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THE EFFECTIVENESS OF CURRENT TREATMENT METHODS FOR COCCIDIOSIS IN PIGS

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Summary. Intestinal dysbiosis and coccidiosis are common conditions in piglets, characterized by disturbances to the microbial community and reduced productivity in animals. Consequently, there is an urgent need to identify effective treatment regimens that eliminate pathogens, restore the intestinal microbiota, and enhance the body's resistance. This study aimed to develop and compare a comprehensive treatment regimen involving sulfadimethoxine and a *Bacillus* spore-forming probiotic for suckling piglets with coccidiosis. Piglets suffering from coccidiosis in the first experimental group were administered sulfadimethoxine orally at a dose of 25 mg/kg once daily for five days. Animals in the second experimental group were administered sulfadimethoxine at the same dose and a *Bacillus* spore-forming probiotic at a dose of two milliliters per animal for 21 days. The results demonstrate that including the *Bacillus* spore-forming probiotic in the treatment regimen normalizes the intestinal microbiota, as evidenced by a reduction in opportunistic microflora and a significant increase in obligate anaerobe populations. Complete clearance of coccidia and elimination of *Candida albicans* was achieved in the second experimental group (sulfadimethoxine + *Bacillus*), confirming a pronounced therapeutic effect compared to monotherapy (sulfadimethoxine)

Keywords: piglets, gut microbiota, sulfadimethoxine, *Bacillus* spore-forming probiotic

Introduction. Coccidiosis is a common parasitic disease in pigs that often goes unnoticed in its early stages. However, its impact on the health of piglets and the overall economics of pig farming can be quite significant. It is a protozoan disease primarily caused by *Cystoisospora suis*, which affects the intestinal mucosa of young piglets, leading to impaired absorption, stunted growth, and increased susceptibility to secondary infections (Hinney et al., 2020; Jankowska-Makosa et al., 2023; Bohach, Paliy and Bogach, 2024).

Despite the low mortality rate of pigs from coccidiosis, it can lead to significant economic losses due to reduced daily weight gain (up to a 20% loss in body weight), poor feed conversion, and deteriorated performance indicators in infected animals during subsequent stages of production (Bogach, Paliy and Bogach, 2022; De Alencar Rezende et al., 2026). Furthermore, coccidiosis directly compromises piglets' immunity. It increases the risk of infection with other enteropathogens, such as rotavirus, transmissible gastroenteritis virus, *Clostridium*, and *Escherichia coli*, leading to a more severe clinical course and increased mortality (Chaudhary, Parajuli and Dhakal, 2023; Bohach et al., 2023; Han et al., 2024).

One of the traditionally used strategies for controlling bacterial and protozoal infections in piglets is the use of sulfonamide antibiotics, such as sulfadimethoxine, a broad-spectrum antimicrobial agent that acts bacteriostatically by competitively inhibiting folic acid synthesis (Joachim and Mundt, 2011). Against the backdrop of antimicrobial use, the use of probiotics has become widespread (Gujvinska and Paliy, 2018; Zhao et al., 2025). Probiotic microorganisms of the genus *Bacillus* are of interest as a means of optimizing the intestinal microbiota, increasing resistance to pathogens, and improving growth parameters. *Bacillus* probiotics — spore-forming strains such as *B. subtilis, B. pumilus, B. amyloliquefaciens*, and others — are capable of surviving in the aggressive conditions of the digestive tract, potentially improving the structure of the intestinal mucosa and enhancing barrier and enzymatic functions (Vieira et al., 2021; Mazur-Kuśnirek et al., 2023; Tang et al., 2024).

The combined use of sulfadimethoxine and *Bacillus* probiotics for piglets is a comprehensive approach. This drug has antiparasitic and antimicrobial effects and is aimed at eliminating or suppressing pathogenic microflora and parasites, while probiotics help maintain and restore beneficial gut microbiota, especially after antibiotic therapy. This strategy may be accompanied by a reduction in the negative consequences of antibiotic-induced dysbiosis, a decrease in clinical manifestations of diarrhea, and a faster restoration of normal digestive tract function, which is critical during the weaning period and contributes to improved performance indicators in young animals.

The study **aimed** to develop and comparatively apply a comprehensive treatment regimen using the drug sulfadimethoxine and the *Bacillus* spore-forming probiotic for coccidiosis in piglets.

Materials and methods. A study was conducted at a pig farm in Poltava Region to determine the therapeutic

efficacy of eimeriostats against coccidiosis in piglets. The efficacy of the drugs was evaluated in 60 infected Large White piglets, aged 14–21 days and weighing 3.5–6.5 kg. The piglets were divided into three groups: two experimental groups of 20 piglets each and one control group of 20 piglets. Ten experimental piglets were selected for coprological and microbiological studies, and 10 g of fecal samples were collected from their rectums. Coccidial oocysts were identified using the Fulleborn flotation method (DSSU, 2009). The intensity of *Cystoisospora suis* infection in the piglets ranged from 88.9 to 92.2 oocysts per gram of feces. Microbiological studies were conducted to determine the intestinal microbiota of the piglets before and after treatment for coccidiosis. Intestinal microbiocenosis in piglets with coccidiosis was determined before and after treatment, in accordance with DSTU 8703-2:2017 (SE 'UkrNDNC', 2018). Laboratory studies were conducted at the Laboratory of Swine Diseases of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Kharkiv, Ukraine) using modern methods.

The piglets in group Experimental I were given the drug sulfadimethoxine orally at a dose of 25 mg/kg once daily for five days. The piglets in group Experimental II were given sulfadimethoxine at the same dose and a *Bacillus* spore-forming probiotic at a dose of two milliliters per animal for 21 days. The control group of piglets received no treatment.

The *Bacillus* spore-forming probiotic contains five strains: *B. amyloliquefaciens* ALB 65, *B. pumilus* UNCSM-026, *B. subtilis* UNCSM-020, *B. subtilis* var. *mesentericus* UNCSM-031, and *B. licheniformis* UNCSM-033.

Helminth and coccidial fecal examinations in animals were conducted before administration and on the 7th, 14th, and 21st days after administration, in accordance with DSTU 5079:2008 (DSSU, 2009). The anticoccidial activity of the drug was assessed based on prevalence reduction (PR) and intensity reduction (IR).

All manipulations with experimental animals were carried out in accordance with the 'European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes' (CE, 1986) and Council Directive 2010/63/EU (CEC, 2010), and under Art. 26 of the Law of Ukraine No. 3447-IV of 21.02.2006 'About protection of animals from cruel treatment' (VRU, 2006) and basic bioethical principles (Simmonds, 2017). Under the current procedure, the research program was reviewed and approved by the Bioethics Committee of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Kharkiv, Ukraine).

The results were processed by methods of variation statistics. To compare mean values Student's *t*-test was used (Van Emden, 2019).

Results and discussion. The treatment of piglets in experimental groups I and II for cystoisosporiasis resulted in a significant reduction in the prevalence of the disease, with a decrease of 1.41 times and 1.38 times, respectively ($p \leq 0.001$), just 7 days after treatment when compared to baseline values. By the 14th day, there was also a notable reduction in the intensity of infection, observed at 6.68 times and 5.3 times, respectively. Three weeks after the initiation of treatment, coproscopy of fecal samples showed no oocysts of the pathogen *Cystoisospora suis* in suckling piglets (Table 1). In contrast, the control group continued to shed oocysts throughout the entire experimental period.

After two weeks, a significant increase in the efficacy of the administered drugs was observed in the two experimental groups, reaching 85.0% and 81.0%, respectively. By the 21st day, this figure had increased to 100% relative to baseline values. Since clinical recovery was not recorded in the experimental animals of the two groups, the overall efficacy of the drugs increased significantly to 80.0% and 85.0% by the 14th day, indicating the onset of widespread clinical recovery among the animals. After 21 days, the therapeutic efficacy of the two treatment regimens for piglets reached 100%.

Table 1 — Comparative efficacy of sulfadimethoxine in the treatment of coccidiosis in piglets aged 14–21 days (M ± m; n = 20)

Animal group	Before treatment	On the 7th day			On the 14th day			On the 21st day		
	MI, oocyst	P, %	PR, %	MI, oocyst	IR, %	PR, %	MI, oocyst	IR, %	PR, %	MI, oocyst
Experimental I	88.9 ± 0.48	28.9	10.0	63.2 ± 0.53***	85.0	80.0	13.3 ± 0.48***	100.0	100.0	0***
Experimental II	92.2 ± 0.31	27.9	10.0	66.5 ± 0.30***	81.0	85.0	17.5 ± 0.38***	100.0	100.0	0***
Control	90.5 ± 0.34	–	–	92.4 ± 0.36***	–	–	97.0 ± 0.55***	–	–	104.3 ± 0.54***

Notes: Experimental I — sulfadimethoxine; Experimental II — sulfadimethoxine + *Bacillus* spore-forming probiotic; *** — the difference in values is statistically significant at $p \leq 0.001$ compared to the corresponding values before treatment.

Coccidial infection significantly reduced bacterial diversity in the intestines of sick piglets. Analysis of the microbial community composition indicates the presence of marked dysbiosis in both experimental groups of infected suckling piglets before treatment. This is confirmed by a significant increase in the levels of opportunistic pathogens, particularly *Staphylococcus* spp. (up to $10.1–14.6\times10^8$ CFU/g), *Proteus* spp. (up to $15.5–19.2\times10^7$ CFU/g), *Shigella* spp. (up to $9.3–12.5\times10^6$ CFU/g), *Klebsiella* spp. (up to $17.3–21.4\times10^6$ CFU/g), as well as the yeast-like fungus *Candida albicans* ($13.1–16.1\times10^4$ CFU/g), anaerobic representatives of the genus *Clostridium* spp. ($4.3–6.2\times10^5$ CFU/g), which significantly exceed the physiological norms for healthy intestinal microflora (Table 2).

