2023), whose time of death must be established for forensic examinations.

Based on the above, it is proposed to calculate the time of death in cats based on the wet spot area obtained from skeletal muscle samples of corpses, using the reference values given in Table 6.

**Table 6**. Reference values of the wet spot area indicator obtained from skeletal muscle samples from cat corpses, M±m

| Days after death | Reference values, $cm^2$ | Days after death | Reference values, $cm^2$ | Days after death | Reference values, $cm^2$ |
|---|---|---|---|---|---|
| 0 | 3.30±0.33 | 10 | 7.120.27 | 20 | 4.70±1.13 |
| 1 | 3.23±0.56 | 11 | 8.64±0.66 | 21 | 4.38±0.54 |
| 2 | 3.87±1.01 | 12 | 5.41±0.13 | 22 | 4.83±0.63 |
| 3 | 7.30±0.74 | 13 | 5.95±0.86 | 23 | 5.43±0.43 |
| 4 | 5.62±0.73 | 14 | 4.61±1.01 | 24 | 8.01±0.96 |
| 5 | 7.06±2.04 | 15 | 3.63±0.24 | 25 | 7.99±0.67 |
| 6 | 7.75±0.46 | 16 | 4.51±0.18 | 26 | 9.13±0.71 |
| 7 | 6.00±0.32 | 17 | 3.70±0.64 | 27 | 10.17±0.39 |
| 8 | 8.46±0.20 | 18 | 4.59±1.11 | | |
| 9 | 6.91±0.23 | 19 | 4.33±0.18 | | |

***Source**: developed by the author*

A specialist who performs a forensic veterinary examination, if representatives of the investigative authorities are tasked with establishing the time of the occurrence of death in a cat, can, after performing a study using the Shkundia method, compare the obtained indicator with the reference value, and establish the time that has passed since the death of the animal, with an accuracy of one day. The data obtained can be compared with data obtained using other methods for determining the time of death, in order to exclude possible errors. Thus, the method of determining the area of a wet spot is informative and can be implemented in forensic veterinary practice. Compared to other methods of determining the time of death, the advantage of such studies is a long time interval – about 1 month from the moment of death, while thermometry is suitable in the time interval of 1-3 days, which is indicated, for example, by S.J. Jeong *et al*. (2020), microscopic examination – 1-18 days, and a method based on determining the state of entomofauna of the corpse, according to J. Byrd & I. Sutton (2021) and S. Matussewski (2021) – no more than 10-15 days. In addition, the equipment required for working with the wet spot method is not difficult to use and expensive, as, for example, for studies using computer or magnetic resonance imaging, which are proposed by E. Watson & J.Kr. Baucom (2020) and M. Zhang (2022).

J. Serdioucov *et al.* (2023) note that one of the most common and accurate methods for determining the time of death – histological examination of postmortem changes in various tissues and organs of the corpse – provides valid results only during the first 18 days after death. It is characteristic that the results presented by J. Serdioucov *et al.* (2023) are obtained specifically on the corpses of domestic cats. The wet spot method proposed by the authors has a number of advantages over the histological method. The postmortem interval, which can be determined by the Shkundia method, is significantly longer than that that can be determined histologically – 27 days and 18 days, respectively. The time required to make a wet spot preparation is approximately 1 hour, while a histological preparation, depending on the latest equipment used by the researcher, takes from 2 to 14 days. Although, according to most researchers, for example, V.K. Sokol (2022), histological examination is a widely available diagnostic method, but the amount of equipment required for histology (automatic filling of material and staining of histoses, microtoms,

microscopes, photo nozzles, etc.) is several times greater than that required for the wet spot method (glass plates, planimeter). In addition, the wet spot method does not require the use of chemical reagents. Wet spot preparations are better preserved than histopreparations. Quantitative data, as noted by D. Shkundia *et al.* (2023), also easier to get by the wet spot method. However, histopreparations have much greater visibility than wet spot preparations. In addition to classic micrographs, as reported by Yu.V. Sarkisova & S.M. Malanchuk (2020), microscopy of histopreparations can produce spectropolarimetric images and reconstruction of their polycrystalline structure, as noted by O.Iu. Lytvynenko (2022).

Measurement of cadaveric cooling rate by thermometry, by definition of C. Henßge & B. Madea (2004), is the most commonly used method for determining the time of death of humans and animals. The features of postmortem cooling in domestic cats have not yet been practically studied. The wet spot method proposed by the authors has a number of advantages over the thermometric method. The most important of these is that the time interval for the onset of death, which can be determined by the Skundia method, is much longer than that which, in particular, according to S.J. Jeong *et al.* (2020), can be recorded by thermometry – 27 days and 1-3 days, respectively. Domestic cats are small animals and therefore a complete equalisation of the temperature of the corpse with the ambient temperature occurs at most during the 2nd day after death. In addition, as noted by S.J. Jeong *et al.* (2020), the dependence of the accuracy of determining the time of death by the thermometric method on climatic conditions is very significant, which affects the measurement results, often not allowing to establish a correlation between the obtained and tabular data. The results of the thermometric study are particularly negatively affected by the air temperature both in open areas and in various rooms, the humidity level and speed of air movement, weather conditions (rain, snow, etc.). The data obtained by the wet spot method are much less affected by these factors, especially when preparations are obtained from cadaveric skeletal muscle, as recommended by D. Shkundia *et al.* (2023). In addition, there is a dependence of the validity of indicators obtained using thermometry on the causes of death, especially diseases, the symptoms of which, in particular, are characterised by a pronounced increase or decrease in body temperature. The authors are not aware of any such influence on the results obtained by the wet spot method. In addition, the wet spot preparation can be used as physical evidence, while the reliability of temperature measurements, especially in the field, sometimes raises doubts among law enforcement officials. Thermometry also has a number of advantages over the wet spot method. This is significantly less time, as noted by C. Henßge & B. Madea (2004), which takes from 1 to 5 minutes depending on the design features of the thermometers, while the production time of the wet spot preparation is approximately 1 hour; simple portable equipment that allows on-site measurements, etc.

Entomological method, according to J. Byrd & L. Sutton (2021), is extremely common in forensic and veterinary medicine. When comparing it with the wet spot method, the obvious advantage of the latter is one thing: an even greater dependence on climatic conditions and, even, the time of year than with the thermometric method, which is associated with the seasonality of the insect life cycle, as noted by S. Matuszewski (2021). For these two methods, the measurement range of the postmortem interval and the time spent on conducting research are approximately the same. The advantage of the entomological method is also the low need for equipment.

This study presents a proprietary method. It is based on determining the moisture-retaining ability of animal body tissues, which changes after the death of an animal, since liquid is released during the breakdown of cellular structures and macromolecules. When comparing the wet spot method with other widely used methods for determining the time of death, a number of advantages over each one are revealed, however, as well as some disadvantages. Therefore, the authors consider it appropriate to use it widely, in particular, in the practice of forensic veterinary examination.

## Conclusions

The proposed method for determining the time of death by measuring the area of a wet spot obtained from skeletal muscle tissue samples from domestic cat corpses is informative and has a number of advantages over other methods for determining the time of death. The most important advantage is the long time interval in which results can be obtained that correlate with the time of death. In addition, the validity of this method is much less dependent on climatic conditions, such as temperature and humidity, weather conditions in the place where the corpse of the animal was found, being in the room, packing corpses in plastic bags, wrapping in blankets, clothing elements, etc. Wet spot preparations obtained when working with fabrics using this method can be stored for a long time and serve as material evidence in the investigation and judicial review of criminal cases. The method provides quantitative data that allows determining the time of death much more accurately than, for example, visual and palpation, and is subjected to statistical processing.

An explanation is found for fluctuations in the values of the wet spot area indicator depending on the time that has passed since death. Statistical studies have shown that the data obtained by measuring the area of a wet spot correlate with the time of death. Reference values of the wet spot area obtained from samples of skeletal muscle of cat corpses have been developed, which forensic veterinarians can use in their practical activities to compare with the data obtained by this method of examining material from cat corpses in specific cases that become the basis for investigating criminal proceedings.

The disadvantages of the method for determining the time of death by measuring the area of a wet spot obtained from skeletal muscle tissue samples from cat corpses are its specificity for certain animal species and certain tissues. In addition, to perform research using this method, it is necessary to have a device (planimeter), which is not widely used equipment. The influence of climatic and other conditions in which the animal's corpse is located on the reliability of the results obtained by applying this method remains unclear. The prospects for further research are to determine the time of death by measuring the area of the wet spot from the material of various organs, species, breeds, sex and age categories of animals, developing amendments to reference values that take into account climatic conditions and the environment in which the corpse can be located, etc.

## Acknowledgements

None.

## Conflict of Interest

None.

## References

[1] Ave, M.T., Ordonez-Mayan, L., Camina, M., Febrero-Bande, M., & Munoz-Barus, J.I. (2021). Estimation of thepost-mortem interval: Effect of storage conditions on the determination of vitreous humour [K+]. *Science & Justice*, 61(5), 597-602. doi: 10.1016/j.scijus.2021.07.005.

[2] Byrd, J., & Sutton, L. (2020). Forensic entomology for the investigator. *WIREs Forensic Science*, 2(4), article number e1370. doi: 10.1002/wfs2.1370.

[3] Criminal Code of Ukraine. (2001, September). Retrieved from https://zakon.rada.gov.ua/laws/show/2341-14#Text.

[4] Doukas, D. (2022). Non-accidental injuries in dogs and cats: Review of post-mortem forensic evaluations and the social significance of small animal practice. *Journal of the Hellenic Veterinary Medical Society*, 73(1), 3543-3552. doi: 10.12681/jhvms.23296.

[5] Emerson, R.W. (2022). ANOVA Assumptions. *Journal of Visual Impairment & Blindness*, 116(4), article number 0145482X2211241. doi: 10.1177/0145482X221124183.

[6] European convention for the protection of vertebrate animals used for research and other scientific purposes. (1986). Retrieved from https://zakon.rada.gov.ua/laws/show/994_137#Text.

[7] Garland, J., Olds, K., Rousseau, G., Palmiere, C., Ondruschka, B., & Kesha, K., Glenn, C., & Morrow, P. (2020). Using vitreous humour and cerebrospinal fluid electrolytes in estimating post-mortem interval – an exploratory study. *Australian Journal of Forensic Sciences*, 52(6), 626-33. doi: 10.1080/00450618.2019.1642956.

[8] González-Estrada, El., Villaseñor, J.A., & Acosta-Pech, R. (2022). Shapiro-Wilk test for multivariate skew-normality. *Computational Statistics*, 37(3), 1985-2001. doi: 10.1007/s00180-021-01188-y.

[9] Heba El-Sayed, M., El-Shafei, D.A., Abouhashem, S.N., & Alaa El-Din, E.A. (2023). Could skeletal muscle changes provide a reliable method for estimating the time since death: A histological, biochemical, and DNA study. *Australian Journal of Forensic Sciences*, 55(1), 46-58. doi: 10.1080/00450618.2021.1921272.

[10] Henßge, C., & Madea, B. (2004) Estimation of the time since death in the early post-mortem period. *Forensic Science International*, 144(2-3), 167-175. doi: 10.1016/j.forsciint.2004.04.051.

[11] Hryhorian, E.K. (2019). Content analysis of the forensic medical assessment of postmortem interval. *Ukrainian Journal of Medicine, Biology and Sports*, 4, 6(22), 265-270. doi: 10.26693/jmbs04.06.265.

[12] Huang, W.-Hs., Kuo, Ch.-Ch., Hu, Hs.-Y., Pan, Ch.-Hs., Taiching, L.A., & Liu, Ch.-Hs. (2020). Manual strangulation of a stray cat: Linking pathologic findings with the crime. *Journal of Veterinary Forensic Sciences*, 1(2), 11-14. doi: 10.32473/jvfs.v1i2.128634.

[13] Jeong, S.J., Park, S.H., Park, J.E., Park, S.H., Moon, T.Y., Shin, S.E., & Lee, J.W. (2020). Extended model for estimation of ambient temperature for postmortem interval (PMI) in Korea. *Forensic Science International*, 309, article number 110196. doi: 10.1016/j.forsciint.2020.110196.

[14] Law of Ukraine No. 3447-IV "On Protection of Animals from Cruelty". (2006, February). Retrieved from https://zakon.rada.gov.ua/laws/show/3447-15#Text.

[15] Lytvynenko, O.Iu. (2022). Method of reconstruction of polycrystalline structure of histological cuts in determination of the stature of formation of damages of internal bodies. *Forensic Sciences Research*, 1, 81-86. doi: 10.24061/2707-8728.1.2022.12.

[16] Matuszewski, S. (2021). Post-mortem interval estimation based on insect evidence: Current challenges. *Insects*, 12(4), article number 314. doi: 10.3390/insects12040314.

[17] Merck, M., & Miller, D. (2013). Postmortem changes and the postmortem interval. In *Veterinary forensics: Animal cruelty investigations* (pp. 255-271). New Jersey: John Wiley. doi: 10.1002/9781118704738.ch14.

[18] Muggenthaler, H., Sinicina, I., Hubig, M., & Mall, G. (2012). Database of post-mortem rectal cooling cases under strictly controlled conditions: A useful tool in death time estimation. *International Journal of Legal Medicine*, 126(1), 79-87. doi: 10.1007/s00414-011-0573-6.