Table 2 — Species composition of the large intestine microbiome in suckling piglets before treatment (M ± m; n = 20)

Microorganisms	Animal group	
	Microbiota indicators, CFU/g	
	Experimental I	Experimental II
E. coli	$28.1\pm0.52\times10^7$	$34.2\pm0.47\times10^7$
Shigella spp.	$9.3\pm0.44\times10^6$	$12.5\pm0.46\times10^6$
Proteus spp.	$15.5\pm0.50\times10^7$	$19.2\pm0.45\times10^7$
Klebsiella spp.	$21.4\pm0.39\times10^6$	$17.3\pm0.47\times10^6$
Clostridium spp.	$6.2\pm0.32\times10^5$	$4.3\pm0.34\times10^5$
Staphylococcus spp.	$14.6\pm0.47\times10^8$	$10.1\pm0.49\times10^8$
Enterococcus spp.	$28.3\pm0.49\times10^7$	$34.3\pm0.52\times10^7$
Candida albicans	$16.1\pm0.52\times10^4$	$13.1\pm0.44\times10^4$
Lactobacillus spp.	$5.4\pm0.37\times10^4$	$8.2\pm0.42\times10^4$
Bifidobacterium spp.	$31.3\pm0.45\times10^5$	$29.3\pm0.54\times10^5$

Notes: Experimental I — sulfadimethoxine; Experimental II — sulfadimethoxine + *Bacillus* spore-forming probiotic.

At the same time, a reduced content of obligate microflora — *Lactobacillus* spp. ($5.4–8.2\times10^4$ CFU/g) — and a relatively unstable level of *Bifidobacterium* spp. ($29.3–31.3\times10^5$ CFU/g) were observed, which is a characteristic sign of an imbalance in the intestinal microbiota. Furthermore, the imbalance between different groups of microorganisms (an increase in opportunistic flora against a background of decreased protective microflora) confirms a disruption in the gut's colonization resistance.

The established state of the microbiota justifies the need for anticoccidial and corrective therapy aimed at eliminating opportunistic flora and restoring the normal microbiota.

A comparative analysis of the results indicates that the use of sulfadimethoxine in Experimental I is less effective in regulating the intestinal microbiota compared to its combination with the *Bacillus* spore-forming probiotic (Experimental II).

In Experimental I, significantly higher levels of opportunistic microflora persist. Specifically, the number of *Staphylococcus* spp. in the first experimental group is 6.3×10^7 CFU/g vs 19.2×10^3 CFU/g in the second group; *Proteus* spp. — 7.1×10^6 vs 26.3×10^5 CFU/g; *Klebsiella* spp.— 5.2×10^6 vs 14.4×10^5 CFU/g, and *Shigella* spp.—16.6×10^5 vs 10.2×10^5 CFU/g. Similarly, the level of *Enterococcus* in Experimental I (37.4×10^5 CFU/g) exceeds the corresponding value in Experimental II (31.5×10^4 CFU/g). *Candida albicans* fungi were detected only in the first experimental group (15.2×10^4 CFU/g), whereas they were absent in the second experimental group. In Experimental I, a high level of *E. coli* (35.2×10^6 CFU/g) persists compared to Experimental II (21.4×10^5 CFU/g), which may indicate incomplete normalization of the animals' intestinal microbiota (Table 3).

Table 3 — Species composition of the large intestine microbiome in suckling piglets after treatment (M ± m; n = 20)

Microorganisms	Animal group	
	Microbiota indicators, CFU/g	
	Experimental I	Experimental II
E. coli	$35.2\pm0.61\times10^{6***}$	$21.4\pm0.68\times10^{5***}$
Shigella spp.	$16.6\pm0.52\times10^{5***}$	$10.2\pm0.45\times10^{5***}$
Proteus spp.	$7.1\pm0.36\times10^{6***}$	$26.3\pm0.67\times10^{5***}$
Klebsiella spp.	$5.2\pm0.40\times10^{6***}$	$14.4\pm0.51\times10^{5***}$
Clostridium spp.	$11.4\pm0.46\times10^{3***}$	$8.1\pm0.45\times10^{3***}$
Staphylococcus spp.	$6.3\pm0.39\times10^{7***}$	$19.2\pm0.58\times10^{3***}$
Enterococcus spp.	$37.4\pm0.58\times10^{5***}$	$31.5\pm0.66\times10^{4**}$
Candida albicans	$15.2\pm0.45\times10^4$	absent
Lactobacillus spp.	$3.3\pm0.36\times10^{5***}$	$28.7\pm0.65\times10^{8***}$
Bifidobacterium spp.	$24.2\pm0.52\times10^{6***}$	$37.5\pm0.68\times10^{10***}$

Notes: Experimental I — sulfadimethoxine; Experimental II — sulfadimethoxine + *Bacillus* spore-forming probiotic; **, *** — the difference in values is statistically significant at $p \le 0.01$ and $p \le 0.001$, respectively, compared to pre-treatment values.

Monotherapy with sulfadimethoxine (Experimental I) is insufficiently effective for normalizing the intestinal microbiota, as evidenced by the persistence of high levels of opportunistic microorganisms and low numbers of obligate flora. In contrast, combined use with the *Bacillus* spore-forming probiotic (Experimental II) ensures marked suppression of pathogenic microflora and active restoration of the normal microbiota, confirming the advantage of complex therapy in correcting dysbiosis.

Coccidial infection directly or indirectly affects the composition of the gut microbiota in animals (Gong et al., 2021; Hinney et al., 2021). Dysbiosis in animals with coccidiosis was characterized by reduced microbiota diversity, a deficiency of obligate anaerobes, and excessive growth of opportunistic microorganisms (Lu et al., 2021; Buffoni et al., 2026). The pathogen *Cystoisospora suis* enriches the bacterial population,

including *Enterococcus* spp., *Escherichia* spp., *Shigella* spp., *Staphylococcus* spp., and others (Cui et al., 2017). This was precisely the pattern observed in the baseline state, which was partially maintained after treatment in Experimental I, where high levels of *Staphylococcus* spp., *Proteus* spp., *Klebsiella* spp., and other opportunistic bacteria were noted.

In Experimental II, conversely, a significant decrease in the abundance of pathogenic microflora and a simultaneous increase in obligate microorganisms (*Lactobacillus* spp., *Bifidobacterium* spp.) were noted. This is consistent with the literature, according to which *Bacillus* probiotics are capable of inhibiting the growth of pathogenic bacteria, restoring microbial balance, and increasing the colonization resistance of the intestine (Hu et al., 2014; Jiang et al., 2022). The therapy administered to the two experimental groups facilitated the effective treatment of piglets for coccidiosis. Complete clearance of coccidia from the animals' bodies was observed, indicating the high therapeutic efficacy of the treatment regimen used.

Bacteria of the genus *Bifidobacterium* play a key role in maintaining intestinal homeostasis, and a decrease in their numbers is one of the most characteristic markers of dysbiosis (Tojo et al., 2014; Kim et al., 2025). The sharp increase in their numbers observed in the second experimental group indicates effective restoration of the normal microbiota and stabilization of the microbial ecosystem. Similarly, members of the genus *Lactobacillus* are key probiotic microorganisms widely used to correct dysbiotic disorders (Sanders et al., 2019). It is known that anticoccidial therapy without the use of probiotics often leads to a disruption of the microbial balance, as it suppresses both pathogenic and normal microflora, which explains the insufficient effectiveness of Experimental I, where the restoration of the obligate microbiota was limited and dysbiotic changes persisted.

The inclusion of the *Bacillus* spore-forming probiotic in the treatment regimen (Experimental II) demonstrated high efficacy in correcting dysbiotic disturbances of the gut microbiota. It was established that the use of the probiotic contributed to a marked suppression of opportunistic and pathogenic microflora, in particular a significant reduction in the levels of *Staphylococcus* spp., *Proteus* spp., *Klebsiella* spp., *Shigella* spp., as well as the complete elimination of *Candida albicans* fungi. This effect may be associated with the antagonistic activity of bacteria of the genus *Bacillus*, which produce a wide range of biologically active substances (bacteriocins, enzymes, organic acids) capable of inhibiting the growth of pathogenic microorganisms (Khalid et al., 2021; Liu et al., 2023). At the same time, in Experimental II, a sharp increase in the abundance of obligate microflora — *Lactobacillus* spp. and *Bifidobacterium* spp. — was observed, indicating the restoration of the intestinal microbiota. Probiotic *Bacillus* strains contribute to the normalization of the microbiota not only through direct antagonistic action but also by creating favorable conditions for the growth of indigenous microflora, including lowering the pH of the environment and competing for adhesion receptors (Wang et al., 2021).

Recent studies (Elisashvili, Kachlishvili and Chikindas, 2019; Sudan, Zhan and Li, 2022) have shown that *Bacillus* spore-forming probiotics are highly stable in the gastrointestinal tract. They can undergo transitory colonization and have a pronounced immunomodulatory effect. These characteristics ensure the effectiveness of *Bacillus* spore-forming probiotics in restoring microbial balance, even against the backdrop of anticoccidial therapy, and contribute to increased intestinal colonization resistance. This is manifested by the displacement of pathogenic microflora and the stabilization of microbial homeostasis.

These findings suggest the advisability of incorporating *Bacillus* spore-forming probiotics into complex therapies for dysbiosis, with the goal of normalizing microbiota and enhancing intestinal colonization resistance.

Conclusions: 1. Research has shown that suckling piglets have pronounced dysbiotic disturbances in their gut microbiota, manifested by increased levels of opportunistic pathogens (e. g., *Staphylococcus, Proteus, Klebsiella, Shigella*, and *Candida albicans*) and decreased numbers of obligate microflora (e. g., *Lactobacillus* and *Bifidobacterium*).

2. Treating piglets with sulfadimethoxine led to a full recovery from coccidiosis, but it did not fully normalize the microbiocenosis. This is evidenced by the persistence of dysbiotic changes and the insufficient restoration of normal microflora. The combined use of sulfadimethoxine and the *Bacillus* spore-forming probiotic is significantly more effective. It promotes treatment of coccidiosis, suppresses pathogenic and opportunistic microflora, eliminates *Candida albicans* fungi, and restores symbiotic microorganisms.

Declaration of competing interest. The authors declare that they have no conflict of interest.

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DOG BODY LANGUAGE (LITERATURE REVIEW)

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Summary. The article is devoted to a comprehensive analysis of contemporary scientific research exploring canine body language as a complex system of nonverbal communication. Based on a review of domestic and international publications indexed in PubMed, Scopus, Web of Science, Google Scholar, and other databases, the study summarizes findings on the development of dogs' communicative abilities in the course of domestication and coevolution with humans. Special attention is given to the evolutionary foundations of interspecies interaction, as well as to the role of morphological and breed differences and the influence of sex and age on behavioral signals. The paper also examines modern objective methods for analyzing canine facial expressions and postures, including the DogFACS (Dog Facial Action Coding System) system and machine learning technologies CNN (Convolutional Neural Network), DeepLabCut (Deep Learning-based Markerless Pose Estimation), used for the automatic recognition of body postures and emotional states in dogs. The study systematizes the main components of canine body language, including facial expressions, ear position, eye expression, mouth posture, body stance, and tail movements, emphasizing their integrated interpretation within various social contexts. The importance of correctly interpreting canine signals for conflict prevention, safety enhancement, and strengthening trust-based relationships between humans and dogs is highlighted. It is concluded that understanding canine body language is an acquired skill based on observation, learning, and interspecies empathy, and that it has significant practical value in veterinary medicine, as well as for dog owners, trainers, and animal behavior specialists

Keywords: nonverbal communication, animal behavior, human–dog interaction

Introduction. Communication with animals is not a language in the human sense, but a complex system of signals (acoustic, visual, and olfactory). Interpreting these signals helps strengthen the bond, resolve behavioral problems, and ensure a better quality of life for pets. If we consider the ways a dog 'communicates' with a human, posture and body language come first. Body language is the primary channel of communication (Hasegawa, Ohtani and Ohta, 2014). Experienced pet owners can read these signals and use them as powerful tools. In dogs, this 'language' is expressed particularly vividly (Firnkes et al., 2017).

Dogs do not know how to lie. Their bodies accurately reflect everything they experience. Emotions can change very quickly (for example, when another dog or a person approaches), but in the moment, dogs are sincere in their expressions. Understanding canine body language is essential for dogs' well-being, health, safety, and trust (Brugarolas et al., 2013). For humans, it offers significant advantages in establishing a two-way relationship based on empathy, understanding, and mutual trust, as well as in developing a lifelong bond with the dog. If we learn to read our dog's postures and signals, we will better understand its feelings and motivations and more accurately predict what it is likely to do. Interpreting the signals sent by a dog requires attentiveness and experience (Walsh et al., 2024).

The systematization and analysis of contemporary scientific data on canine body language are important for understanding its role in both interspecies and intraspecies communication, as well as for assessing its practical significance in veterinary medicine, cynology, and human–animal interaction, enabling people to understand dogs more naturally and effectively (Miklósi and Topál, 2013; Crosby and Rider, 2019).

Analysis of recent research and publications. According to recent studies conducted by the Canine Science Research Center at Arizona State University, people do not understand dogs' body language and the emotions associated with it as well as they believe they do (Derra S., 2025). Why is it important to understand canine body language? Dogs are highly expressive animals with a wide range of emotions (Hasegawa, Ohtani and Ohta, 2014). They communicate when they feel happy, sad, nervous, frightened, or angry, using their bodies and facial expressions to convey most of this information (Ballantyne, 2018; Gähwiler et al., 2020). Many owners dream of genuine mutual understanding with their pets but do not always know how to correctly interpret their behavior (Molinaro and Wynne, 2025).

In addition, there are scientific studies devoted to analyzing canine body language using machine learning. Brugarolas et al. (2013) developed an application for behavior recognition that requires data from a wireless sensor worn by the dog. According to Raman, Maskeliūnas and Damaševičius (2022), CNN (Convolutional Neural Network) and DeepLabCut (Deep Learning-based Markerless Pose Estimation) were used for the automatic recognition of a dog's body parts and postures.

Dogs communicate through numerous body language signals, adapting to life with humans. Dogs and humans perceive body language in similar ways (Borgi and Cirulli, 2016). This conclusion was reached by

Austrian researchers in a study involving 40 participants and 15 domestic dogs who underwent magnetic resonance imaging (MRI), as reported by Interesting Engineering. It was found that, like humans, dogs have a specialized area in the temporal lobe responsible for the visual perception of body posture. However, unlike humans, when observing faces and bodies, dogs also activate brain regions associated with olfactory processing. Thus, despite different evolutionary paths, dogs and humans have developed similar mechanisms for mutual understanding and communication (Karl et al., 2020).

In addition to obvious distance-increasing signals such as whining, barking, growling, snapping, baring teeth, and biting, there exists a world of subtle communication that expands the ways we can better interact with this species. Canine body language is often presented in overly simplified narratives, whereas in reality it is a highly developed and complex system.

By noticing the signals expressed through a dog's body, an owner can support and protect the dog when it shows discomfort. This strengthens the trusting relationship between owner and dog and helps the dog remain calmer in uncomfortable environments, as trust in the owner increases the dog's confidence and sense of security. It is also good practice to share information about canine body language with colleagues who may not be familiar with the signals dog display.

The **aim** of the research is to present a comprehensive review of current knowledge on canine body language.

Materials and methods. This review synthesizes findings from relevant literature sources. The research methods determined the direction of this systematic review by identifying gaps in the existing literature. The materials for the study consisted of published works by both domestic and international authors.

To achieve the stated objective, the following methods were applied: the dialectical method, the chronological method, and the methods of analysis and synthesis.

The search strategy was designed to include all relevant studies published up to 2026. A comprehensive literature search was conducted across several databases to identify studies relevant to this systematic review, using the following keywords: canine body language, nonverbal communication, communication, animal behavior, and combinations of these terms. Online databases searched included PubMed, Scopus, Web of Science Theses, Google Scholar, and ResearchGate (to identify potentially significant studies published by authors). In addition, the findings were supplemented by the authors' knowledge of the literature in the field of human-dog interaction.

Presentation of the main research material. ***How canine language was formed: evolution and domestication.*** For many thousands of years, dogs have closely coexisted and interacted with humans. In recent years, social cognition in dogs has been intensively studied (Grewal et al., 2020). It appears that during the process of domestication, dogs acquired unique abilities for interspecific social behavior with humans (Miklósi and Topál, 2013; Serpell, 2017).

The bond between dogs and humans is unique and serves as a defining indicator of the evolution of human cultures. Since ancient times, the animal known as the dog has forged its path through numerous challenges. Humans began domesticating dogs approximately 15,000–16,000 years ago. In fact, it was not dogs that were initially domesticated, but wolves — some of which were less aggressive and more tolerant of humans (Serpell, 2017). Some researchers suggest that the domestication and transformation of wolves into dogs began even earlier, possibly around 30,000 years ago (Virányi et al., 2008). From that point, a gradual restructuring of brain architecture began. However, the initial steps that led to wolf domestication remain unknown (Kaminski et al., 2019).

The estimate of 15,000–16,000 years is currently the most widely accepted. There is greater disagreement regarding the geographic origin of domestication. Comparing genetic and archaeological data, some scholars point to the Middle East, others to Western Europe, and some propose that domestication occurred independently in multiple regions. It is believed that the cold and dry climate of that era facilitated interaction between wolves and humans. At the same time as the wolf was simplified into the domestic dog, domestication occurred in a broader sense, which can be described as 'humanization'. This process has even given rise to the concept of coevolution — the joint historical development of dogs and humans (Kubinyi, Virányi and Miklósi, 2007; Tebelmann and Ganslosser, 2024).

Charles Darwin also noted how domestication influenced the behavior of domestic dogs by reducing their fear of humans. He argued that dogs possess emotions such as love, fear, shame, and rage, as well as the capacity for dreams, imitation, and reasoning (Darwin, 1871).

During domestication, dogs lost some of the complex communicative signals characteristic of their wild ancestors — the Gray wolf. Instead, they developed an enhanced ability to understand humans and adapted their body language and behavior for human interaction. Dogs recognize and interpret human communicative cues and interact with people in ways not observed in their wild ancestors (Miklósi and Topál, 2013).

As a result, dogs developed a kind of 'human–dog dialect', which makes them more understandable to their owners. They use their entire bodies to communicate, conveying information both intentionally and unintentionally (Siniscalchi et al., 2018).

Modern researchers actively study similarities between animals and humans in terms of language and communication (Gábor et al., 2026). However, the main reason dogs have not 'developed speech' may be social rather than biological. Humans evolved language as a tool for cooperation in complex group tasks, enabling the transmission of highly specific messages. Dogs, by contrast, coordinate effectively without words —

through gaze, movement, scent, and vocal signals. There was simply no evolutionary need for speech to develop in dogs (Marshall-Pescini and Kaminski, 2014; Elgier et al., 2009).

Thus, the domestication of wolves may have occurred not only as their populations became more tame, but also as they adapted to human preferences. These findings enhance our understanding of early dog domestication as a communicative evolution toward refined human comprehension and effective nonverbal interaction (Mandal, 2014; Kaminski and Marshall-Pescini, 2014).

Canine body language. The fact that dogs cannot verbally tell us what they think or feel does not mean they cannot communicate with us. Instead of verbal language, dogs often rely on body language to give humans or other animals clues about their emotions (Elgier et al., 2009). In dogs, body language plays a central role in maintaining social hierarchies, preventing conflicts, and initiating affiliative interactions such as play (Górski, Kondracki and Kępka-Borkowska, 2026). Understanding canine body language is a fundamental skill of critical importance for preventing injuries to the public, dog owners, veterinarians, and people working in dog-related environments, as well as for helping dogs avoid negative emotional states such as fear, anxiety, and stress.

Unlike humans, dogs rely heavily on body postures and olfactory (scent) signals, while vocal communication is less significant. Humans are primarily listeners, whereas dogs are observers. Another key difference between human and canine communication lies in the type of information transmitted (Carson, 2007). Your dog can communicate a lot through its posture. According to Ertürk (2006), differences in behavior and body language in dogs are influenced by sex and age. One review noted that males tend to be more aggressive and bold, and to show more social contact with humans during play. Females, on the other hand, are more sociable during cooperative tasks with humans. Differences in body language also partly depend on breed, height, and weight (Mota-Rojas et al., 2021; McGreevy et al., 2013; Duffy, Hsu and Serpell, 2008; Eken Asp et al., 2015).

These differences are explained by the fact that dog morphology, shaped through artificial selection, has led to the loss of certain components of their body language (Zepeda et al., 2024). However, body language is often misunderstood, which can predispose to behavioral problems and conflicts.

But what exactly is canine body language? It is a complex and elegant system of nonverbal communication. Nonverbal communication is a channel of interaction between humans and animals, with human body language often expressed at a subconscious level (Hasegawa, Ohtani and Ohta, 2014).

Some amusing manifestations of canine body language often noticed by humans include yawning and lip-licking, which are considered calming signals and have been observed in studies assessing animal welfare (Palestrini et al., 2010). Body language is also used to convey threats, aggression, submission, and attempts to regulate distance during social interactions, both with other animals and humans (Walsh et al., 2024).