[19] Nioi, M., Napoli, P.E., Demontis, R., Locci, E., Fossarello, M., & d'Aloja, E. (2021). Postmortem ocular findings in the optical coherence tomography era: A proof of concept study based on six forensic cases. *Diagnostics*, 11(3), article number 413. doi: 10.3390/diagnostics11030413.

[20] Novak, S.Y. (2022). On the T-test. *Statistics & Probability Letters*, 189, article number 109562.

[21] Piegari, G., De Pasquale, V., d'Aquino, I., De Biase, D., Caccia, G., Campobasso, P.C., Tafuri, S., Russo, V., & Paciello, O. (2023). Evaluation of muscle proteins for estimating the post-mortem interval in veterinary forensic pathology. *Animals*, 13(4), article number 563. doi: 10.3390/ani13040563.

[22] Rodrigues de Souza, R., Toebe, M., Mello, A. Ch., & Bittencourt, K.Ch. (2023). Sample size and Shapiro-Wilk test: An analysis for soybean grain yield. *European Journal of Agronomy*, 142, article number 126666. doi: 10.1016/j.eja.2022.126666.

[23] Romano, C.M., Dorman, C.D., & Gaskill, L.C. (2020). Postmortem veterinary toxicology: Animal death investigation and the veterinary diagnostic laboratory. *WIREs Forensic Science*, 2(5), article number e1375. doi: 10.1002/wfs2.1375.

[24] Sarkisova, Yu.V. (2021). Diagnostic possibilities of accurate determination of the time since death on the concentration of electrolytes in the vitreous body of the human eye. *Forensic Medical Examination*, 1, 79-85. doi: 10.24061/2707-8728.1.2021.10.

[25] Sarkisova, Yu.V., & Malanchuk, S.M. (2020). Spectral-selective laser-induced autofluorescent microscopy of polycrystalline fraction of the human vitreous body in diagnostics time since death. *Forensic Medical Examination*, 1, 61-69. doi: 10.24061/2707-8728.1.2020.8.

[26] Savka, I.G., Kozan N.M., Dunaev, O.V., & Oliynyk, I.Y. (2021). *Establishment of accurate criteria for diagnosis of the time since death in forensic medical practice.* Retrieved from http://dspace.bsmu.edu.ua/bitstream/123456789/17806/1/SME_2021_1_018.pdf.

[27] Serdioucov, J., Shkundia, D., & Kruchynenko, O. (2023). Identification of time of death of cats according to histological changes in some organs. *Regulatory Mechanisms in Biosystems*, 14(3), 399-406. doi: 10.15421/10.15421/022359.

[28] Shkundia, D.Yu., Serdioucov, J.K., Malyuk, M.O., & Bokotko, R.R. (2023). *The method of determining the time of death by measuring the area of the wet spot obtained from the tissue samples of cat corpses* (Patent of Ukraine No. 153614). Retrieved from https://iprop-ua.com/inv/h0zfzhbd/.

[29] Sokol, V.K. (2022). *Peculiarities of microscopic changes in the tissue of the human organism at different periods of death*. Retrieved from http://forensic.bsmu.edu.ua/article/view/262428/258860.

[30] Viciano, J., Lopez-Lazaro, S., & Tanga, C. (2022). Post-mortem dental profile as a powerful tool in animal forensic investigations – a review. *Animals*, 12(16), article number 2038. doi: 10.3390/ani12162038.

[31] Wang, Y., Tang, M., Wang, P., Liu, B., & Tian, R. (2022). The Levene test based-leakage assessment. *Integration*, 87, 182-193. doi: 10.1016/j.vlsi.2022.06.013.

[32] Watson, E., & Baucom, J.Kr. (2020). Two case studies in veterinary forensic imaging and a brief literature review. *Forensic Imaging*, 21(4), article number 200382. doi: 10.1016/j.fri.2020.200382.

[33] Yatsenko, I., & Parilovsky, O. (2022) Animal mutilation as a subject of forensic veterinary expertise. *Bulletin of the Black Sea Littoral*, 102-103, 71-86. doi: 10.37000/abbsl.2022.102.13.

[34] Zhang, M. (2022). Forensic imaging: A powerful tool in modern forensic investigation. *Forensic Sciences Research*, 7(3), 385-392. doi: 10.1080/20961790.2021.2008705.

## Визначення давності настання смерті кота свійського методом вимірювання площі вологої плями

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**Анотація.** Жорстоке поводження з тваринами, відповідно до чинного законодавства України, є кримінальним злочином. Під час розслідування кримінальних проваджень, пов'язаних із жорстоким поводженням із тваринами, в тому числі котами свійськими, часто виникає необхідність встановлення давності настання смерті. Тому надзвичайно актуальними є розробка нових методів її визначення та вдосконалення вже існуючих. Метою дослідження було встановлення залежності між отриманими значеннями показників площі вологої плями та часом настання смерті. Для досягнення поставленої мети авторами запропонований новий метод визначення давності настання смерті котів свійських за результатами вимірювання площі вологої плями, отриманої зі зразків тканини скелетних м'язів трупів (метод Шкунді). Для цього відбирали від трупів котів зразки м'язової тканини, за допомогою пресування отримували препарат вологої плями та вираховували її площу, з метою встановлення кореляції значень цього показника з часом, який пройшов з настання смерті. Дослідженням отриманих показників встановлено, що ці значення є сталими, встановлено і показано графічно особливості коливань значень даного показника з часом, що пройшов з моменту смерті тварин. За допомогою низки статистичних методів визначено відсутність залежності показників площі вологої плями від м'язів, із яких відбирають зразки для дослідження, та порід тварин. Встановлено характер змін значень показника площі вологої плями залежно від часу і проаналізовано особливості їх коливань у різні періоди протягом 27 діб з моменту загибелі тварини. Представлено еталонні значення даних показників залежно від часу, що пройшов з моменту смерті тварин, які судово-ветеринарні експерти можуть використовувати в практичній роботі для встановлення давності настання смерті котів свійських. Завдяки низці переваг даний метод може бути широко впроваджений у практику судової ветеринарної медицини

**Ключові слова:** судово-ветеринарна експертиза; постмортальний інтервал; *Felis silvestris catus*; метод Шкунді; м'язова тканина

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# Informativeness of postmortem dynamics of skeletal muscles of dog and cat corpses for forensic veterinary diagnosis of death due to acute hypoxia

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**Abstract.** The relevance of the study is conditioned by the need in the practice of forensic veterinary expertise to establish the statute of limitations for the occurrence of death of dogs and cats during the investigation of crimes against animal health and life. In this regard, the purpose of the study is to solve diagnostic forensic veterinary problems regarding the informative value of postmortem disorganisation of skeletal neck muscles of dog and cat corpses within 72 hours after the death of animals. The main methods of investigating the diagnostic information content of postmortem dynamics of skeletal muscles of dog and cat corpses for forensic veterinary determination of the conditions and prescription of death due to acute hypoxia are those that can be comprehensively considered the identified problem, in particular, Papenheim-Kryukov staining of smears, slides – hematoxylin and eosin, fluorescein isothiocyanate, and Shabadash staining. The forensic veterinary diagnostic significance of two expert criteria is justified: "the number of patterns of disorganisation of the neck muscles of dog and cat corpses" and "glycogen content in the neck muscles of dog and cat corpses". It is proved that biotransformation of skeletal muscles occurs in the following sequence: during the first 24 hours after the death of the animal, postmortem stiffness of the

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muscles of corpses occurs, accompanied by dehydration, compaction, contraction of myofibrils; muscle relaxation is observed from 24 to 48 hours after death, myofibrils are stretched and swollen; from 48 to 72 hours, autolysis of muscles is recorded under the influence of saprotrophs. It was found that the destruction of skeletal muscle tissue is more intense in the case of death due to suffocation compared to death from poisoning, and the destruction of muscle tissue is more dynamic in the case of death due to freezing compared to death caused by drowning in cold water. For the first time, the results obtained will contribute to solving diagnostic forensic veterinary tasks, in particular, establishing the statute of limitations and conditions for the occurrence of death of dogs and cats during the investigation by law enforcement agencies of crimes related to animal cruelty

**Keywords:** asphyxia; cytomorphological examination; histotopograms; destruction patterns; thanatogenesis; abuse

## Introduction

The study of postmortem dynamics of organs and tissues of animal corpses is important for forensic veterinary diagnostics, as can accurately determine the time and cause of death of the animal. Analysing these changes also helps to identify possible cases of abuse or improper animal care. In addition, this study is relevant for ensuring justice, as it can reveal evidence of criminal acts, such as animal cruelty or illegal hunting. It helps in determining the exact time of death, which can be a key element in criminal investigations. Thus, post-mortem diagnostics of veterinary and forensic medicine is a necessary tool in the protection of animals and ensures the maintenance of law and order.

Questions of informative postmortem dynamics of various organs and tissues of animal corpses for forensic veterinary diagnosis of death for various reasons were analysed by a large number of researchers. Among them: J.J. Paltian *et al.* (2019) evaluated the duration of the postmortem interval by determining the activity of catalase and aminolevulinate dehydratase in the liver, kidney, skeletal muscle, and brain tissues of Swiss mice; J. Abbate *et al.* (2022) found the informativeness of postmortem changes in skeletal muscle electrical conductivity of *Dicentrarchus labrax* to estimate the duration of the postmortem period; J. Geissenberger *et al*. (2021) investigated postmortem degradation of skeletal limb protein in pigs; T. Du *et al*. (2018) characterised the metabolic profile of rat femoral muscle at different time periods after death; F.X. Shi *et al*. (2020) observed the expression of autophagy-related protein in rat muscle tissue after ante-and postmortem trauma; Z. Zheng *et al.* (2019) substantiated the relationship between electrical conductivity and chemical content of rat skeletal muscle impregnation solution to determine the duration of the postmortem period.

The achievements obtained contributed to solving some problems. According to reports R. Kazantsev & I. Yatsenko (2022) and M. Skrypka *et al.* (2023), the issue of finding informative criteria remains relevant, the use of which will allow a forensic veterinary expert to come to certain categorical conclusions when solving situational problems and argumentation of causal relationships. The lack of prospective morphological studies of muscle tissue of animal corpses, clarification of the significance of marker changes in its postmortem disorganisation during forensic veterinary diagnosis of causes of death and systematisation of scientifically based information in scientometric databases on these issues, necessitates in-depth research in this area. As noted by M. Lamri *et*

*al*. (2023) and G. Piegari *et al*. (2023), the study of skeletal muscles is promising in this context due to their histomorphological (fibre density) and histochemical (glycogen depot) features.

Thus, the lack of developed expert criteria for determining the time of death of animals of various species for postmortem phenomena negatively affects the practice of conducting forensic veterinary expertise, causing unjustified refusals to conduct it; substitution of forensic expertise for other procedural actions; raising questions that go beyond the competence of a forensic expert, etc.; and determined the purpose of this study – to substantiate the diagnostic information content of postmortem biotransformation of skeletal muscles of dog and cat corpses to establish the conditions and duration of the postmortem period for hypoxic thanatogenesis. In this regard, the experimental solution of these issues is of both theoretical and practical significance.

## Materials and Methods

The subject of the study was the dynamics of post-mortem biotransformation of skeletal muscles within 72 hours after death from acute hypoxia in 28 animals (*Felis silvestris catus,* n=12; *Canis lupus familiaris,* n=16) in the absence of anamnesis data on zooanthroponoses. The category of violent death was known in advance. The study was conducted at the State Biotechnological University during 2021-2023 in compliance with the requirements of DSTU EN ISO/IEC 17025:2019 (2021). During the modelling of hypoxic thanatogenesis, environmental climatic factors were reproduced in which, with a high probability, the corresponding type of animal death occurred.

All the cases under study were divided into four experimental groups according to the principle of the immediate cause of death of the animal (n=7 in the group). Each group contained corpses with the following distribution: by taxonomic species (cats, n=3; dogs, n=4), sex (females, n=3; males, n=4), age (neonatal, n=2; mature, n=3; geriatric, n=2).

Group 1 included the corpses of animals whose death occurred due to intoxication with carbon monoxide or isoniazid. They were stored at a temperature of 18°C and a humidity of 59%. Group 2 included the corpses of animals that died from mechanical asphyxia or general overheating. They were placed in a thick plastic bag and stored at a temperature of 18°C and a humidity of 64%. Group 3 consisted of corpses of animals whose death occurred as a result of fluid aspiration during drowning or perinatal pneumopathy. They were stored in tap water at a temperature of plus 2°C and a humidity of 73%. Group 4 includes the corpses of animals that died from a general cold injury. They were stored at negative 18°C and 92% humidity.

Skeletal muscle samples from *mm. proprii colli.* were taken from the corpses of animals of all experimental groups after forensic veterinary necropsy. The choice of object for this study is justified by three reasons. Firstly, the relatively easy availability of material selection. Secondly, even in fragmented animal corpses, there is a certain possibility of obtaining the necessary number of tissue samples. Thirdly, the muscles are sufficiently isolated from the destructive effects of the environment and putrefactive transformation by endogenous saprotrophs.

During the first day after the onset of animal death, a series of preparations were made from muscle necropsies. For cytomorphological examination, they were stained using the Papenheim-Kryukov method. For histological studies of muscles, samples from corpses were taken at the 48$^{th}$ and 72$^{nd}$ hours of follow-up using a special punch. Histological slides with a thickness of 6 microns from samples were made on a rotary Microtome MPS-2 (Ukraine) in accordance with the generally accepted method (Horalskyi *et al.*, 2015).