Dogs communicate visually by altering the position of various parts of their bodies. Control of voluntary muscles allows dogs to display a wide range of postures and positions that convey different information about the signaler's internal state and intentions (Mariti et al., 2012). Consequently, communication can be extremely complex for some dogs, both in terms of accurately sending and interpreting visual information (Simpson, 1997).

A dog can and will use its entire body to communicate with humans or conspecifics (Völter et al., 2025). Dogs can express confidence, alertness, or threat by making themselves appear larger, stretching to full height, and increasing muscle tension. Canine body language signals can be divided into facial expressions (mimicry), gestures, and the posture and movement of different body parts. Gestures involve the ears and tail, while facial expressions primarily rely on the eyes and mouth (Quaranta, Siniscalchi and Vallortigara, 2007). Finally, posture also plays a crucial role. These nonverbal signals are combined to create a coherent message that the dog intends to convey.

Facial expressions (mimicry) in dogs. Facial expressions are involuntary reactions that serve to convey the emotions dogs experience (Darwin, 1872). They are considered sensitive indicators of emotional states in humans and many other animals (Bremhorst et al., 2019). Due to their close coexistence with humans, significant attention has been given to identifying these expressions, particularly in dogs (Bloom and Friedman, 2013). Consequently, facial expressions are increasingly studied as potential indicators of subjective states in research on animal emotions and welfare (Mota-Rojas et al., 2021).

Machado and da Silva (2019) assert that animals can produce facial movements — especially with their ears and eyes — linked to specific emotional states. Scientific tools and automated analysis of facial expressions help make such interpretations more objective (Borgi and Cirulli, 2016). To reduce subjectivity, researchers developed the DogFACS system (Dog Facial Action Coding System), which functions as a kind of 'dictionary' of canine facial expressions (Waller et al., 2013). It breaks down facial movements into discrete 'units', such as raised eyebrows, flattened ears, downward corners of the mouth, and so on (Martvel et al., 2025).

In experiments, dogs are exposed to different situations: a treat (joy), anticipation without a reward (frustration or annoyance), a loud noise (fear) (McPeake et al., 2019). The findings show that facial expressions change differently depending on the context, yet the patterns are generally consistent across breeds (Burrows et al., 2021). This demonstrates that a dog's face can reliably indicate what it is feeling (Boneh-Shitrit et al., 2022).

Humans tend to interpret canine expressions through an anthropocentric lens. For example, a dog's 'averted gaze' might be perceived as embarrassment, similar to how a human looks when caught doing something forbidden. However, canine facial expressions function differently: what appears as embarrassment to us may mean something entirely different for the dog. It is as if we are using the same alphabet but speaking different languages (Correia-Caeiro, Guo and Mills, 2023).

Breed differences further complicate interpretation. The same facial expression in a Collie and a Pug may look very different to humans. In Pugs, Huskies, or Dobermans, identical muscle movements can produce different visual effects. What appears as 'furrowed brows' in one breed may simply be a normal anatomical feature in another (Duffy, Hsu and Serpell, 2008).

In conclusion, when interpreted correctly, facial expressions play a crucial role in successful human–dog interactions (Mota-Rojas et al., 2021; Pickersgill, Mills and Guo, 2023).

Canine body language — calming signals. What are calming signals, and why do dogs use them? Turid Rugaas, a Norwegian dog trainer and behaviorist, coined the term 'calming signals' (Rugaas, 2006). This type of body language is used by dogs to diffuse stressful, uncomfortable, or frightening situations.

Dogs use calming gestures, such as yawning or lip-licking, to communicate to humans or other animals that they come in peace. For example, if another dog approaches, a dog may avert its gaze and lick its lips. This signals to the approaching dog that it does not intend to fight. You may also notice your own dog doing this if you scold or punish it — the dog is simply trying to convey that it poses no threat to you (Siniscalchi et al., 2013).

Dogs may also use calming signals to redirect attention in certain situations, a behavior often called 'displacement behavior' (Firnkes et al., 2017). For instance, when a dog is learning a new command it does not yet understand, it might start scratching itself excessively. This action expresses the dog's tension and nervousness. Once the dog understands the command, the scratching stops.

Calming signals are therefore an important part of canine communication, helping dogs manage social interactions and reduce potential conflicts while expressing their emotional state (Firnkes et al., 2017).

Ears as an indicator of a dog's mood. A dog's ears can reveal a great deal about its emotional state. Body language and ear position play a crucial role in expressing emotions in dogs (Machado and da Silva, 2019). Dogs' ears are highly mobile: about 20 muscles control the movement of the ear, allowing not only precise localization of sounds but also the expression of emotional states.

Ear position serves as an important indicator of mood: slightly raised ears indicate a sense of security and calmness (Siniscalchi et al., 2018), while upright ears help with sound localization. Flattened ears, pressed close to the head, most often signal pronounced fear. When a dog is interested in something, the ears are directed toward the stimulus — usually forward, as the dog is looking in that direction. The ears become pointed, and the dog often holds its head high.

Flattened or lowered ears indicate insecurity, fear, or sometimes submission. If the ears are pulled backward, it can signal aggressive intent (Pedretti et al., 2022).

By observing ear positions alongside other body signals, owners can better understand their dog's feelings and intentions in any given situation.

Mouth and facial expressions in dogs. A relaxed and slightly open mouth usually indicates that a dog is calm and content (Ertürk, 2006). When a dog is very happy or excited, it may raise the corners of its mouth — essentially smiling. Yes, dogs can smile too!

Observing the mouth, along with the eyes, ears, and overall posture, helps owners interpret a dog's emotional state more accurately and strengthens the bond between human and pet (Burrows et al., 2021; Siniscalchi et al., 2018).

Baring teeth and mouth tension in dogs. A dog's baring of teeth differs significantly from a relaxed, slightly open mouth. When a dog bares its teeth, the lips are tense and raised, revealing the teeth. Sometimes the dog also wrinkles its nose (Burrows et al., 2021). The more pronounced the baring, the more aggressive the dog is likely to be. Often, this is accompanied by growling and raised hair along the neck and shoulders.

A tightly closed mouth is a sign of insecurity, which may escalate into aggression or fear. Lip licking is another important signal, indicating that the dog is scared or anxious. This gesture is sometimes combined with a nervous yawn, showing that the dog is experiencing significant stress or discomfort (Ertürk, 2006).

Observing these subtle cues is essential for understanding a dog's emotional state and preventing potential conflicts.

Eyes in canine body language. Eyes play a key role in canine body language, serving not only as sensory organs but also as important tools for social communication (Savalli, Resende and Gaunet, 2016). Eye contact, especially in dogs, is one of the most significant and effective channels for conveying information (Vas et al., 2005). Direct staring usually occurs during close interactions. Most often, it can be a challenge, but some dogs will gaze intently at a beloved owner to anticipate their wishes (Topál, Kis and Oláh, 2014). It is important to consider other signals to accurately interpret what a stare means (Borgi and Cirulli, 2016).

Eye shapes vary by breed: some dogs have round eyes, others almond-shaped. Eyes that appear larger than usual typically indicate that the dog feels threatened, stressed, or frightened. Aggressive dogs also often have eyes that seem larger than normal (Bremhorst et al., 2021). Conversely, small or squinted eyes can indicate fear or stress.

Dogs frequently avert their gaze to show that they are not seeking conflict. This gesture can be exaggerated by turning the muzzle or lowering the head. Some owners interpret this as guilt, but in reality, the dog simply senses displeasure and attempts to appease humans.

Eyes are also used to communicate emotional states to other animals. Extremely wide eyes can signal that the dog feels threatened, whereas gaze aversion serves as a calming signal, reducing tension (Crosby and Rider, 2019). Observing eye behavior in conjunction with other body signals provides a more complete understanding of a dog's emotional state.

Body posture in dogs. A dog's body posture communicates a wide range of emotions and intentions. When a dog lowers its front body to the ground, it is inviting you or another dog to play. In this posture, the dog may eagerly chase a ball or engage in playful wrestling.

A tense body — especially with raised fur along the neck or spine — indicates alertness and can signal aggression (Duffy, Hsu and Serpell, 2008). Highly nervous dogs may pace or walk in circles (Siniscalchi et al., 2018).

When a dog is scared, it often tries to make itself appear smaller: lowering its body, dropping its head, and hunching its back. Conversely, when demonstrating dominance, the dog's legs stiffen, the back straightens, and the head is held high. If a dog in this posture looks at another animal or person and moves toward them, it may be preparing to attack.

Rolling onto the back shows trust and submission, indicating that the dog recognizes the human's authority. Sometimes, dogs also do this to request belly rubs. Submissive behavior can also be displayed when a dog lies down with its head and tail lowered.

By observing a dog's body posture alongside other signals such as facial expressions, ears, and tail movements, owners can better interpret the dog's emotional state and intentions.

Talking tails in dogs. The tail is a true indicator of a dog's mood and plays an important communicative role. Its position and movement, combined with facial expressions, create complex signaling patterns that reflect different emotional states (Machado and da Silva, 2019). Due to its length and high mobility, the tail serves as a highly visible means of communication, effective even over considerable distances and allowing a wide range of interpretations (Leonetti et al., 2024). Tail position and dynamics provide insight into a dog's intentions and emotional state. A high tail usually signals confidence, excitement, or motivation to initiate interaction, such as during greetings or play. When anxious, the tail lowers — the lower it goes, the more fearful the dog (Siniscalchi et al., 2013). In cases of extreme fear, the tail is tucked tightly between the hind legs. Many people assume that a wagging tail always indicates happiness, but this is not necessarily true. A slightly lowered tail moving slowly can indicate confusion (Stellato et al., 2017). Quaranta, Siniscalchi and Vallortigara (2007) studied asymmetry in tail movement control in dogs, as well as differences in movement amplitude to the left and right depending on visual stimuli. They found that when seeing their owners, dogs showed a pronounced rightward bias with greater amplitude in tail movement. A similar reaction was observed toward strangers, though the range of motion was less pronounced (Quaranta, Siniscalchi and Vallortigara, 2007).

Paws also play a role in communication. Placing a paw on another dog's head, withers, or back can signal a claim to leadership; if the other dog disagrees, a fight may occur. Owners should be attentive when noticing this gesture. Dogs may also touch their human's hand or body with a paw to get attention, request a treat or toy, or lead the person somewhere.

Practical advice: How to learn to understand your dog. When trying to understand dogs, the first thing to pay attention to is their body language and posture, including the position of the head and neck, ear placement, tail position and activity, raised fur along the shoulders or back, eye and ear orientation, facial expressions, and vocalizations (Darwin, 1872; Rugaas, 2006). Learning to understand canine body language is primarily a matter of practice. It is evident that building good mutual understanding with a dog should begin at a young age, and a quality puppy school often includes training in these skills. Take time to observe dogs in various situations and pay close attention even to small changes in their posture and facial expressions. You can consult an animal behaviorist or professional dog trainer, who can provide advice and exercises to help you better interpret what your dog is trying to communicate. These activities will reinforce your understanding of how dogs interact with one another, leading to more positive experiences when meeting new puppies. Through observation and experience, you will learn to interpret more accurately what your dog is really trying to tell you (Ertürk, 2006; Siniscalchi et al., 2018).

Understanding canine body language is an ongoing learning process that offers numerous benefits. By carefully attending to your dog's signals, you can improve communication, enhance safety, and strengthen the bond with your four-legged friend. Use this knowledge to ensure a happy and harmonious relationship with your dog (Firnkes et al., 2017; Górski, Kondracki and Kępka-Borkowska, 2026).

Conclusions and future research perspectives. Dogs are sincere in expressing their emotions, but they do not always experience the same feelings as humans in similar situations. The ability to read canine body language helps recognize when a dog is relaxed, tense, fearful, or ready to respond, allowing for appropriate reactions and ensuring the safety of everyone involved.

Communicating with a dog is an art of attentiveness. Their signals require sensitivity from us. By learning to understand them, one can discover a remarkable world of loyalty, joy, and complex emotions that can reside in even the smallest paw. This is a dialogue in which one

must listen not only with the ears, but also with the eyes and the heart.

Understanding canine body language is not an innate skill but a result of observation, learning, and love for your pet. By studying signals, postures, and sounds, it is possible to establish a strong bond with a dog and create a more harmonious shared life. Do not interpret individual signals in isolation; pay attention to how they manifest together as a whole.

As we continue to uncover the mysteries of the animal world, it becomes increasingly clear that our furry companions have much more in common with us than previously thought.

Thus, by applying knowledge of canine body signals, opportunities arise to understand how dogs communicate with each other, to interact with a dog on its own terms, to strengthen the bond with your dog, and to give the dog confidence through trust in its owner.

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THE INFORMATIVE VALUE OF HISTOMORPHOLOGICAL EXAMINATION OF MAMMARY GLAND NEOPLASIA FOR PREDICTING METASTASIS IN DOGS AND CATS

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Summary. Significant morphological diversity and varying degrees of metastatic potential have been demonstrated in mammary tumors in dogs and cats. Histomorphological verification is a key step in determining the biological behavior of neoplasms. A statistically significant association has been demonstrated between clinical stage, as defined by the TNM system, and histological malignancy of tumors. Malignant neoplasms are significantly more often associated with advanced stages of the disease and the presence of metastases. Regional lymph node involvement and distant metastasis are decisive prognostic criteria. Benign tumors predominantly correspond to early stages and show no signs of metastatic growth. This study demonstrates the feasibility of using χ^2 and Fisher's criteria to analyze small samples in veterinary oncology studies. The metastatic potential of a tumor is emphasized as having greater prognostic significance than the size of the primary tumor. A comprehensive approach that takes into account clinical and morphological parameters is shown to improve the accuracy of predicting disease progression. The need for further research using standardized methods and larger samples to advance knowledge in veterinary oncology is emphasized

Keywords: histomorphological identification, TNM stratification, metastatic potential, prognostic factors

Introduction. Approximately 200 scientific papers on mammary tumors in cats have been published since the First Symposium on Mammary Neoplasia in Dogs and Cats was held at the Ninth Annual Congress of the British Small Animal Veterinary Association in London in 1966 (Guirguis and Beggs, 2025). Mammary tumors are common in cats and account for approximately 17% of all neoplasms in these animals. Most of them are hormone-independent carcinomas (80–90%) of the simple type (involving a single neoplastic cell component — the ductal epithelium), which are characterized by aggressive biological behavior (Rodrigues-Jesus et al., 2025).

It is reported that the metastasis rate ranges from 50% to 90%, with regional lymph nodes (83%), lungs (83%), liver (25%), and pleura being the most commonly affected sites (Vazquez et al., 2023). The median survival time after initial tumor detection in untreated cats is approximately 12 months, although it varies depending on the clinical stage following tumor removal (Barbagianni and Gouletsou, 2023).

The clinical, pathomorphological, and molecular characteristics studied to identify prognostic indicators and therapeutic prognostic markers for mammary tumors in cats include: disease stage (based on tumor size, lymph node status, and the presence of metastases); histological grade of malignancy; molecular markers, such as cell cycle proteins, estrogen and progesterone receptors (ER and PR), and the epidermal growth factor receptor 2 (HER-2) (Feliciano et al., 2023).

In addition, numerous retrospective studies indirectly link the prognostic role of the parameter under study to already known prognostic factors (e. g., grade of malignancy). This approach does not allow for an accurate assessment of whether the parameter under study directly affects tumor progression and the outcome. Approximately 3% of all studies are dedicated to the study of therapeutic prognostic markers. Furthermore, most prognostic factors were evaluated using univariate analysis (e. g., Kaplan–Meier curves). Multivariate analysis is often either absent or results in the loss of statistical significance of the studied parameter (Zhelavskyi and Dmytriv, 2023).

A detailed review of the literature on mammary tumors in cats was conducted, evaluating various proposed prognostic parameters in accordance with the recently published Recommended Guidelines for the Conduct and Evaluation of Prognostic Studies in Veterinary Oncology (Webster et al., 2010). These principles reflect the current consensus among veterinary pathologists and oncologists regarding the evaluation of the most important prognostic factors in animal neoplasms. Based on these criteria, statistically significant prognostic parameters were identified, and recommendations for predicting disease progression were formulated (Pastor et al., 2022).

The median age at which mammary tumors develop in cats is 10–12 years, with an increased risk up to 14 years of age. Carcinomas are more common in older cats than benign tumors. Although there is evidence of a poorer prognosis in older animals, the results remain inconclusive. The age threshold of 10 years was not associated with recurrence, recurrence-free interval, or lifespan. Age also had no significant effect on 1- and 2-year survival after surgery and is generally not considered an independent prognostic factor. At the

same time, one study found an association between age and survival; however, the large number of variables (n = 35) reduced the statistical power of the analysis, and the data collection methods remained insufficiently described (Hillaert et al., 2022). In some studies, older age was associated with shorter survival based on univariate analysis, but this association lost significance in multivariate analysis (Massimini et al., 2022).

Although mammary tumors most commonly occur in female cats, they have also been reported in males, and their aggressiveness is similar to that in females. No difference in tumor behavior has been observed between spayed and unspayed animals. However, spaying before 6 months of age and before 1 year of age reduces the risk of tumor development by 91% and 86%, respectively. An increased risk is also associated with long-term use of progestins (Choi et al., 2022).

Siamese cats have an increased risk of developing mammary carcinomas and are more likely to develop the disease at a younger age (up to 9 years) compared to other breeds (up to 14 years). They also more frequently develop invasive tumor forms, including micropapillary carcinoma. However, reliable data on disease incidence by breed are limited (Yitbarek and Dagnaw, 2022). Long-haired breeds (Siamese and Persian) showed a poorer prognosis in univariate analysis, but this effect was not confirmed in multivariate analysis. Overall, breed is not considered an independent prognostic factor (Koo et al., 2022). Unfortunately, only about 15% of the studies included clinical data from follow-up observations, and almost all of these were retrospective. Thus, recurrence-free survival, one of the most important predictors of clinical behavior, was often not assessed. Recurrence-free survival is the time from surgery to the development of recurrence and/or metastasis. Furthermore, the methods used to collect follow-up data are often unclear, and the number of cases included is typically small (15–65 animals in studies published after 1990) (Greco et al., 2023).

Mammary tumor staging in cats is based on the TNM system, which is widely used in clinical practice despite certain differences in its application. However, there is significant variability in tumor measurement methods and lymph node assessment, highlighting the need to standardize this system (Klaengkaew et al., 2021).

Staging is not always included in studies because information on tumor size or the presence of distant metastases is often lacking. Therefore, these indicators are often analyzed separately. Nevertheless, the prognostic significance of clinical stage has been confirmed; survival decreases significantly as the disease stage increases. However, stage does not always remain an independent prognostic factor in multivariate analysis (Petrucci et al., 2021).

Histologically confirmed lymph node metastases are an independent prognostic factor associated with reduced overall survival (less than nine months) (Soultani et al., 2021). Compared to more conservative interventions, radical mastectomy in cats without distant metastases provides a longer recurrence-free period (Moraes and Borges, 2021), although these differences are not always confirmed in multivariate analysis (Hart et al., 2020).

Tumor size is an important prognostic factor included in the TNM system (Rose and Worley, 2020). In most studies, tumor size was assessed as a continuous variable; however, the methods used to measure it are often not described (Stan et al., 2020). Tumor volume also correlates with survival; however, it is tumor diameter that remains an independent prognostic indicator in multivariate analyses (Sánchez et al., 2019).

Changes are also occurring in the nosological structure of mammary tumors in dogs. Numerous studies and various approaches to morphological diagnosis have sparked discussions about their classification (Tanaka et al., 2020).

Thus, mammary tumors in cats are among the most common neoplasms and are characterized by high malignancy and a high rate of metastasis. Most of them are aggressive carcinomas, which result in a relatively short survival time for the animals after diagnosis. It has been established that key prognostic factors include tumor size, the presence of metastases (especially in lymph nodes), and surgical treatment strategy.