For general histomorphological examination at the final stage, the slides were stained

with hematoxylin and eosin. Fluorescein isothiocyanate staining with neutral glycerin immersion was used to indicate bacterial colonies. For the purpose of histochemical determination of muscle glycogen content, the slides were stained using the Shabadash method. All the glasses were air-dried. Using an optical microscope *Granum R50* (China), skeletal muscle cytograms were examined in 10×10 and 10×100 visual fields, and 10×20 and 10×40 microscope visual fields were used to study muscle histotopograms.

The results were evaluated within each day, comparing the previous data with the next and the first with the last in each study group. The most representative changes were photographed using the ToupCam UCMOS03100KPA digital nozzle (China) integrated with the microscope. The obtained information was analysed on a personal computer with the Windows 10 operating system. The resulting photos were processed using the Photo Frame Studio 3.0 software suite and the destruction patterns were evaluated according to the recommendations of R.E. Raskin *et al*. (2022). Patterns in this study were considered any elements of muscle fibre decomposition, in particular, cell destruction and bacterial colonies, which were analysed in dynamics on the obtained cytograms and histotopograms. Then, in the image editor Adobe Photoshop CS6, histograms of slides were created, additionally coloured according to Shabadash, which automatically displayed the perimeter of glycogen distribution over the image, expressed in pixels, according to the tinkorial brightness level. On the histograms, attention was drawn to the last two indicators of the gradation scale: the total number of pixels, which identifies the outlined area in logical units, and the median of the integrated density, which is equivalent to the average value of colour brightness. In statistical data, using the MS Office Excel 2016 software suite, arithmetic mean indicators were determined (M±m) and evaluated by the level of reliability (P).

All studies at different levels of structural organisation were conducted in Ukraine for the first time, in compliance with the principles of evidence-based veterinary medicine in the context of forensic veterinary expertise and in accordance with the principles of European Association for Animal Research (n.d.).

## Results and Discussion

Applying a retrospective analysis of the nosological structure of *exitus letalis* among cats and dogs in veterinary medical institutions, it was found that decompensated hypoxia of various aetiologies is fatal in 7% of all cases of death of various categories, including due to acute respiratory distress syndrome, neonatal lung atelectasis, and aspiration pneumonitis. Two hours after the death of the animals, during their forensic veterinary sectional examination, it was determined that *mm. proprii colli* are dense to the touch, and the joints of the cervical vertebrae are almost not mobile.

Cytomorphological studies of muscle specimens from the corpses of cats from group 1, obtained 8 hours after death, revealed the preservation of their vital structure. Cytograms showed clusters of striated skeletal muscle fibres in the form of spindle-shaped strands located tightly (Fig. 1).

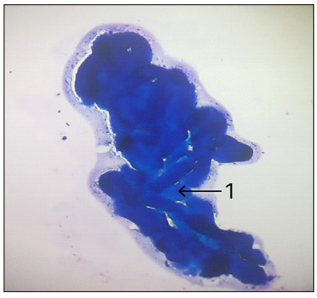



**Figure 1.** Cytogram of the skeletal muscle of the cat corpse from group 1, 8 hours after death
***Notes:*** *1 – fibres of striated skeletal muscle. Eyepiece 10×lens 10, stained according to Papenheim-Kryukov*
***Source****: developed by the authors*

Myosymplasts are dark blue in colour, with pronounced transverse striation and clear edges. The nuclei of cells are oblong in shape, their chromatin is concentrated in the depths. The background of drugs is represented by singular erythrocytes around clusters of fibres. 16 hours after the death of cats from group 1, there are no optical changes in skeletal muscle. Cytological preparations contained accumulations of striated muscle fibres. Nuclei of elongated myosymplasts without morphological changes in karyolema, karyoplasm, and chromatin (Fig. 2). Transverse striation was found with clearly structured boundaries of their cytoarchitecture (Fig. 2). The cells were dark blue in colour, and some of them showed indistinct membranes. The background of drugs is a small number of erythrocytes.

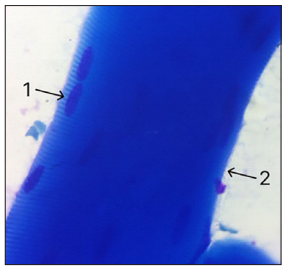



**Figure 2.** Cytogram of skeletal muscle of the cat corpse from group 1, 16 hours after death
***Notes:*** *1 – nuclei in the middle of the muscle fibres; 2 – edges of the muscle fibre with clear borders. Eyepiece 10 × lens 100, stained according to Papenheim-Kryukov*
***Source:*** *developed by the authors*

24 hours after the death of cats from group 1, changes in muscle striation were visualised. On cytological preparations, muscle fibres are arranged in clusters. The shape of individual myosymplast nuclei is fragmented (Fig. 3). The cytoplasm of cells is coloured dark blue, and there is mostly no striation. The indistinct edges of the fibres were stained eosinophilic pink. Against the background of such destructive changes in the structure of muscle fibre cells, isolated nuclei located outside the cytoplasm were observed.

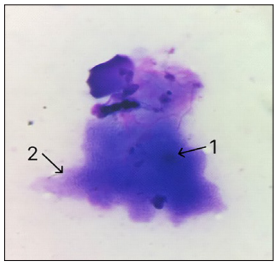



**Figure 3.** Cytogram of the skeletal muscle of the cat corpse from group 1, 24 hours after death
***Notes:*** *1 – fragments of the muscle fibre nucleus; 2 – part of the muscle fibre with an altered internal structure. Eyepiece 10 × lens 100, stained according to Papenheim-Kryukov*
***Source****: developed by the authors*

8 hours after the onset of death of dogs of the group 1, the fibres of their skeletal muscle looked morphologically typical and retained their intravital structure. Dense accumulations of muscle fibres in the form of elongated strands were recorded on cytological preparations (Fig. 4). Myosymplasts are dark blue in colour, with well-defined transverse striation and edges. The nuclei are elongated in shape with tightly concentrated chromatin. The background of cytopreparations is represented by singular erythrocytes around clusters of fibres.

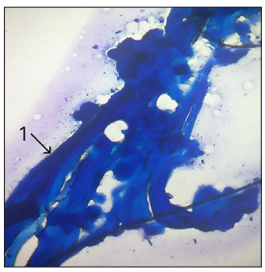



**Figure 4.** Cytogram of the skeletal muscle of the corpse of a dog from group 1, 8 hours after death
***Notes:*** *1 – fibres of striated skeletal muscle. Eyepiece 10 × lens 10, stained according to Papenheim-Kryukov*
***Source****: developed by the authors*

In cytological preparations of muscle tissue from dogs from group 1, obtained 16 hours after death, there were almost no morphological changes in the muscles. The muscle fibres retained their striated structure and were arranged in clusters. Myosymplasts were coloured dark blue, and their edges remained clear (Fig. 5). The transverse striation is well expressed, the nuclei are oblong in shape without noticeable morphological changes in karyoplasm, karyolema, and chromatin. The background of the print preparation is represented by a small number of erythrocytes and isolated nuclei of myosymplasts.

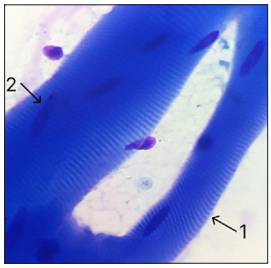



**Figure 5.** Cytogram of the skeletal muscle of the corpse of a dog from group 1, 16 hours after death
***Notes:*** *1 – edges of the muscle fibre; 2 – nucleus in the middle of the muscle fibre. Eyepiece 10 × lens 100, stained according to Papenheim-Kryukov*
***Source:*** *developed by the authors*

24 hours after the onset of death of dogs from group 1, significant changes in the structure of muscle fibres were observed, which were placed in clusters on the slides. The cytoplasm of myosymplasts is coloured dark blue, their striation is mostly absent. The indistinct edges of the fibres change the tinkorial properties towards eosinophilic staining (Fig. 6). The sarcoplasm and individual nuclei in the middle of the fibres changed shape and structure. The background of cytological preparations is protein, grey in colour, represented by isolated nuclei of myosymplasts.

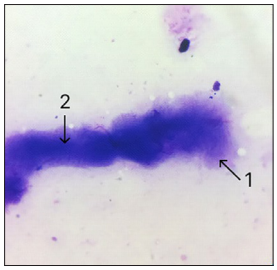



**Figure 6.** Cytogram of the skeletal muscle of the corpse of a dog from group 1, 24 hours after death
***Notes:*** *1 – indistinct edges of the muscle fibre with pink cytoplasm; 2 – part of the muscle fibre with changes in the internal structure. Eyepiece 10 × lens 100, stained according to Papenheim-Kryukov*
***Source:*** *developed by the authors*

During cytomorphological examination of skeletal muscles of corpses of cats from group 2, it was found that as early as 8 hours after death, the muscle fibres looked morphologically typical (Fig. 7). However, a significant number of fibre nuclei located in isolation were found. Cytological preparations are represented by accumulations of striated skeletal muscle fibres in the form of elongated strands. The cells are coloured dark blue, with a slightly altered transverse striation. The boundaries of the sarcoplasm are clearly defined, the nuclei inside are oblong in shape with tightly concentrated chromatin. The background is represented by singular erythrocytes and a large number of extra-fibre nuclei.

16 hours after the death of cats from group 2, changes in skeletal muscle were visualised, which characterised the destruction of cell structure. In addition to compactly arranged striated muscle fibres, numerous nuclei located outside of them were found (Fig. 8). Myosymplasts are coloured dark blue, their edges are blurred, stained in light pink. The transverse striation was almost not observed in comparison with the samples obtained in the previous interval of the experiment. The cell nuclei were mostly not morphologically altered, but single

nuclei with a modified shape were also found. The background of cytological preparations is represented by a significant number of isolated nuclei and blood cells.

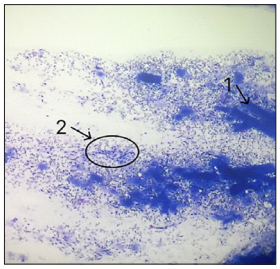



**Figure 7.** Cytogram of the skeletal muscle of the cat corpse from group 2, 8 hours after death
***Notes:*** *1 – fibres of striated skeletal muscle; 2 – accumulations of separated nuclei of muscle fibres. Eyepiece 10 × lens 10, stained according to Papenheim-Kryukov*
***Source:*** *developed by the authors*

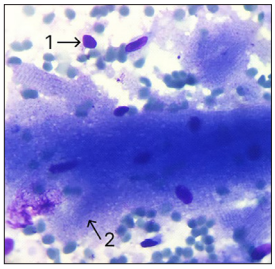



**Figure 8.** Cytogram of the skeletal muscle of the cat corpse from group 2, 16 hours after death
***Notes:*** *1 – isolated nucleus of a muscle fibre; 2 – part of a muscle fibre with changes in its internal structure. Eyepiece 10 × lens 100, stained according to Papenheim-Kryukov*
***Source:*** *developed by the authors*

24 hours after the death of cats in group 2, significant changes in the structure of muscle fibres were observed. The preparations obtained during this period of time showed clusters of muscle fibres with dark blue cells. Their characteristic striation is completely absent, and the edges of the fibres are indistinct (Fig. 9). The shape of the nuclei in the middle of the fibres is morphologically altered. The background of cytological preparations is protein, grey-blue in colour with a large number of fragmented myosymplast nuclei.

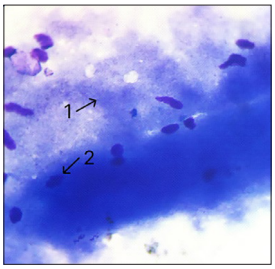



**Figure 9.** Cytogram of the skeletal muscle of the cat corpse from group 2, 24 hours after death
***Notes:*** *1 – muscle fibre with complete loss of internal structure; 2 – fragments of the nucleus in the middle of the muscle fibre. Eyepiece 10 × lens 100, stained according to Papenheim-Kryukov*
***Source:*** *developed by the authors*

During the cytomorphological study of skeletal muscle imprints of corpses of dogs from group 2, it was found that 8 hours after death, the fibres are represented morphologically typically, but with minor destruction of myosymplasts and the formation of extra-fibre nuclear formations (Fig. 10). Cytological preparations contained accumulations of striated

skeletal muscle fibres in the form of elongated strands, in some of them the shape was destructively changed. Skeletal muscle fibres are coloured dark blue, mostly with clear transverse striation and edges. Myosymplast nuclei are elongated in shape with tightly concentrated chromatin. The background of cytological preparations is represented by singular erythrocytes and isolated non-destroyed nuclei.

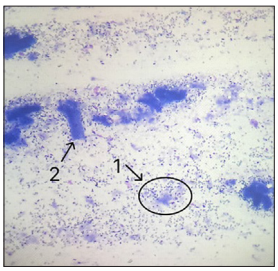



**Figure 10.** Cytogram of the skeletal muscle of the dog corpse from group 2, 8 hours after death
***Notes:*** *1 – accumulation of isolated nuclei of muscle fibres; 2 – fibres of striated skeletal muscle with an altered shape. Eyepiece 10 × lens 10, stained according to Papenheim-Kryukov*
***Source:*** *developed by the authors*

At the 16th hour of the experiment, significant destructive changes in the shape of muscle fibres were detected in the corpses of dogs in group 2. The imprinted preparations contained clusters of striated muscle fibres and separated nuclei arranged in small clusters (Fig. 11). Myosymplasts are coloured dark blue, with blurred fibre edges and a light pink background around the edges. The transverse striation is somewhat blurred compared to samples obtained in previous time intervals. Local fibre regions were observed with significant structural changes in the form of a complete loss of internal structure. The nuclei are mostly without noticeable changes, but some of them are deformed. The background of cytological preparations is represented by isolated muscle fibre nuclei, erythrocytes, and endotheliocytes.

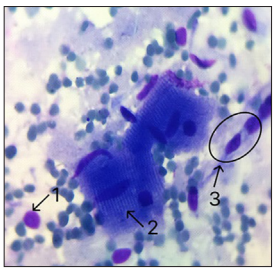



**Figure 11.** Cytogram of the skeletal muscle of the corpse of a dog from group 2, 16 hours after death
***Notes:*** *1 – isolated nucleus of the muscle fibre; 2 – part of the muscle fibre with minor changes in the internal structure; 3 – accumulation of endotheliocytes. Eyepiece 10 × lens 100, stained according to Papenheim-Kryukov*
***Source:*** *developed by the authors*

After 24 hours of the experiment, it was found that myosymplasts of skeletal muscle corpses of dogs from group 2 underwent significant destructive changes not only in terms of shape, but also in their internal structure. Thus, microscopic examination of preparations revealed that the muscle fibres completely lost their transverse striation (Fig. 12). Myosymplasts are dark blue in colour, their edges are blurred, and the preserved nuclei are deformed. The background of such cytological preparations is grey-blue, represented mainly by fragments of isolated nuclei and protein debris.

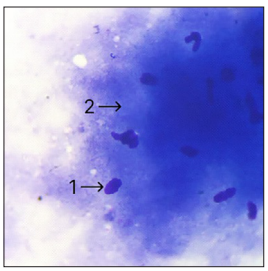



**Figure 12.** Cytogram of the skeletal muscle of the dog corpse from group 2, 24 hours after death
***Notes:*** *1 – accumulation of destroyed muscle fibres; 2 – deformed nucleus of the muscle fibre. Eyepiece 10 × lens 100, stained according to Papenheim-Kryukov*
***Source:*** *developed by the authors*

Cytomorphological examination of the skeletal muscle tissue of the corpses of cats from group 3 revealed that at 8 hours after their death, the muscle fibres looked morphologically typical, but showed an increased number of extra-fibrous, separately located myosymplastic nuclei. The cellular composition of preparations is represented by clusters of myosymplasts of muscle fibres in the form of elongated strands (Fig. 13) and destroyed fibres with separately located nuclei.

Myosymplasts are dark blue in colour, with well-defined transverse striation and clear edges. The nuclei are elongated, and chromatin is placed compactly in them. The background of cytological preparations is represented by singular erythrocytes and isolated nuclei of muscle fibres. 16 hours after the death of cats from group 3, characteristic changes in the structure of myosymplasts began to appear. Cytological preparations contained clusters of striated muscle fibres against the background of a small number of nuclei located outside them (Fig. 14). The fibres are turned dark blue, the contouring of their edges is reduced, the colour is eosinophilic, light pink. The severity of transverse striation is slightly reduced compared to previous samples. The nuclei of myosymplasts located in the sarcoplasm are oblong in shape without noticeable changes in karyolema, chromatin, and karyoplasm.

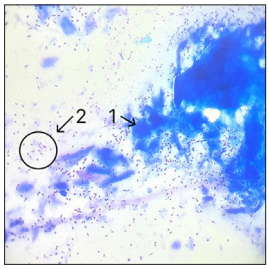



**Figure 13.** Cytogram of the skeletal muscle of the cat corpse from group 3, 8 hours after death

***Notes:*** *1 – skeletal muscle fibre; 2 – cluster of detached myosymplastic nuclei. Eyepiece 10 × lens 10, stained according to Papenheim-Kryukov*

***Source:*** *developed by the authors*

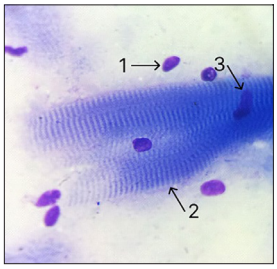



**Figure 14.** Cytogram of the skeletal muscle of the cat corpse from group 3, 16 hours after death

***Notes:*** *1 – isolated nucleus of the muscle fibre; 2 – indistinct edges of the muscle fibre; 3 – nucleus in the middle of the muscle fibre. Eyepiece 10 × lens 100, stained according to Papenheim-Kryukov*

***Source:*** *developed by the authors*

24 hours after the death of cats in group 3, there were significant changes in the clarity of the edges of muscle fibres and the number of nuclei outside them (Fig. 15), and the severity of transverse striation. On the print preparations, a cluster of muscle fibres with sarcoplasma coloured dark blue is visible. Their visual striation is almost invisible, and the edges of the fibres are indistinct. The fibre nuclei are fragmented. The cytological background of such drugs is represented by a large number of nuclei isolated from sarcoplasm.

With regard to the dynamics of changes in the muscle tissue of dogs from group 3, 8 hours after death, it was proved that the muscle fibres were morphologically typical. However, a significant number of extra-fibre nuclei separated from the cytoplasm were observed. On such preparations, accumulations of striated skeletal muscle fibres in the form

of elongated strands are visually noticeable. The cytoplasm of myosymplasts is coloured dark blue. The transverse striation and edges are clearly defined (Fig. 16). The nuclei are elongated in shape, and their chromatin is densely located. The background of cytopreparations is represented by erythrocytes in combination with separated nuclei.

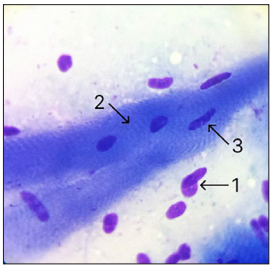



**Figure 15.** Cytogram of the skeletal muscle of the cat corpse from group 3, 24 hours after death
***Notes:*** *1 – nucleus of the muscle fibre separated from the sarcoplasm; 2 – striation of the muscle fibre; 3 – nucleus in the middle of the muscle fibre. Eyepiece 10 × lens 100, stained according to Papenheim-Kryukov*
***Source:*** *developed by the authors*

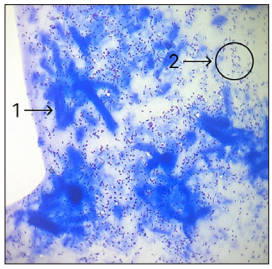



**Figure 16.** Cytogram of the skeletal muscle of the dog corpse from group 3, 8 hours after death
***Notes:*** *1 – fibres of striated skeletal muscle; 2 – accumulations of isolated nuclei of myosymplasts. Eyepiece 10 × lens 10, stained according to Papenheim-Kryukov*
***Source:*** *developed by the authors*

16 hours after the death of dogs in group 3, patterns of cell destruction began to appear in the skeletal muscle specimens. Cytological preparations contained clusters of individual muscle fibres with a significant number of nuclei separated from sarcoplasm (Fig. 17). The fibres are coloured dark blue, and the clarity of their edges is reduced. The severity of transverse striation is slightly reduced or absent in some fibres. Areas with a modified structure and a light pink background along the contour were visualised. Most myosymplast nuclei are elongated without noticeable morphological changes in karyolema and chromatin. The cytological background is represented by isolated nuclei of muscle fibres.

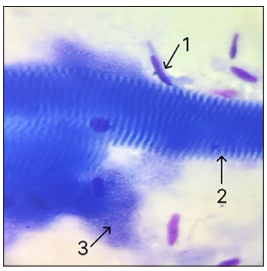



**Figure 17.** Cytogram of the skeletal muscle of the dog corpse from group 3, 16 hours after death
***Notes:*** *1 – the nucleus separated from the sarcoplasm; 2 – transverse striation of the muscle fibre; 3 – part of the muscle fibre with destructive changes in the internal structure. Eyepiece 10 × lens 100, stained according to Papenheim-Kryukov*
***Source:*** *developed by the authors*

The destructive changes that occurred in the skeletal muscle tissue of the corpses of dogs from group 3, 24 hours after death, were characterised by the presence of separated vascular endothelial cell nuclei from the cytoplasm (Fig. 18) and tintorial properties around the edges of fibres and their internal structure. The preparations contained accumulations of muscle fibres. Myosymplasts are dark blue in colour, with reduced striation, indistinct fibre edges, a small number of altered fibre nuclei and nucleols. The cytological background is represented by isolated nuclei of muscle fibres and fragments of blood capillaries.

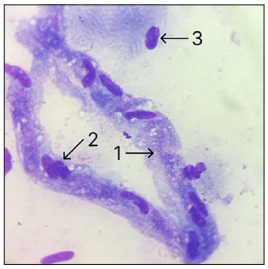



**Figure 18.** Cytogram of the skeletal muscle of the dog corpse from group 3, 24 hours after death
***Notes:*** *1 – cytoplasm of capillary endotheliocytes; 2 – nuclei of capillary endotheliocytes; 3 – nucleus of muscle fibre with destructive changes. Eyepiece 10 × lens 100, stained according to Papenheim-Kryukov*
***Source:*** *developed by the authors*

According to cytomorphological changes in the muscle tissue of corpses of cats in group 4, it was found that 8 hours after their death, skeletal muscle fibres retained a morphologically typical structure. The preparations contained accumulations of striated skeletal muscle fibres in the form of elongated strands (Fig. 19). Myosymplasts are dark blue in colour, with pronounced transverse striation and clear edges. The nuclei are elongated, and their chromatin is densely located. The cellular background of such drugs is represented by singular erythrocytes.

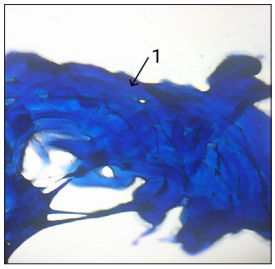



**Figure 19.** Cytogram of the skeletal muscle of the cat corpse from group 4, 8 hours after death
***Notes:*** *1 – fibres of striated skeletal muscle. Eyepiece 10 × lens 10, stained according to Papenheim-Kryukov*
***Source:*** *developed by the authors*

At the same time, 16 hours after death, characteristic changes in the structure of skeletal muscle fibres of corpses of cats in group 4 were observed. Striated muscle fibres located in dense clusters were determined on the print preparations. Sarcoplasma is stained in a dark blue colour with a light pink background around the edges. The transverse striation is not clearly expressed (Fig. 20). The nuclei are oblong in shape without noticeable morphological changes in karyolema and chromatin. The background of cytological preparations is represented by nuclei located outside the visual contour of the sarcolemma of muscle fibres.

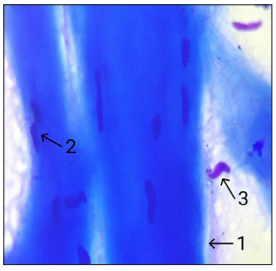



**Figure 20.** Cytogram of the skeletal muscle of the cat corpse from group 4, 16 hours after death
***Notes:*** *1 – indistinct edges of the striated skeletal muscle fibre; 2 – nucleus of the muscle fibre; 3 nucleus of the muscle cell outside the skeletal fibre. Eyepiece 10 × lens 100, stained according to Papenheim-Kryukov*
***Source:*** *developed by the authors*

Twenty-four hours after the onset of death in cats from group 4, significant destructive changes were observed in their muscle tissue compared with the previous interval of the experiment. Thus, tightly placed and deformed muscle fibres were found on the impression preparations. Their sarcoplasm is coloured blue with completely lost banding (Fig. 21). The contours of muscle fibres were characterised by changes in tinctorial properties, a characteristic eosinophilic hue, and indistinct edges. The nuclei were visualised with altered karyolema, irregular geometric shape. However, their number against previously obtained samples is insignificant.

Cytological preparations of skeletal muscles of dogs in group 4 (8 hours after the death of dogs) contained dense clusters of striated skeletal muscle fibres in the form of elongated strands (Fig. 22). Sarcoplasma is coloured dark blue with pronounced transverse striation and clear edges. The nuclei of myosymplasts are also elongated, and the chromatin in the nucleoli is represented by condensed structures.

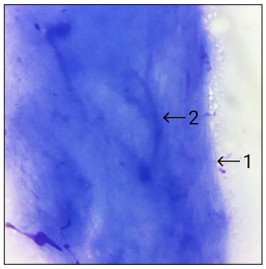



**Figure 21.** Cytogram of the skeletal muscle of the cat corpse from group 4, 24 hours after death
***Notes:*** *1 – indistinct edges of skeletal muscle fibre; 2 – muscle fibre with lost striation. Eyepiece 10 × lens 100, stained according to Papenheim-Kryukov*
***Source:*** *developed by the authors*

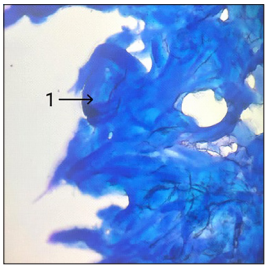



**Figure 22.** Cytogram of the skeletal muscle of the dog corpse from group 4, 8 hours after death
***Notes:*** *1 – fibres of striated skeletal muscle. Eyepiece 10 × lens 10, stained according to Papenheim-Kryukov*
***Source:*** *developed by the authors*

Muscle tissue specimens obtained 16 hours after the death of dogs from group 4 are represented by clusters of striated muscle fibres. Their sarcoplasm turned dark blue, but the contours became eosinophilic. The striation and boundaries of the fibres are not clearly expressed (Fig. 23). A small number of karyol-altered myosymplast nuclei located in the middle of the fibres were recorded. The background of cytological preparations is represented only by isolated nuclei of skeletal muscle fibres.