Clinical staging according to the TNM system is of great importance, but its prognostic value is not always confirmed in multifactorial models. At the same time, molecular and histological markers are considered promising prognostic tools, but their role requires further clarification. The age and breed of animals do not have a clear impact on prognosis, although some studies indicate certain associations. There is also an evolution in classifications and approaches to morphological diagnosis, reflecting the development of veterinary oncology. However, the following issues have been identified:

— lack of prospective studies — most publications are retrospective in nature, which reduces the reliability of the conclusions;

— small sample size — in many studies, the number of cases is limited, which affects the statistical significance of the results;

— incomplete clinical data — information on staging, metastasis, or follow-up is often missing;

— lack of standardization — different methods for assessing tumor size, lymph node status, and the application of the TNM system make it difficult to compare results;

— limited use of multifactorial analysis — a significant portion of the conclusions is based solely on univariate methods, which may distort the true prognostic value of the factors;

— uncertainty regarding the role of individual factors — such as age, breed, and hormonal status — due to conflicting results;

— insufficient study of molecular markers — despite their potential, only a small fraction of studies is devoted to their prognostic significance;

— controversy surrounding classifications — the lack of a unified approach to the morphological classification of tumors complicates the standardization of diagnosis and prognosis.

Based on this, it can be concluded that despite a significant number of studies, current knowledge about mammary tumors in cats and dogs remains fragmented. Further progress in this field requires standardized approaches and larger prospective studies aimed at predicting metastasis and oncological stratification of affected animals.

In light of the above, our **aim** is to evaluate the prognosis of breast tumor metastasis in dogs and cats based on histomorphological verification results.

Materials and methods. The study material consisted of biopsied samples of mammary gland neoplasms obtained from dogs and cats of various ages and breeds that were referred for surgical treatment to the veterinary clinic 'Dovira' veterinary clinic (Kharkiv, Ukraine). The prospective study included 10 dogs and 10 cats with clinically diagnosed mammary gland tumors aged 6 to 17 years. In all cases, on the day before surgery, an assessment of the local tumor status and determination of the clinical stage of the disease according to the TNM system were performed.

Surgical treatment involved mastectomy (zonal in dogs or unilateral in cats) with mandatory dissection of regional lymph nodes. After removal of the neoplasms, biopsies were taken (tumor, adjacent tissues, lymph nodes), which were labeled according to the location and type of sample. The obtained biopsies were fixed in 10% neutral buffered formalin for 24–48 hours to ensure preservation of the morphological structure of the tissues. After fixation, the material underwent standard histological processing: dehydration in alcohols of increasing concentration and embedding in paraffin. Histological sections 4–5 μm thick were prepared from paraffin blocks using a rotary microtome. The sections were mounted on slides and stained with hematoxylin and eosin for subsequent light microscopic examination.

Microscopic examination was performed using a light microscope (× 400). The tumor architecture, cellular composition, degree of differentiation, mitotic activity, presence of necrosis, invasion into surrounding tissues and vessels, as well as the status of regional lymph nodes, were assessed.

The histomorphological classification of tumors was performed in accordance with generally accepted criteria, determining the type of neoplasm (benign or malignant) and the degree of differentiation (G1–G3). The results were compared with the clinical stage of the disease according to the TNM system to establish a correlation between the clinical and morphological characteristics of mammary tumors in dogs and cats.

All manipulations with experimental animals were carried out in accordance with the 'European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes' (CE, 1986) and Council Directive 2010/63/EU (CEC, 2010), and under Art. 26 of the Law of Ukraine No. 3447-IV of 21.02.2006 'About protection of animals from cruel treatment' (VRU, 2006) and basic bioethical principles (Simmonds, 2017). Under the current procedure, the research program was reviewed and approved by the Bioethics Committee of the State Biotechnology University.

Statistical analysis of the results in the experimental animal groups was performed using the χ^2 criterion and Fisher's exact test. These methods were used to assess the significance of differences between groups and to identify statistically significant associations between the studied parameters.

Results and discussion. Based on the analysis of medical history data from dogs and cats in the experimental groups, it was noted that some animals with mammary gland neoplasms had previously received hormonal medications to suppress estrus, suggesting an estrogen-dependent etiology of the neoplasms. Mammary gland neoplasms in dogs and cats were observed in various areas of the mammary gland without any specific localization.

An analysis of the veterinary clinic's medical records showed that cases of mammary gland tumors in dogs are being recorded with increasing frequency. Of 20 mammary gland tumors, histomorphological studies confirmed that only 6 were benign (30%).

It should be noted that the incidence of malignant mammary gland tumors in the study group of dogs was 60% (6 cases), with a mean age of 9.5 years, while the incidence of malignant mammary tumors in the study group of cats was 80% (8 cases), and the average age was 9.4 years. This suggests that the progression of mammary tumors depends on the age of the animals.

The results of the analysis of the postoperative period and follow-up of animals after zonal or unilateral mastectomy indicate that the most effective approach is simultaneous mastectomy with regional lymph node dissection and hysterovarioectomy. Clinical observation of the animals over the course of a year revealed the following: tumor recurrence and distant metastasis within the first three months after surgery were detected in animals diagnosed with poorly differentiated carcinomas (G3) or carcinosarcomas, while other malignant tumors metastasized within 3–6 months to either the contralateral mammary gland or the nearest lymph nodes. Inguinal and axillary lymph nodes were palpable as hardened masses during examination.

Regarding benign lesions, it should be emphasized that no recurrences were recorded during the observation period; only one animal showed inflammation and induration of the inguinal lymph node. The results of the studies indicate that the surgical treatment of cats and dogs with mammary gland tumors via zonal or unilateral mastectomy with lymph node removal reduces the incidence of postoperative recurrence and the occurrence of inflammation and induration of lymph nodes, unlike the removal of the tumor alone without lymphadenectomy.

We have detailed the relationship between the clinical stage of mammary gland neoplasms and their histomorphological features in the experimental dogs (Table 1).

Table 1 — The relationship between the clinical stage of mammary gland tumors and their histological verification in dogs

The animal's name and age	Clinical stage by TNM	Histological diagnosis
Texas, 10 years old	$T_2N_0M_0$	Well-differentiated breast carcinoma (G1)
Businka, 10 years old	$T_1N_0M_0$	Simple breast adenoma
Gina, 7 years old	$T_1N_0M_0$	Mixed benign breast tumor
Kapa, 10 years old	$T_3N_1M_1$	Solid breast carcinoma with metastatic spread to a regional lymph node
Lusia, 10 years old	$T_1N_0M_0$	Simple, well-differentiated breast carcinoma (G1)
Pusha, 9 years old	$T_1N_0M_0$	Ductal carcinoma of the breast; lymph node with no signs of metastatic growth
Tina, 11 years old	$T_1N_0M_0$	Mixed benign breast tumor
Faya, 17 years old	$T_3N_0M_0$	Soft tissue sarcoma, complex breast adenoma, breast carcinoma, well-differentiated (G1)
Funia, 11 years old	$T_1N_0M_0$	Benign complex breast tumor
Hasya, 11 years old	TNM	Well-differentiated simple breast carcinoma (G1)

Thus, the mammary gland neoplasm in the male dog named 'Texas' is encapsulated, round in shape, measuring 7×6 cm, with clear borders, and localized in the projection of the 5th pair; the right inguinal lymph node is enlarged; the surrounding tissue is normal. Histomorphological verification was performed on biopsy specimens removed by excision, which were appropriately labeled. Analytical macroscopic description: 1k — a gray-colored skin lesion measuring 4.3×3.8 cm, with a hypodermis thickness of 1.4 cm. A 0.5×0.4 cm area of indentation, 0.3 cm deep, is visible on the surface. On sectioning, a gray-colored nodule measuring 2.0×1.6×1.5 cm. The resection margins are marked with black dye; 2k — a lymph node measuring 5.0×4.8×2.0 cm with a small amount of fatty tissue. Microscopic description: Microscopically, a well-defined formation is observed, composed of tubular structures lined with epithelial cells. The cells range in shape from rounded and oval to polygonal. They have well-defined borders and a small amount of eosinophilic cytoplasm. Nuclei are oval, normochromic or hyperchromic, with granular chromatin and a central nucleolus. Anisocytosis and anisokaryosis range from moderate to marked. Mitotic activity is up to 4–6 mitoses per 10 high-power fields. A large number of dilated ducts filled with eosinophilic contents are present. The stroma is moderate, containing lymphocytic-plasmacytic infiltrates. Necrotic areas are present. The slides show normal adipose tissue under the microscope. Histomorphological diagnosis: well-differentiated breast carcinoma (G1).

From the pathological material removed from a female dog named 'Businka': left inguinal lymph node, 5th pair of mammary glands on the left, and a morphologically unchanged cranial mammary gland. The lymph node is slightly enlarged, adherent to the subcutaneous tissue; the tumor is an encapsulated formation, up to 5 mm in diameter, rounded; the cranial mammary gland with nipple is unchanged. No changes in tissues or organs adjacent to the lesion are identified. The material, appropriately labeled, was obtained during a lateral mastectomy and lymph node dissection. Macroscopic description: 1k — 2 gray-colored tissue fragments with a total size of 2×2×0.7 cm; 2k — a gray-colored skin flap, measuring 2.6×1.7×0.4 cm. A dome-shaped, gray-colored formation measuring 1×0.8×0.5 cm with a finely nodular surface is visible on the surface. A nodular formation measuring 0.5×0.5×0.4 cm is visible on the section. The resection margins are marked with blue dye; 3k — a gray skin excision, measuring 0.9×0.9×0.4 cm. On the surface, a dome-shaped formation is observed, gray in color, measuring 0.7×0.5×0.4 cm, with a finely nodular surface. The resection margins are marked with blue dye. Microscopic description: Microscopically significant changes are present only near the nipple. A well-defined lesion is observed; it has sclerotic changes in the center and consists of tubular structures lined with epithelial cells. The cells are oval and polygonal in shape, with well-defined borders, sparse eosinophilic cytoplasm, an oval nucleus, granular or open chromatin, moderate anisocytosis and anisokaryosis, and sporadic mitoses. Portions of a lymph node characterized by follicular hyperplasia are observed in the adipose tissue. Histomorphological diagnosis: simple breast adenoma.