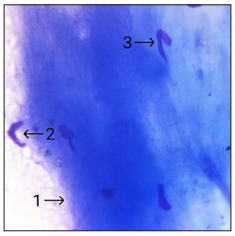



**Figure 23.** Cytogram of the skeletal muscle of the dog corpse from group 4, 16 hours after death
***Notes:*** *1 – indistinct edges of the skeletal muscle fibre; 2 – nucleus outside the muscle fibre; 3 nucleus in the middle of the muscle fibre. Eyepiece 10 × lens 100, stained according to Papenheim-Kryukov*
***Source:*** *developed by the authors*

It was found that within a day after the death of dogs from group 4, significant destructive changes in the structure of skeletal fibres occurred. It was found that myosymplasts were located in the form of significantly deformed clusters. The sarcoplasm was coloured dark blue, its banding and optical boundaries were not visualised (Fig. 24). The shape of the indestructible fibre nuclei is oval. The background of cytological preparations is represented by single, fragmented nuclei of polygonal myosymplasts located outside their visual contour.

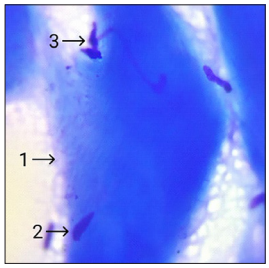



**Figure 24.** Cytogram of the skeletal muscle of the dog corpse from group 4, 24 hours after death
***Notes:*** *1 – indistinct edges of the skeletal muscle fibre; 2 – intact nucleus of the muscle fibre; 3 – nucleus outside the muscle fibre. Eyepiece 10 × lens 100, stained according to Papenheim-Kryukov*
***Source:*** *developed by the authors*

According to the results of microscopy of skeletal muscles of corpses of dogs and cats of all experimental groups, it was proved that the degree of development of destructive changes after 24 hours of the postmortem period does not allow using the cytomorphological method to monitor further biotransformation of myosymplasts. Therefore, the focus of research was shifted to the tissue level of structural organisation of animal corpses.

On the 48$^{th}$ hour after the onset of death in cats from group 1, individual areas of slightly swollen, relaxed myofibrils were found in their skeletal muscles, which were tightly adjacent to each other (Fig. 25), with small gaps between fibres. Total destruction of myofibrils was not observed. In the process of disorganisation of muscle tissue, there was a fairly rapid transition from their jamming to the stage of relaxation. As a result, some areas of myofibrils underwent pronounced postmortem contraction. Marginalisation of myosymplast nuclei in the form of their deformation was observed mainly among clusters located in sarcoplasm. Bacterial colonies formed around such clusters, which apparently enhanced further skeletal muscle autolysis (Fig. 26).

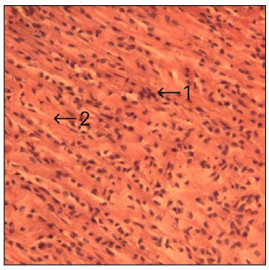



**Figure 25.** Histotopogram of the skeletal muscle of the cat corpse from group 1, 48 hours after death
***Notes:*** *1 – densely located myofibrils; 2 – accumulation of myosymplast nuclei in the middle of the fibres. Eyepiece 10 × 40 lens, hematoxylin and eosin staining*
***Source:*** *developed by the authors*

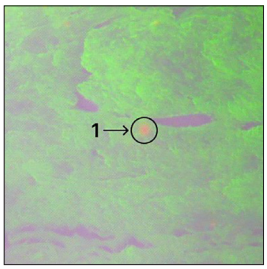



**Figure 26.** Histotopogram of the skeletal muscle of the cat corpse from group 1, 48 hours after death
**Notes:** *1 – bacterial colony. Eyepiece 10 × lens 20, immersion, fluorescein isothiocyanate staining*
***Source:*** *developed by the authors*

At 48 hours after the onset of death in dogs from group 1, significant structural changes were recorded in their skeletal muscles in the form of focal ruptures of individual myofibrils and individual muscle bundles. Isolated myosymplast nuclei were observed between deformed and preserved muscle fibres (Fig. 27), which also underwent destruction with a significant change in the shape of karyolema.

Loci of total destruction of muscle fibres changed their own tinctorial properties, which was reflected in the eosinophilic staining of such histological preparations. There was a natural loss of parallel arrangement of fibres and their significant deformation and fragmentation. Such destroyed areas were somewhat compacted (Fig. 27). Completely destroyed myofibrils also underwent fragmentation. Disorganisation of myofibrils was aggravated by the activation of enzymes, under the influence of which an amorphous mass was formed. The mass fraction in the rapid development of skeletal muscle autolysis processes was played by the bright development of saprotrophs (Fig. 28), which colonised not only on the surface of tissues, but also penetrated deep.

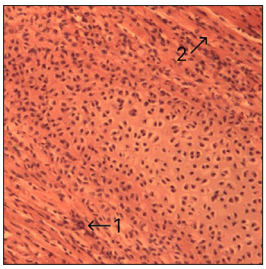



**Figure 27.** Histotopogram of the skeletal muscle of the corpse of a dog from group 1, 48 hours after death
***Notes:*** *1 – accumulation of myosymplast nuclei in the middle of the fibres; 2 – densely placed myofibrils. Eyepiece 10 × 40 lens, hematoxylin and eosin staining*
***Source:*** *developed by the authors*

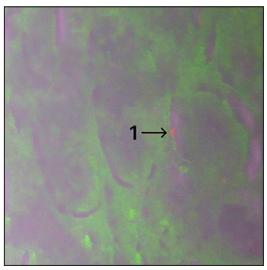



**Figure 28.** Histotopogram of the skeletal muscle of the corpse of a dog from group 1, 48 hours after death
***Notes:*** *1 – bacterial colony. Eyepiece 10 × lens 20, immersion, fluorescein isothiocyanate staining*
***Source:*** *developed by the authors*

The histomorphological examination of skeletal muscle obtained from the corpses of cats and dogs from group 2, 48 hours after death, revealed patterns of destruction typical of the processes of deep autolysis of individual myofibril sections. Marginalisation of myosymplast nuclei was observed among clusters located mainly separately from the sarcoplasm, in the form of loss of their natural rounded shape. Bacterial colonies were formed around such clusters due to cross-contamination (Fig. 29, 30), which increased further disorganisation of muscle fibres. Most of the myofibrils were placed randomly on the area of histological preparations and underwent total decay. However, it should be noted that fragmentation of myofibrils covered only a small part of their total number.

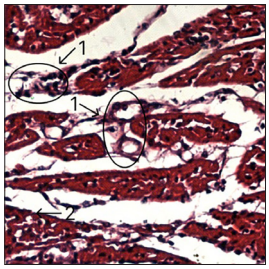



**Figure 29.** Histotopogram of the skeletal muscle of the cat corpse from group 2, 48 hours after death
***Notes:*** *1 – bacterial colonies around marginalised myosymplast nuclei; 2 – fragmented myofibrils. Eyepiece 10 × 40 lens, hematoxylin and eosin staining*
***Source:*** *developed by the authors*

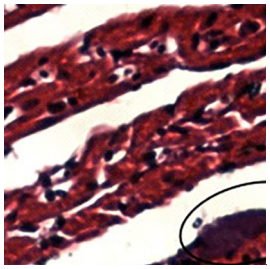


**Figure 30.** Histotopogram of the skeletal muscle of the dog corpse from group 2, 48 hours after death
***Notes:*** *1 – bacterial colonies. Eyepiece 10 × 40 lens, hematoxylin and eosin staining*
***Source:*** *developed by the authors*

It is worth noting that histotopograms of skeletal muscles of cats and dogs 48 hours after the start of the experiment showed changes characteristic of the autolysis stage. Some of the fibres were represented by an "amorphous mass", homogenised, and their transverse striation was not visually manifested. Destructive processes led to the destruction of myofibrils. A significant number of them acquired somewhat thickened, wavy shapes. Their sarcomeric structure remained partially preserved. Further intensive growth of saprotrophs occurred to the same extent as in cat corpses (Fig. 31), and in the dog corpses (Fig. 32).

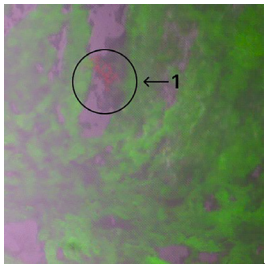



**Figure 31.** Histotopogram of the skeletal muscle of the cat corpse from group 2, 48 hours after death
***Notes:*** *1 – bacterial colonies. Eyepiece 10 × lens 20, immersion, fluorescein isothiocyanate staining*
***Source:*** *developed by the authors*

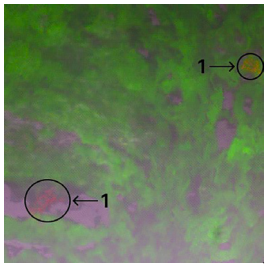



**Figure 32.** Histotopogram of the skeletal muscle of the dog corpse from group 2, 48 hours after death
***Notes:*** *1 – bacterial colonies. Eyepiece 10 × lens 20, immersion, fluorescein isothiocyanate staining*
***Source:*** *developed by the authors*

The study of skeletal muscle samples from cats and dogs from group 3, 48 hours after death, revealed the formation of large gaps due to the crystallisation of fluid localised between myofibril cords and separated muscle fibres. Isolated myosymplast nuclei were arranged in clusters (Fig. 33), marginalised (Fig. 34). Liquid crystals created unevenly placed cavities of different areas in the layers of sarcoplasm, which were deformed due to compression.

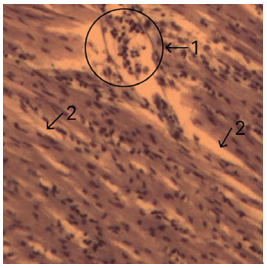



**Figure 33.** Histotopogram of the skeletal muscle of the cat corpse from group 3, 48 hours after death
***Notes:*** *1 – clusters of isolated, marginalised nuclei of myosymplasts; 2 – gaps between muscle filaments. Eyepiece 10 × 40 lens, hematoxylin and eosin staining*
***Source:*** *developed by the authors*

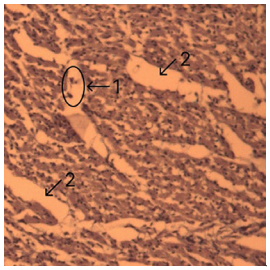



**Figure 34.** Histotopogram of skeletal muscle of a dog corpse from group 3, 48 hours after death
***Notes:*** *1 – isolated nuclei of myosymplasts; 2 –gaps between muscle filaments. Eyepiece 10 × 40 lens, hematoxylin and eosin staining*
***Source:*** *developed by the authors*

The histotopograms of muscle fibre samples from the corpses from group 3 cats, 48 hours after death, looked "loose". The nuclei located in the sarcoplasm of muscle fibres were in a state of total pycnosis. During histochemical identification of glycogen, it was found that it is represented by a fine-grained, deep substance with inherent oxyphilic properties (Fig. 35a), which evenly filled most of the area of histological slides not only in the cavities, but also was located directly in the sarcoplasm of myosymplasts. Gaps formed in the liquid crystallisation sites were observed between the fibres of individual filaments (Fig. 35b). At the stage of muscle relaxation, the phenomenon of «loosening» of fibres was observed, which is explained by the action of autolytic processes.

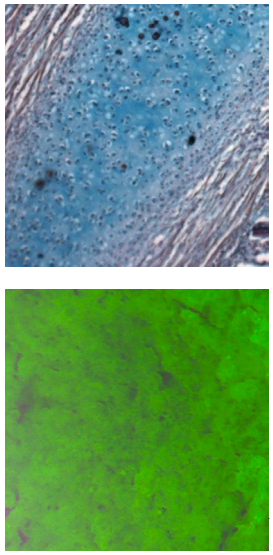



**Figure 35.** Histotopogram of the skeletal muscle of the cat corpse from group 3, 48 hours after death
***Notes:*** *eyepiece 10 × lens 20; a – hematoxylin and eosin staining, Shabadash post-staining; b – immersion, fluorescein isothiocyanate staining*
***Source:*** *developed by the authors*

The histotopograms of muscle fibre samples obtained 48 hours after the death of dogs in group 3 showed some loosening of their structure. The nuclei of myosymplasts were pycnotic, the contours of karyolema were not visually distinguished. The damaging effect of tissue fluid crystals on the sarcolemma was manifested by the development of gaps around the contours of individual muscle filaments. During histochemical identification of glycogen, it was noted that it is evenly distributed over almost the entire area of histological slides and is

represented by a fine-grained lumpy substance with a characteristic oxyphilic colour (Fig. 36a).

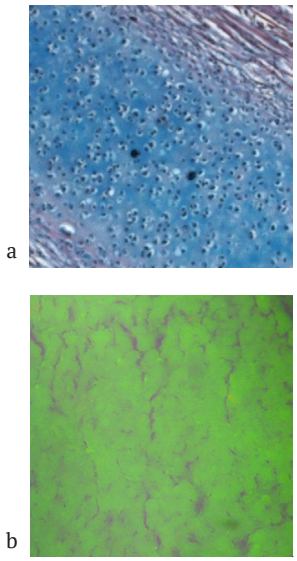


**Figure 36.** Histotopogram of skeletal muscle of a dog corpse from group 3, 48 hours after death
***Notes:*** *a eyepiece 10 × lens 20, hematoxylin and eosin staining, Shabadash post-staining; b eyepiece 10 × lens 20, immersion, fluorescein isothiocyanate staining*
***Source:*** *developed by the authors*

Small gaps were observed between the individual fibres of deformed myofibrils, which formed in the areas of crystallisation of tissue fluid, as a result of which the muscles looked somewhat "loose" (Fig. 36b). 48 hours after death, rapid development of numerous liquid crystals was recorded in tissue samples from the corpses of cats and dogs in group 4, which caused a pronounced total deformation of myofibrils with their rough fragmentation (Fig. 37a). However, their transverse striation was well defined. The muscle fibres were subjected to significant compression, as a result of which some of them looked torn (Fig. 37b).

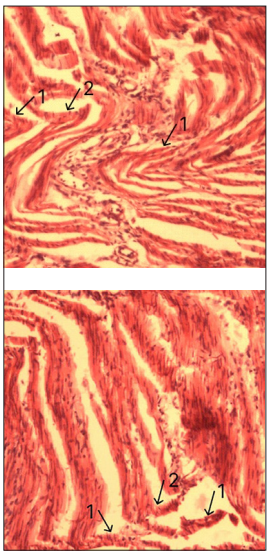



**Figure 37.** Histotopogram of the skeletal muscle of the cat corpse from group 4, 48 hours after death
***Notes:*** *a – 1 – fragments of torn myofibrils; 2 – gaps between myofibrils. Eyepiece 10 × lens 40, hematoxylin and eosin staining; b – 1 – fragments of torn myofibrils; 2 – gaps between myofibrils. Eyepiece 10 × 40 lens, hematoxylin and eosin staining*
***Source:*** *developed by the authors*

Total crystallisation was accompanied by development both inside and outside the sarcoplasm. As a result of the destructive effect of liquid crystals, in the deep layers of skeletal muscles, there was a significant deformation of both muscle fibre bundles and tissue layers between the bundles of myofibrils. The marginal nuclei of myosymplasts remained outside the visual contour of the sarcolemma and were located on histological slides in the form of isolated clusters. Histochemical studies of striated skeletal muscle of cats from group 4, 48 hours after death, revealed that the glycogen substance was localised on histological slides in some limited areas and was a

fine-grained substance with a characteristic oxyphilic colour (Fig. 38).

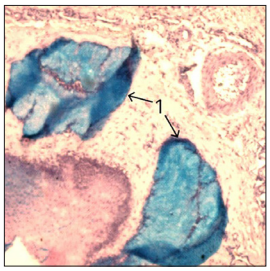



**Figure 38.** Histotopogram of the skeletal muscle of the cat corpse from group 4, 48 hours after death
***Notes:*** *1 – deep structures of glycogen. Eyepiece 10 × lens 20, hematoxylin and eosin staining, Shabadash post-staining*
***Source:*** *developed by the authors*

Significant morphological changes were recorded not only in the surface, but also in the deep layers of muscle tissue. The destructive effect of liquid crystals formed a significant number of large voids (Fig. 39) between myofibrils. Histomorphological examination of skeletal muscles revealed a significant amount of not only surface deformed muscle fibres, but also intramuscular crystallisation with increasing spaces between fibres and sarcolemma breaks.

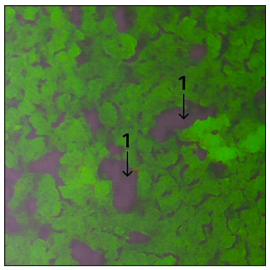



**Figure 39.** Histotopogram of the skeletal muscle of the cat corpse from group 4, 48 hours after death
***Notes:*** *1 – gaps between myofibrils. Eyepiece 10 × lens 20, immersion, fluorescein isothiocyanate staining*
***Source:*** *developed by the authors*

Histochemical studies of striated skeletal muscle from the corpses of dogs in group 4, 48 hours after death, showed that the glycogen substance on histological slides was localised in some limited areas and was represented by a fine-grained substance with a characteristic oxyphilic colour (Fig. 40).

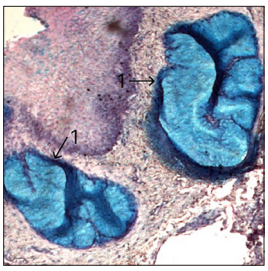



**Figure 40.** Histotopogram of skeletal muscle of a dog corpse from group 4, 48 hours after death
***Notes:*** *1 – deep structures of glycogen. Eyepiece 10 × lens 20, hematoxylin and eosin staining, Shabadash post-staining*
***Source:*** *developed by the authors*

During histomorphological microscopy of skeletal muscles, homogenisation of muscle fibres, a significant number and sufficiently large voids were recorded (Fig. 41) between bundles of muscle fibres in places of fluid crystallisation.

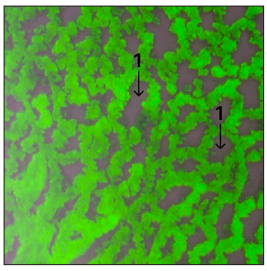



**Figure 41.** Histotopogram of skeletal muscle of a dog corpse from group 4, 48 hours after death
***Notes:*** *1 – gaps between myofibrils. Eyepiece 10 × lens 20, immersion, fluorescein isothiocyanate staining*
***Source:*** *developed by the authors*

Microscopy of histotopograms of skeletal muscles obtained from the corpses of cats and dogs from group 1 at 72 hours after death was similar. The identified patterns indicated deep autolytic processes. Diffuse destruction of the architectonics of muscle fibres caused the development of homogeneous, fragmented, with no signs of histological differentiation, shapeless structures. Areas of destructive changes were found in the form of significant gaps in myofibrils with the development of wide layers between the endomysium and lysis of marginal karyoplasma residues, which had the same general character in the skeletal muscles of cats and dogs. Individual bacteria (Fig. 42) against the background of chaotic enzymatic reactions contributed to further disorganisation of the tissue and manifestly indicated the stage of putrefactive biotransformation of skeletal muscles.

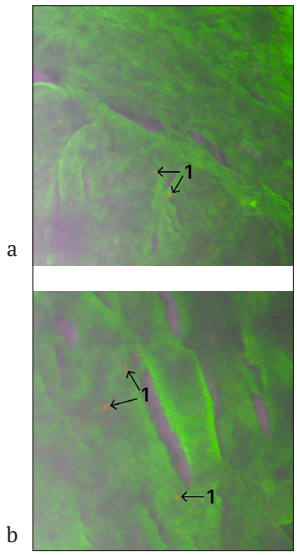



**Figure 42.** Histotopogram of skeletal muscle of corpse from group 1, 72 hours after death
***Notes:*** *a – cat corpse; b – dog corpse; 1 – individual bacteria. Eyepiece 10 × lens 20, immersion, staining with fluorescein isothiocyanate*
***Source:*** *developed by the authors*

72 hours after the onset of death of cats and dogs from group 2, the manifestation of cross-contaminated saprotrophs covered with a common film was observed (Fig. 43).

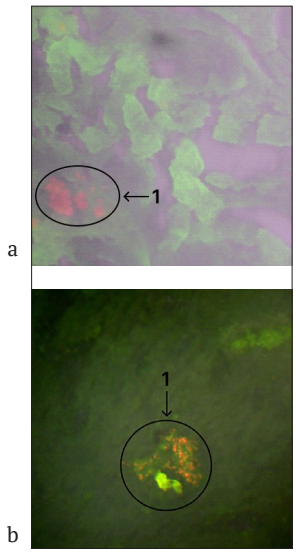



**Figure 43.** Histotopogram of skeletal muscle of corpse from group 2, 72 hours after death
***Notes:*** *a – cat corpse; b – dog corpse; 1 – bacterial colonies. Eyepiece 10 × lens 20, immersion, fluorescein isothiocyanate staining*
***Source:*** *developed by the authors*

Such colonies were recorded in the expanded layers between myofibrils in the same way in histotopograms of muscle tissue from cats and dogs. Patterns of putrefactive biotransformation were found in the form of significant changes in histoarchitectonics and the order of placement of deformed muscle fibres that are subject to diffuse fragmentation and total destruction. In the nuclei of myosymplasts, lysis of the remnants of the totally destroyed karyoplasm and karyolema was observed. Microscopy of histological specimens revealed deep processes of autolysis on the part of the sarcoplasm with structurally destroyed

endomysium and significant ruptures of muscle fibre bundles. This degree of autolysis indicates the final stage of disorganisation of muscle tissue, characterised by diffuse deep homogenisation of the tissue with the formation of an amorphous substance.

According to the results of histomorphological studies of skeletal muscles of corpses of cats from group 3, 72 hours after death, it was found that liquid crystals formed under the influence of low temperature were characterised by significantly smaller sizes, were found in larger quantities and were evenly located over the entire area of histological slides. There were practically no breaks in the integrity of muscle fibres, and the myofibrils themselves were placed tightly, sometimes with slight undulation (Fig. 44). Bacterial contaminants and fibre breaks in the transverse direction were not observed, and the layers between them were insignificant.

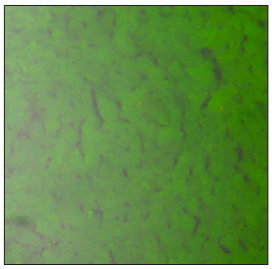


**Figure 44.** Histotopogram of skeletal muscle of the cat corpse from group 3, 72 hours after death

***Notes:*** *eyepiece 10 × lens 20, immersion, fluorescein isothiocyanate staining*

***Source:*** *developed by the authors*

During histochemical identification of glycogen, it was found that it is localised mainly in the centre of the histological slide area in the form of a fine-grained deep substance with oxyphilic staining (Fig. 45).

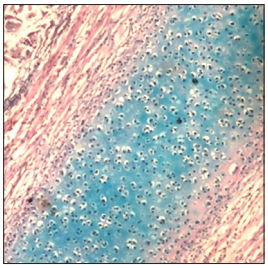


**Figure 45.** Histotopogram of skeletal muscle of the cat corpse from group 3, 72 hours after death

***Notes:*** *eyepiece 10 × lens 20, hematoxylin and eosin staining, Shabadash post-staining*

***Source:*** *developed by the authors*

According to the results of histomorphological studies of skeletal muscles of dog corpses from group 4, 72 hours after death, it was found that liquid crystals formed under the influence of low temperature caused structural changes, as a result of which myofibrils loosened. Between the bundles of muscle fibres, slits filled with liquid crystals were formed, followed by deformation of the fibres, which acquired more rounded shapes (Fig. 46).

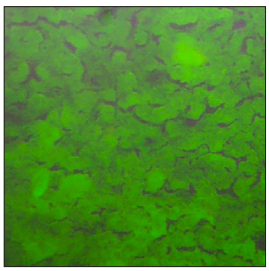


**Figure 46.** Histotopogram of skeletal muscle of a dog corpse from group 3, 72 hours after death

***Notes:*** *eyepiece 10 × lens 20, immersion, fluorescein isothiocyanate staining*

***Source:*** *developed by the authors*

There were practically no breaks in the integrity of muscle fibres, and the myofibrils themselves were placed tightly, sometimes with slight undulation. The number of fibre deformations and sarcolemma breaks in the deep layers of the muscles was significantly less than in the surface muscles. Bacterial contamination and fibre breaks in the transverse direction were not observed, and the gaps between them remained insignificant. During histochemical identification of glycogen, it was found that it is localised mainly in the centre of the histological slide area and is represented by a fine-grained deep substance with oxyphilic staining (Fig. 47).

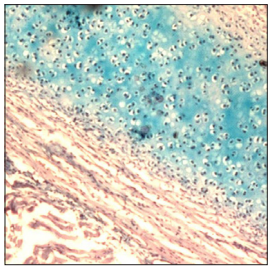


**Figure 47.** Histotopogram of skeletal muscle of a dog corpse from group 3, 72 hours after death
***Notes:*** *eyepiece 10 × lens 20, hematoxylin and eosin staining, Shabadash post-staining*
***Source:*** *developed by the authors*

As a result of histomorphological studies of skeletal muscles of cats from group 4, after 72 hours of postmortem period, it was found that there were no patterns of bacterial contamination at the onset of death under such conditions. However, numerous fluid crystals were formed evenly between the muscle fibres and bundles, which damaged the sarcolemma and caused severe deformation of the myofibrils (Fig. 48).

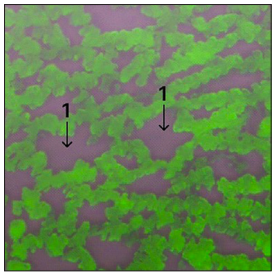



**Figure 48.** Histotopogram of the skeletal muscle of the cat corpse from group 4, 72 hours after death
***Notes:*** *1 – gaps between myofibrils. Eyepiece 10 × lens 20, immersion, fluorescein isothiocyanate staining*
***Source:*** *developed by the authors*

The residues of the deep glycogen substance on histological slides were localised in areas with a small area and defined as a fine-grained substance with an oxyphilic colour (Fig. 49).