A neoplasm in a female dog named 'Gina' is located in the area of the second mammary gland, is encapsulated, measures 7×4 cm, is non-invasive, has an irregular oval shape, and the surrounding tissues are unchanged. Histomorphological verification was performed on biopsy specimens removed by excision, which were appropriately labeled. Macroscopic description: 1k — a gray-colored skin flap measuring 4.0×3.0 cm, with a hypodermis thickness of 3.0 cm. A dome-shaped, gray-colored formation measuring 0.8×0.7×0.7 cm with a finely nodular surface is visible on the surface. On sectioning, a dense, nodular, gray-colored formation measuring 4.0×3.5×3.0 cm is visible. The resection

margins are marked with black dye. Microscopic description: The specimen consists of hairy skin containing an intradermally demarcated lesion composed of three components — epithelial, myoepithelial, and mesenchymal. The epithelial component consists of tubules lined with epithelial cells; the cells are columnar, with moderately defined eosinophilic cytoplasm, an oval normochromic nucleus, granular or open chromatin, and a central nucleolus. Anisocytosis and anisokaryosis are not pronounced Mitoses are sporadic. The myoepithelial component consists of spindle-shaped cells with indistinct eosinophilic cytoplasm, reticulated chromatin, and a central nucleolus. Anisocytosis and anisokaryosis are minimal. No mitotic figures are observed. The cells are embedded in an extracellular basophilic matrix. The mesenchymal component is represented by cartilage. Histomorphological diagnosis: mixed benign tumor of the breast.

A tumor on the mammary gland, collected from a dog named 'Kapa', is encapsulated and oval in shape; the surrounding tissues are normal. Macroscopic description: 1k — 5 irregularly shaped tissue fragments, ranging in size from 2×2×0.7 cm (without skin) to 2.5×2×1.8 cm. Four of them are partially covered with skin; one has a nipple. All fragments are irregular in shape. The subcutaneous tissues are dense; in 3 of them, they form whitish nodules. Microscopic description: Hairy skin is present; intradermally, there is a non-encapsulated, ill-defined, infiltrative formation consisting of solid, irregular islands of neoplastic cells. Cells range in shape from rounded and oval to polygonal. They have well-defined borders and a small amount of eosinophilic cytoplasm. Nuclei are oval, clear, with deep chromatin and a central nucleolus. Anisocytosis and anisokaryosis range from moderate to marked. Mitotic activity is approximately 50 mitotic figures per 10 high-power fields. The stroma is markedly reactive, infiltrated with plasma cells and lymphocytes, and contains reactive fibroblasts. The lymph node contains a carcinoma metastasis. Histomorphological diagnosis: solid breast carcinoma with metastatic spread to a regional lymph node.

A neoplasm of the fifth mammary gland on the left side, excised from a dog named 'Lusia'; it is round, firm, and small in size; the surrounding tissues are unaltered. Following a regional mastectomy, the following histomorphological findings were obtained from the labeled biopsy specimens. Macroscopic description: 1k — 5 fragments of gray-brown tissue, partially covered by skin, with a total size of 4.5×3.0×1.5 cm; on sectioning, areas of induration are observed in the dermis and adjacent tissues; marking is not possible due to fragmentation. Microscopic description: A mammary gland structure is present, multilobular, and relatively well-defined. Composed of tubular and papillary structures lined with epithelial cells. Cells range in shape from rounded and oval to polygonal. They have well-defined borders and a small amount of eosinophilic cytoplasm. Nuclei are oval, normochromic, with deep chromatin and a central nucleolus. Anisocytosis and anisokaryosis range from moderate to marked. Mitotic activity is approximately 6–7 mitotic figures per 10 high-power fields. A large number of dilated ducts filled with eosinophilic or amphophilic contents are present. The stroma is moderate and contains lymphocytic-plasmacytic infiltrates. Necrotic areas and marked neutrophilic infiltration are present. Structures of regular lobular hyperplasia are present around the lesion. Histomorphological diagnosis: breast carcinoma, simple, well-differentiated (G1).

During a mastectomy performed on a dog named 'Pusha', the following labeled biopsy specimens were obtained: specimen 1 — tumor, caudal mammary gland; specimen 2 — inguinal lymph node. The tumor is cluster-like, consisting of encapsulated round nodules that are firm to the touch. Lymph node: size and shape are normal. Surrounding tissues show no noticeable morphological changes. Macroscopic description: 1k — gray tissue fragments with a total size of 2.7×1.9×1.2 cm. Resection margins cannot be marked due to prior fragmentation; 2k — gray tissue fragments with a total size of 3.8×2.4×1.3 cm. The resection margins cannot be marked due to prior fragmentation. Microscopic description: a mammary gland structure is present, unencapsulated, multilobular, consisting of tubular structures with ductal differentiation (filled with keratin). Cells range in shape from rounded and oval to polygonal. They have well-defined borders and a small amount of eosinophilic cytoplasm. Nuclei are oval, hyperchromatic, with deep chromatin and a central nucleolus. Anisocytosis and anisokaryosis range from moderate to marked. Mitotic activity is approximately 35–40 mitotic figures per 10 high-power fields. Stroma is moderate. Enlarged ducts filled with eosinophilic contents are present. Microscopically, normal adipose tissue and a lymph node without signs of metastatic growth are present. Histomorphological diagnosis: ductal carcinoma of the breast, lymph node without signs of metastatic growth.

The specimen collected from a dog named 'Tina' consisted of a mammary gland with a localized, encapsulated, homogeneous, oval-shaped mass. The surrounding tissues were not affected. Macroscopic description: 1k — skin flap fragments with overall dimensions of 5.5×4.0×1.0 cm. Dome-shaped formations measuring 0.3×0.3×0.2 cm and 0.5×0.4×0.3 cm are visible on the surface. Within the thickness of one of the fragments, a mass measuring 2.0×1.6×0.5 cm is identified; it cannot be marked due to the fragmentation of the pieces. Microscopic description: In the dermis, there is an encapsulated formation consisting of three components: epithelial, myoepithelial, and mesenchymal. The epithelial component consists of tubules lined with epithelial cells; the cells are columnar, with moderately defined eosinophilic cytoplasm, an oval normochromic nucleus, granular or open chromatin, and a central nucleolus. Anisocytosis and anisokaryosis are not pronounced. Mitoses are sporadic. Slightly dilated ducts

with eosinophilic or amphophilic contents are present. The myoepithelial component consists of spindle-shaped cells with indistinct eosinophilic cytoplasm, reticulated chromatin, and a central nucleolus. Anisocytosis and anisokaryosis are minimal. No mitotic figures are observed. The cells are embedded in an extracellular myxoid matrix. The mesenchymal component is represented by cartilage. Histomorphological diagnosis: mixed benign tumor of the breast.

The material for histological examination, collected during a mastectomy performed on a dog named 'Faya', consisted of a large, dense mammary gland mass and a right inguinal lymph node. The surrounding tissues were edematous. Macroscopic description: 1k — fragments with a total size of 5.7×5.0×0.4 cm. On sectioning of some fragments, a nodular gray-colored mass is identified, ranging in size from 0.8 to 1.5 cm; 2k — fragments with a total size of 4.0×3.0×0.4 cm. Microscopic description: a section is presented containing a non-encapsulated, ill-defined, infiltrative mass consisting of irregular, disorganized bundles and streams of neoplastic cells, spindle-shaped, with indistinct borders of scant eosinophilic cytoplasm, round or oval, large, somewhat clear nuclei, reticulated chromatin, and prominent purple nucleoli (1–3). Anisocytosis and anisokaryosis are pronounced; mitoses are frequent. The lesion contains large necrotic areas. Microscopically, it represents a mammary gland lesion consisting of two components — epithelial and myoepithelial. The epithelial component consists of tubules and papillary structures lined with epithelial cells; the cells are columnar, with moderately defined eosinophilic cytoplasm, an oval normochromic nucleus, granular or open chromatin, and a central nucleolus. Anisocytosis and anisokaryosis are not pronounced. Mitoses are sporadic. Slightly dilated ducts with eosinophilic or amphophilic contents are present. The myoepithelial component consists of spindle-shaped cells with indistinct eosinophilic cytoplasm, reticulated chromatin, and a central nucleolus. Anisocytosis and anisokaryosis are minimal. No mitotic figures are observed. The cells are embedded in an abundant basophilic extracellular myxoid matrix. Structures of regular lobular hyperplasia are present around the lesion. Microscopically, altered breast tissue is present, characterized by the growth of epithelial neoplastic cells forming tubules and papillary growths with ductal differentiation (filled with keratin). The neoplastic cells are rounded, with hyperchromatic nuclei, granular chromatin; anisocytosis and anisokaryosis are moderately pronounced; mitotic activity is up to 20 mitotic figures per 10 high-power fields. Histomorphological diagnosis: soft tissue sarcoma, complex breast adenoma, breast carcinoma, well-differentiated (G1).

A round-shaped breast neoplasm, firm to the touch and mobile on palpation, was obtained during a mastectomy performed on a dog named 'Funya' and sent for histomorphological examination. The surrounding tissues are unaltered. Macroscopic description: 1k — a skin flap measuring 1.5×1.5×1.2 cm. On sectioning, a round formation with clear borders, 0.5 cm in diameter, is visible in the dermis and has been marked. Microscopically, the formation is relatively well-defined and consists of two components intermingled with each other. The epithelial component consists of tubules lined with epithelial cells; the cells are columnar, with moderately defined eosinophilic cytoplasm, an oval normochromic nucleus, granular or open chromatin, and a central nucleolus. Anisocytosis and anisokaryosis are not pronounced. Mitoses are sporadic. Slightly dilated ducts with eosinophilic or amphophilic contents are present. The myoepithelial component consists of spindle-shaped cells with indistinct eosinophilic cytoplasm, reticulated chromatin, and a central nucleolus. Anisocytosis and anisokaryosis are minimal. No mitotic figures are observed. The cells are embedded in an extracellular basophilic myxoid matrix. Histomorphological diagnosis: complex benign breast tumor.

A mammary gland neoplasm in a dog named 'Hasya' was located in the region of the third pair of mammary glands; it was oval in shape, heterogeneous, and encapsulated. The surrounding tissues were unaltered. Microscopic description: 1k — a gray skin flap measuring 3.8×3.3 cm, with a hypodermis thickness of 1.9 cm. A dome-shaped, gray formation measuring 0.8×0.5×0.5 cm with a finely nodular surface is visible on the surface. On sectioning — a dense, nodular, gray-colored formation measuring 4.0×3.0×1.5 cm with a cavity filled with liquid white contents. The margins of the resection are marked with black dye. Microscopically, hairy skin with a nipple is observed; intradermally, extending into the hypodermis, there is an encapsulated formation consisting of tubular and solid structures (islands, solid shields) lined with epithelial cells. Cells range in shape from rounded and oval to polygonal. They have well-defined borders and a small amount of eosinophilic cytoplasm. Nuclei are oval, hyperchromatic, with deep chromatin and a central nucleolus (areas containing large, vacuolated epithelial cells are present). Anisocytosis and anisokaryosis are moderately pronounced. Mitotic activity is approximately 15–20 mitotic figures per 10 high-power fields. Dilated ducts filled with amphiphilic contents are present. The stroma is moderate and contains lymphocytic-plasmacytic infiltrates. Areas of necrosis are present. The lesion appears to have been completely excised. Histomorphological diagnosis: well-differentiated simple breast carcinoma (G1).