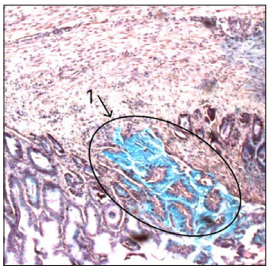



**Figure 49.** Histotopogram of the skeletal muscle of the cat corpse from group 4, 72 hours after death
***Notes:*** *1 – condensed glycogen structures. Eyepiece 10 × lens 20, hematoxylin and eosin staining, Shabadash post-staining*
***Source:*** *developed by the authors*

In the case of histomorphological studies of skeletal muscles of dog corpses from group 4, no bacterial contamination was detected after 72 hours of postmortem. Numerous liquid crystals formed in the fabric under such conditions

caused compression of the fibres, their breaks and deformation. Due to prolonged exposure to excessively low temperatures, shrinkage processes occurred in the muscle tissue, which contributed to the development of large voids between the muscle fibres (Fig. 50).

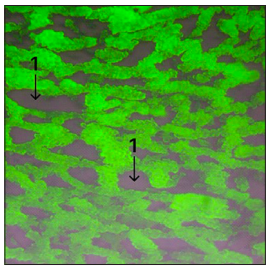



**Figure 50.** Histotopogram of the skeletal muscle of the dog corpse from group 4, 72 hours after death
***Notes:*** *1 – gaps between myofibrils. Eyepiece 10 × lens 20, immersion, fluorescein isothiocyanate staining*
***Source:*** *developed by the authors*

Glycogen residues in the deep substance on histological slides were localised in areas with a small area as a fine-grained substance with oxyphilic staining (Fig. 51).

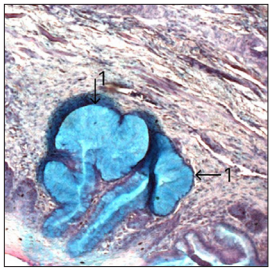



**Figure 51.** Histotopogram of the skeletal muscle of the dog corpse from group 4, 72 hours after death
***Notes:*** *1 – condensed glycogen structures. Eyepiece 10 × lens 20, hematoxylin and eosin staining, Shabadash post-staining*
***Source:*** *developed by the authors*

As a result of the conducted studies, it was established that disorganisation of skeletal muscles of corpses of cats and dogs of all experimental groups developed in the following sequence and in the corresponding time ranges of the postmortem period: muscle stiffening, muscle relaxation, and muscle destruction.

Researchers like T. Du *et al.* (2018), B.P.H. Righetti *et al.* (2022) and R. Liu *et al.* (2022), note the importance of morphological changes in animal organisms observed in various types of injuries and poisoning. However, their research shows that these morphological changes are mainly caused by chaotic enzymatic reactions and a lack of energy resynthesis in cells. This means that after damage or poisoning, the body's cells can go into a state of chaos, where enzymatic reactions become disorganised and uncontrolled. This can lead to the destruction of cellular structures and organs. in addition, the influence of saprotrophs (organisms that decompose organic substances) can play an important role in the processes of thanatogenesis. Saprotrophs can actively decompose organic substances, which can lead to the build-up of toxic products and further deterioration of the body's condition.

Despite the variety of factors that can cause injury or poisoning, the researchers also emphasise that the main link of thanatogenesis in animals is the development of mixed hypoxia and cerebral anoxia, as stated by G. Piegari *et al.* (2019), A.F.M. Botelho *et al.* (2020) and M.A. King *et al.* (2021). Hypoxia indicates insufficient oxygen supply to the cells, and cerebral anoxia means a lack of oxygen for the brain. These conditions can result from various types of injuries or poisoning and can lead to serious disorders in the body. It is proved that the intensity of the manifestation of patterns of postmortem disorganisation of skeletal neck

muscles of corpses of dogs and cats was directly dependent on the influence of environmental conditions and the nature of fatal damaging factors, which is reflected in Table 1.

**Table 1.** Dynamics of skeletal neck muscle disorganisation patterns (units) of dog and cat corpses in the time ranges of the postmortem period

| Time range (hours) | Dog corpses (n=16) | | | | Cat corpses (n=12) | | | |
|---|---|---|---|---|---|---|---|---|
| | Group 1 | Group 2 | Group 3 | Group 4 | Group 1 | Group 2 | Group 3 | Group 4 |
| 2-8 | - | 2.66 ±0.20 | 10.07 ±0.11 | 16.53 ±0.09 | - | 2.56 ±0.16 | 11.83 ±0.22 | 14.87 ±0.25 |
| 8-16 | 0.91 ± 0.07 | 6.71 ±0.34*** | 16.07 ±0.09*** | 18.59 ±0.12*** | 0.70 ±0.12 | 6.64 ±0.36*** | 13.86 ±0.08*** | 22.37 ±0.14*** |
| 16-24 | 1.50 ±0.09** | 11.36 ±0.71*** | 16.70 ±0.06*** | 22.93 ±0.06*** | 1.50 ±0.15** | 11.77 ±0.58*** | 16.16 ±0.05*** | 23.40 ±0.54 |
| 24-48 | 2.49 ±0.13*** | 16.69 ±0.89* | 17.79 ±0.07*** | 27.93 ±0.41*** | 1.66 ±0.10 | 13.76 ±0.29* | 18.33 ±0.04 *** | 27.09 ±0.55** |
| 48-72 | 3.17 ±0.10** ^^ | 22.10 ±0.78*** ^^^ | 18.47 ±0.04*** ^^ | 38.54 ±0.16*** ^^^ | 2.91 ±0.32** ^^ | 22.84 ±1.15*** ^^^ | 18.71 ±0.04*** ^^ | 36.74 ±0.43*** ^^^ |

***Notes:*** **P<0.05, **P<0.01, ***P<0.001 compared to the indicators of the previous study interval within the experimental group; ^P<0.05, ^^P<0.01, ^^^P<0.001 compared between the first and last indicators within the experimental group*

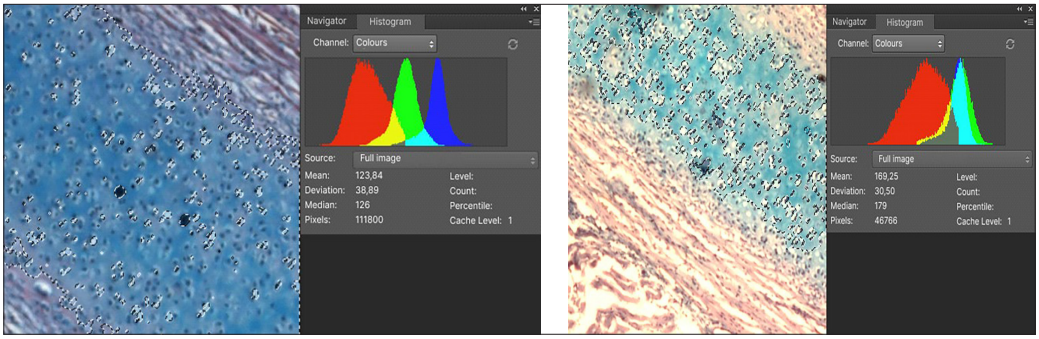
***Source:*** *developed by the authors*

The data in Table 1 show that there are common patterns of postmortem neck muscle disorganisation in dog and cat corpses. In all experimental groups of dog corpses, quantitative indicators significantly increased during the corresponding time intervals of observation, but with different intensity. Similar to changes in dog corpses, in all study groups of cat corpses, muscle fibre biotransformation rates also increased significantly during the corresponding follow-up time periods. The intensity of such growth is variable and was directly dependent on the conditions of death of the animal.

W. Li *et al*. (2023) note that under the influence of hydrolases, the hydrogen index of muscle tissue changes, favourable conditions are created for contamination with bacteria, which cause further biotransformation and autolysis of skeletal muscles, which explains these phenomena. W. Zhu *et al*. (2021) argue that post-mortem autolysis of muscle tissue is caused by the destructive effects of a number of enzymes that cause skeletal muscle stiffness, their progressive destruction, and accumulation of catabolism products. However, the study suggests that the destructive effects of enzymes are interrelated. Their role in the corresponding time ranges for the development of postmortem disorganisation of cellular and tissue components of muscles varies.

Based on the results of histochemical staining according to Shabadash, the pattern of a gradual decrease in the glycogen content by the indicator of its distribution over the area of the histotopogram is proved. N. Wenzlow *et al*. (2023) note that glycogen, especially in hypothermia, is sensitive to impaired muscle oxygenation, and, therefore, it is a predictor of the development of tissue hypoxia, which is accompanied by a slowdown in phosphorylation, which is consistent with obtained data. The variability of glycogen content directly depended on the duration of the postmortem period, if the death of the animal was

caused by excessive exposure to low ambient temperatures. In connection with the results obtained, it can be assumed that at the beginning of general hypothermia of the animal, the changes that occur are reversed, which is consistent with the results obtained by C-C. Liao *et al*. (2016) and P. Listos *et al.* (2017). Based on the results of histogram evaluation, it was detailed that the enzymatic processes of glycogenolysis during prolonged exposure to excessively low temperatures were slower in dog corpses (Fig. 52 a, b) and cats (53 a, b) from group 3 compared to such changes in the corpses of animals from group 4.

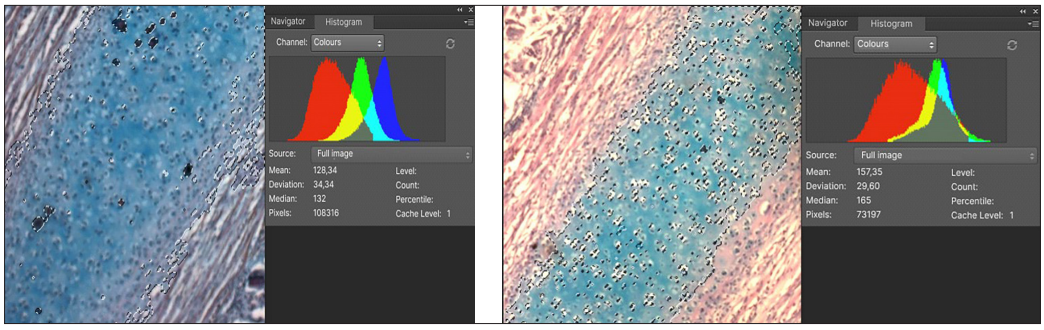



a b

**Figure 52.** Histological preparation of skeletal muscle of a dog corpse from group 3 and histogram of glycogen distribution

***Notes:*** *a – 48 hours after death, b – 72 hours after death.*

***Source:*** *developed by the authors*



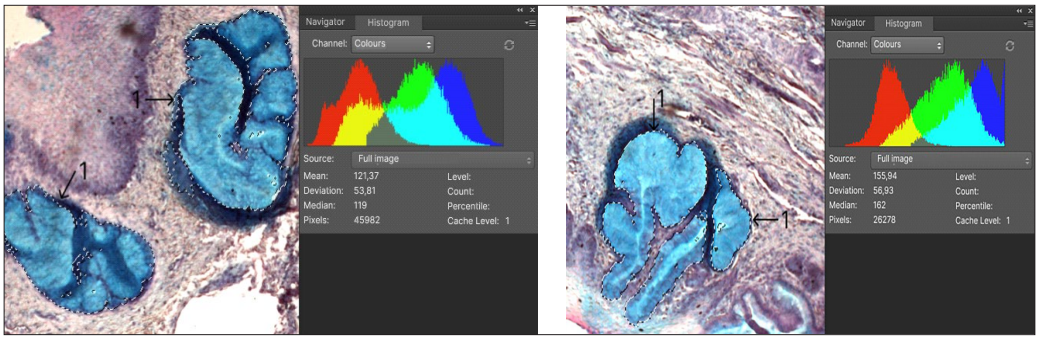

a b

**Figure 53.** Histological specimen of skeletal muscle from a cat corpse from group 3 and histogram of glycogen distribution

***Notes:*** *a – 48 hours after death, b – 72 hours after death*

***Source:*** *developed by the authors*

According to histochemical studies of skeletal muscles of dog (Fig. 54 a, b) and cat (Fig. 55 a, b) corpses from group 4 found that the vast majority of glycogen was decomposed to low-molecular-weight compounds over the time range – from 48 up to 72 hours after the death of animals from freezing.

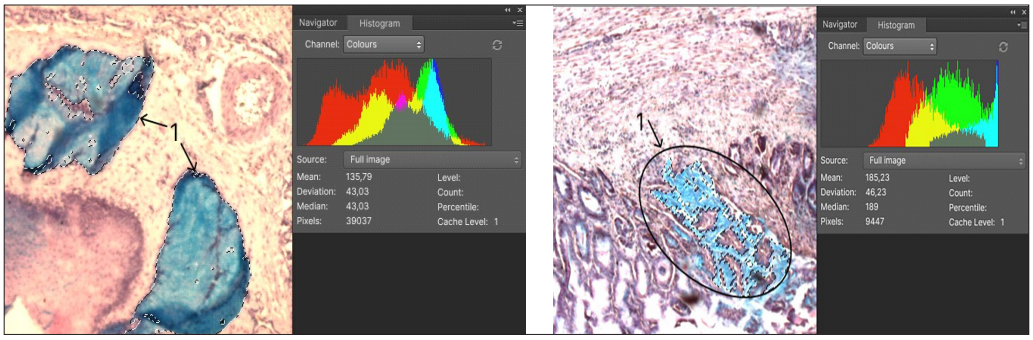



a b

**Figure 54.** Histological preparation of skeletal muscle of a dog corpse from group 4 and histogram of glycogen distribution

***Notes:*** *a – 48 hours after death, b – 72 hours after death; 1 – condensed glycogen structures*

***Source:*** *developed by the authors*




a b

**Figure 55.** Histological preparation of skeletal muscle of the cat corpse from group 4 and histogram of glycogen distribution

***Notes:*** *a – 48 hours after death, b – 72 hours after death; 1 – condensed glycogen structures*

***Source:*** *developed by the authors*

The results of quantitative changes in glycogen content in the neck myosymplasts of dogs and cats whose death occurred under conditions of general hypothermia were characterised by a tendency to a rapid decrease in the level of the latter. They were directly dependent on the increase in the duration of death. Therefore, their authors considered them basic. The established facts about the informative value of postmortem biotransformation of skeletal muscles of animal corpses to find out the causes and duration of the post-mortem period for hypoxic thanatogenesis should be applied in the practical activities of a forensic veterinary expert to solve situational problems with similar adverse consequences.

## Conclusions

The results of postmortem biotransformation of skeletal muscles of animal corpses are important for elucidating the causes and duration of the postmortem period for hypoxic thanatogenesis in dogs and cats. The "intermediate" values of the dynamics of the indicators of the patterns of disorganisation of the skeletal muscles of the neck of dogs and cats, statistically reflected in different time ranges of the early postmortem period, outline the identified regular trends and allow diagnosing the time of death of the animal during the first three days of the postmortem period according to two forensic veterinary criteria: "the number of patterns of disorganisation of the neck muscles of dog and cat corpses" and "the level of glycogen content in the neck muscles of dog and cat corpses" with maximum approximation and minimum error, which, according to the principles of evidence-based veterinary medicine, correspond to expert information.

It is proved that the biotransformation of skeletal muscles of all the studied animal corpses proceeds in the same direction from the stage of postmortem stiffness, through the stage of muscle relaxation to their final autolysis. However, the attention is focused on the different duration of such stages, depending on the conditions of death. Postmortem changes in muscle tissue up to 24 hours after the onset of animal death are accompanied by its dehydration, compaction, and contraction of myofibrils. Skeletal muscle relaxation occurs between 24 and 48 hours after death and is characterised by stretching and swelling of the fibres. Under the influence of saprotrophs and autolytic processes, deep changes in the structure and composition of muscle fibres occur in the interval from 48 to 72 hours after the death of animals. For the first time in Ukraine, based on the results of a study of the dynamics of structural and biochemical changes in symplasts of skeletal neck muscles of dogs and cats, an algorithm was developed, the value of which consists of in the use of the identified relationship between the degree of disorganisation and the 72-hour time range after the onset of animal death in order to solve forensic veterinary diagnostic problems.

In the future, a promising area is to investigate the informative value of other thanatognomonic criteria for determining the causes and time of death of dogs and cats during a comprehensive forensic veterinary assessment of the phenomena of biotransformation of corpses, considering their various conditions.

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## Conflict of Interest

None.

## References

[1] Abbate, J., Grifò, G., Capparucci, F., Arfuso, F., Savoca, S., Cicero, L., Consolo, G., & Lanteri, G. (2022). Postmortem electrical conductivity changes of *Dicentrarchus labrax* skeletal muscle: Root mean square (RMS) parameter in estimating time since death. *Animals (Basel)*, 12(9), article number 1062. doi: 10.3390/ani12091062.

[2] Botelho, A.F.M., Machado, A.M.D., da Silva, R.H.S., Faria, A.C., Machado, L.S., Santos, H., Braga, S.D.M., Torres, B.B.J., Miguel, M.P., Chaves, A.R., & Melo, M.M. (2020). Fatal metaldehyde poisoning in a dog confirmed by gas chromatography. *BMC Veterinary Research*, 16(1), article number 139. doi: 10.1186/s12917-020-02348-w.

[3] DSTU ISO/IEC 17025:2019. (2021). *General requirements for the competence of testing and calibration laboratories.* Retrieved from http://surl.li/rmsby.

[4] Du, T., Lin, Z., Xie, Y., Ye, X., Tu, C., Jin, K., Xie, J., & Shen, Y. (2018). Metabolic profiling of femoral muscle from rats at different periods of time after death. *PLoS ONE,* 13(9), article number e0203920. doi: 10.1371/journal.pone.0203920.

[5] European Association for Animal Research. (n.d.). Retrieved from https://www.eara.eu/animal-research-law.

[6] Geissenberger, J., Ehrenfellner, B., Monticelli, F.C., Pittner, S., & Steinbacher, P. (2021). Dismembered porcine limbs as a proxy for postmortem muscle protein degradation. *International Journal of Legal Medicine*, 135(4), 1627-1636. doi: 10.1007/s00414-021-02571-6.

[7] Horalskyi, L.P., Khomych, V.T., & Kononskyi, O.I. (2015). *Fundamentals of histological technique and morphofunctional methods of research in norm and at pathology*. Zhytomyr: "Polissia".

[8] Kazantsev, R., & Yatsenko, I. (2022). Forensic veterinary diagnosis of dog's fatal poisoning with anticoagulant rodenticide – bromadiolone. *Scientific Bulletin of Veterinary Medicine*, 1(173), 120-136. doi: 10.33245/2310-4902-2022-173-1-120-136.

[9] King, M.A., Alzahrani, J.M., Clanton, T.L., & Laitano, O.A. (2021). Preclinical model of exertional heat stroke in mice. *Journal of Visualized Experiments,* 1(173). doi: 10.3791/62738.

[10] Lamri, M., Malva, A.D., Djenane, D., Albenzio, M., & Gagaoua, M. (2023). First insights into the dynamic protein changes in goat Semitendinosus muscle during the post-mortem period using high-throughput proteomics. *Meat Science*, 202, article number 109207. doi: 10.1016/j.meatsci.2023.109207.

[11] Li, W., Xing, Y., Gan, L., Peng, W., & Deng, S. (2023). Exploring the value of microorganisms in the appendix for inferring postmortem interval in Sprague-Dawley rats using high-throughput sequencing. *Journal of Forensic Sciences*, 68(1), 163-175. doi: 10.1111/1556-4029.15173.

[12] Liao, C.C., Chang, Y.S., Yang, S.Y., & Chou, R.G.R. (2016). Post-mortem proteolysis and tenderisation are more rapid and extensive in female duck breast muscle. *British Poultry Science,* 57(6), 734-739. doi: 10.1080/00071668.2016.1209736.

[13] Listos, P., Gryzinska, M., Batkowska, J., Dylewska, M., Dudzinska, E., & Piorkowski, J. (2017). Preliminary study on the estimation of the time of death in animals based on microflora development in a canine's gastrocnemius muscle. *Medycyna Weterynaryjna*, 73(4), 229-233. doi: 10.21521/mw.5677.

[14] Liu, R., Wang, Q., Zhang, K., Wu, H., Wang, G., Cai, W., Yu, K., Sun, Q., Fan, S., & Wang, Z. (2022). Analysis of postmortem intestinal microbiota successional patterns with application in postmortem interval estimation. *Microbial Ecology,* 84(4), 1087-1102. doi: 10.1007/s00248-021-01923-4.

[15] Paltian, J.J., da Fonseca, C.A.R., Pinz, M.P., Luchese, C., & Wilhelm, E.A. (2019). Post-mortem interval estimative through determination of catalase and Δ-aminolevulinate dehydratase activities in hepatic, renal, skeletal muscle and cerebral tissues of Swiss mice. *Biomarkers*, 24(5), 478-483. doi: 10.1080/1354750X.2019.1619837.

[16] Piegari, G., De Pasquale, V., d'Aquino, I., De Biase, D., Caccia, G., Campobasso, C. P., Tafuri, S., Russo, V., & Paciello, O. (2023). Evaluation of muscle proteins for estimating the post-mortem interval in veterinary forensic pathology. *Animals (Basel),* 13(4), article number 563. doi: 10.3390/ani13040563.

[17] Piegari, G., De Biase, D., d'Aquino, I., Prisco, F., Fico, R., Ilsami, R., Pozzato, N., Genovese, A., & Paciello, O. (2019). Diagnosis of drowning and the value of the diatom test in veterinary forensic pathology. *Frontiers in Veterinary Science*, 6, article number 404. doi: 10.3389/fvets.2019.00404.

[18] Raskin, R.E., Meyer, D., & Boes, K.M. (2022). *Canine and feline cytopathology: A color atlas and interpretation*. Amsterdam: Elsevier.

[19] Righetti, B.P.H., Lima, D., Dias, V.H.V. Mattos, J.J., Piazza, C.E., Vilas-Boas, L. O.B., Alves, T.C., Almeida, E.A., Lüchmann, K.H., & Bainy, A.C.D. (2022). Life after death? Exploring biochemical and molecular changes following organismal death in green turtles, *Chelonia mydas* (Linnaeus, 1758). *Chemosphere*, 308, article number 136569. doi: 10.1016/j.chemosphere.2022.136569.

[20] Shi, F.X., Li, P., Lu, H.L., Li, N., Du, Q.X., Wang, Y.Y., & Sun, J.H. (2020). Expression of autophagy-associated protein in rat muscle tissues after antemortem and postmortem injury. *Fa Yi Xue Za Zhi,* 36(3), 293-298. doi: 10.12116/j.issn.1004-5619.2020.03.001.

[21] Skrypka, M., Boyko, Y., Zapeka, I., & Golovan, K. (2023). The practice of forensic-veterinary examination of psycho-emotional stress in the genesis of insufficiency and death of animals. *Agrarian Bulletin Black Sea Littoral*, 106, 14-22. doi: 10.37000/abbsl.2023.106.02.

[22] Wenzlow, N., Mills, D., Byrd, J., Warren, M., & Long, M.T. (2023). Review of the current and potential use of biological and molecular methods for the estimation of the postmortem interval in animals and humans. *Journal of Veterinary Diagnostic Investigation,* 35(2), 97-108. doi: 10.1177/10406387231153930.

[23] Zheng, Z., Zhai, X.D., Xia, Z.Y., Li, Y.L., Zhao, L.L., Liu, H.L., & Mo, Y.N. (2019). Relationship between electrical conductivity and chemical content of rat skeletal muscle impregnating solution and postmortem interval. *Fa Yi Xue Za Zhi*, 35(5), 572-575. doi: 10.12116/j.issn.1004-5619.2019.05.011.

[24] Zhu, W., Zhai, X., Zheng, Z., Sun, K., Yang, M., & Mo, Y. (2021). New contributions to the relationship between sequential changes of ATP-related metabolites and post-mortem interval in rats. *Legal Medicine (Tokyo)*, 48, article number 101809. doi: 10.1016/j.legalmed.2020.101809.

# Інформативність постмортальної динаміки скелетних м'язів трупів собак і котів для судово-ветеринарної діагностики смерті внаслідок гострої гіпоксії

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**Анотація.** Актуальність дослідження зумовлена потребою у практиці судово-ветеринарної експертизи встановити давність настання смерті собак і котів під час розслідування злочинів проти здоров'я і життя тварин. У зв'язку з цим мета роботи – це вирішення діагностичних судово-ветеринарних завдань щодо інформативності постмортальної дезорганізації скелетних м'язів шиї трупів собак і котів упродовж 72 годин після смерті тварин. Основними методами дослідження діагностичної інформативності постмортальної динаміки скелетних м'язів трупів собак і котів для судово-ветеринарного встановлення умов і давності настання смерті внаслідок гострої гіпоксії є ті, за якими можна комплексно розглянути виокремлену проблему, зокрема, фарбування мазків-відбитків за Папенгеймом-Крюковим, слайдів – гематоксиліном та еозином, флуоресцеїна ізотіоцианатом і за Шабадашем. Обґрунтовано судово-ветеринарне діагностичне значення двох експертних критеріїв: «кількість патернів дезорганізації м'язів шиї трупів собак і котів» і «вміст глікогену в м'язах шиї трупів собак і котів». Доведено, що біотрансформація скелетних м'язів відбувається у наступній послідовності: упродовж перших 24 годин після смерті тварини настає посмертне заклякання м'язів трупів, що супроводжується дегідратацією, ущільненням, скороченням міофібрил; розслаблення м'язів спостерігається з 24 до 48 години після смерті, міофібрили розтягнуті й набряклі; з 48 до 72 години під впливом сапротрофів реєструється аутоліз м'язів. Встановлено, що деструкція скелетної м'язової тканини відбувається інтенсивніше у разі смерті внаслідок задушення порівняно зі смертю від отруєння, а деструкція м'язової тканини динамічніша у разі смерті внаслідок замерзання порівняно зі смертю, спричиненою утопленням у холодній воді. Вперше отримані результати сприятимуть вирішенню діагностичних судово-ветеринарних завдань, зокрема, встановленню давності та умов настання смерті собак і котів під час розслідування правоохоронними органами злочинів стосовно жорстокого поводження з тваринами

**Ключові слова:** асфіксія; цитоморфологічне дослідження; гістотопограми; патерни деструкції; танатогенез; жорстоке поводження

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