Concerning neoplastic processes in cats, we have demonstrated the following correlation between the clinical stage of mammary gland tumors and their histological findings (Table 2).

Thus, during a unilateral mastectomy performed on a cat named 'Anfisa', the right mammary glands were removed, including morphologically normal tissue, and a lymph node dissection of the inguinal and axillary lymph nodes was performed.

Table 2 — The relationship between the clinical stage of breast tumors and their histological confirmation in cats

The animal's name and age	Clinical stage by TNM	Histological diagnosis
Anfisa, 9 years old	$T_2N_0M_0$	Tubular, well-differentiated breast carcinoma without evidence of metastatic spread to the lymph nodes (G1)
Bagira, 8 years old	$T_1N_0M_1$	Simple tubular breast carcinoma, poorly differentiated (G3)
Baron, 11 years old	$T_2N_0M_0$	Simple tubular breast carcinoma (G1)
Jaima, 11 years old	$T_2N_1M_0$	Comedocarcinoma of the breast with metastasis to a lymph node
Leia, 7 years old	$T_1N_0M_0$	Simple carcinoma of the breast
Nika, 11 years old	$T_3N_1M_1$	Osteosarcoma of the breast
Plyusha, 11 years old	$T_3N_0M_0$	Apocrine ductal adenoma, follicular hyperplasia of a lymph node
Pusha, 6 years old	$T_3N_0M_0$	Fibroadenomatous changes in the breast; a lymph node with follicular hyperplasia
Sonya, 10 years old	$T_3N_1M_0$	Carcinoma with neoplastic lymph node invasion
Helga, 10 years old	$T_3N_1M_0$	Solid breast carcinoma with metastatic spread to a regional lymph node

All pathological material was labeled: specimen 1 — mammary gland tumor, specimen 2 — lymph nodes (inguinal and axillary). Macroscopic description: 1k — a gray skin excision measuring 2.5×0.5 cm, with a hypodermis thickness of 1.3 cm. On section — an area of gray induration. The resection margins are marked with black dye; 2k — two fragments with a total size of 3.4×2.9×1.0 cm. On sectioning, a gray-colored nodule measuring 0.3×0.2×0.1 cm. Microscopically, a relatively well-defined mammary gland formation is observed, located within a dense stroma. It is composed of tubular structures lined with epithelial cells. Cells range in shape from rounded and oval to polygonal. They have well-defined borders and a small amount of eosinophilic cytoplasm. Nuclei are oval, hyperchromatic, with deep chromatin and a central nucleolus. Anisocytosis and anisokaryosis range from moderate to marked. Mitotic activity is approximately 25 mitotic figures per 10 high-power fields. Dilated ducts filled with eosinophilic contents are present. There is no vascular invasion; the lesion appears completely excised. There are no signs of metastatic growth in the lymph nodes. Histomorphological diagnosis: breast carcinoma, tubular, well-differentiated, with no signs of metastatic growth in the lymph nodes (G1).

A histological examination was performed on a neoplasm located at the level of the second–third mammary gland pair on the left side of a cat named 'Bagira', measuring 8.0–10.0 cm, which exhibited local ulcerative foci. Examination was also performed on a lymph node obtained following lymph node dissection. The samples were labeled as follows: 1 — mammary gland neoplasm (recurrence at the necrosis stage); 2 — axillary lymph node. Macroscopic description: 1k: a soft tissue fragment measuring 5.5×4.0×3.0 cm consisting of a skin flap measuring 5.5×4.0 cm with a bumpy, whitish-grey surface and a subcutaneous, nodular, firm-elastic mass measuring 5.0×3.5×2.3 cm. This mass was partially cut, firm-elastic in consistency, and grey in colour, with necrotic cavities throughout. 2k: a soft tissue fragment measuring 5.0×4.0×2.8 cm presented as a sectioned nodule of whitish-grey colour with an elastic consistency and a cavity of decay. Microscopically, it contains areas of hairy skin with mammary gland formation. The neoplasm is not encapsulated and has no defined borders. It consists of tubular structures lined with epithelial cells. The cells range in shape from rounded and oval to polygonal. They have well-defined borders and a small amount of eosinophilic cytoplasm. Nuclei are oval, hyperchromatic, with deep chromatin and a central nucleolus. Anisocytosis and anisokaryosis range from moderate to marked. Mitotic activity is approximately 70 mitotic figures per 10 high-power fields. The stroma is moderate, abundantly infiltrated with neutrophils. Large necrotic areas are present centrally and around the neoplastic structures. The structure of the lesion is morphologically similar to the previous one; however, lymphoid follicles are present nearby, but they lie outside the tissue, so likely that this neoplasm is completely displaced by lymph node carcinoma structures. Histomorphological diagnosis: simple tubular carcinoma of the breast, poorly differentiated (G3).

During the mastectomy of a cat named 'Baron', which was performed due to the presence of a round-shaped, 1×1 cm, encapsulated neoplasm that was non-invasive to the surrounding tissues, two tissue samples were collected. The first specimen consists of dense, elastic, whitish-gray tissue, measuring 5×4×4 mm. The second is a skin fragment measuring 1.2×1.0 cm, 3 to 7 mm thick; the skin surface is unchanged; on sections, an intradermal formation is visible in the form of a dense nodule of heterogeneous gray-brown color. Microscopically, a relatively well-defined mammary gland formation is observed. It is composed of tubular structures lined with epithelial cells. The cells range in shape from rounded and oval to polygonal. They have well-defined borders and a small amount of eosinophilic cytoplasm. Nuclei are oval, hyperchromatic, with deep chromatin and a central nucleolus. Anisocytosis and anisokaryosis are moderate. Mitotic activity is up to 8–10 mitotic figures per 10 high-power fields. Dilated and markedly dilated ducts filled with eosinophilic contents

are present. Histomorphological diagnosis: simple tubular carcinoma of the breast (G1).

Histological material from the cat 'Jaima', obtained during a mastectomy, has been divided into two samples: No. 1 — mammary gland neoplasm, No. 2 — axillary lymph node. Multiple lesions on the mammary glands. The largest is up to 3.0 cm in diameter and is located at the level of the first mammary gland on the right. Lesions on the 3rd–5th mammary glands up to 1.0 cm; enlarged right axillary lymph node. Macroscopic description: 1k — a 4.5×4.5 cm irregularly shaped skin fragment with subcutaneous tissue ranging from 0.5 to 1.5 cm, with multiple parallel incisions to the skin; on the incisions, a subcutaneous nodular mass of dense consistency, whitish-gray in color, with clear borders, measuring 2.2×2.0×1.3 cm; 2k — a nodular formation measuring 1.5×1.2×0.5 cm, representing an opened cystic formation with a cavity measuring 0.8×0.6×0.3 cm, with whitish walls ranging from 0.1 to 0.3 cm. Lesions are characterized by the formation of fairly large aggregates of densely packed cells containing large central foci of necrosis. The necrotic foci contain eosinophilic material. The tumor mass consists of densely packed aggregates of cells, predominantly in tubules. Tumor cells are round in shape, with a small amount of eosinophilic cytoplasm with distinct borders; the nuclei are large, round, hyperchromatic, and contain 1–3 nucleoli. Anisocytosis and anisokaryosis are pronounced. Mitotic activity is increased, with an average of 3–4 mitotic figures per high-power field. The lymph node contains carcinoma structures. Histomorphological diagnosis: comedocarcinoma of the breast with metastatic spread to a lymph node.

The histological specimens collected during a mastectomy from a cat named 'Leia' are labeled as follows: specimen 1 — right inguinal lymph node; specimen 2 — tissue from the caudal mammary gland; specimen 3 — cranial mammary gland; specimen 1 — vesicles on the lymph node, enlarged in size; specimen 2 — a neoplasm of the caudal lymph node, up to 3 cm in diameter, rounded, with clear borders and a capsule; specimen 3 — morphologically unchanged nipple and mammary gland (cranial). Bubbles are visualized in the subcutaneous tissue and around the neoplasm and lymph node, with inflammation of the subcutaneous fat. Macroscopic description: 1k — a gray tissue fragment measuring 1.9×1.7×1.0 cm. On sectioning — a gray-colored nodule measuring 1.0×0.9×0.7 cm; 2k — gray-colored tissue fragments with a total size of 4.0×3.5×1.0 cm. On sectioning of the fragments, areas of gray-colored induration are identified. The resection margins cannot be marked due to prior fragmentation; 3k — a gray-colored skin flap measuring 2.2×1.3 cm, with a hypodermis thickness of 0.3 cm. A gray, polypoid formation measuring 0.4×0.2×0.2 cm with a finely nodular surface is visible on the surface. The resection margins are marked with black dye. The dermis is present. A non-encapsulated, ill-defined neoplasm localized in the hypodermis, consisting of neoplastic epithelial-type cells arranged in tubular structures that form islands separated by septa of a thin fibroepithelial stroma. Cells range in shape from rounded and oval to polygonal. They have well-defined borders and a small amount of eosinophilic cytoplasm. Nuclei are oval, hyperchromatic, with deep chromatin and a central nucleolus. Anisocytosis and anisokaryosis range from moderate to marked. Mitotic activity is approximately 30 mitotic figures per 10 high-power fields. In the hypodermis, there is mammary gland formation similar to that described above; however, the islands are more massive, the nuclei are lighter, and mitotic activity is slightly higher, up to 35 mitotic figures per 10 high-power fields. Large necrotic foci and lymphoid follicles are noted at the periphery. Vascular invasion is observed. However, the nipple is not affected; structures of regular lobular hyperplasia are observed in the underlying tissue. Histomorphological diagnosis: simple breast carcinoma.

A large formation in the caudal mammary gland (the fifth pair on the right) of a cat named 'Nika' was sent for histological analysis. The neoplasm was found to be adjacent to local areas of erosion and necrosis, as well as disseminated small skin neoplasms in the region of the cranial mammary glands and hyperplasia of the axillary lymph node. Macroscopic description: 1k — two unmarked fragments. The first fragment is dense-elastic and partially spongy with a grey colour. It measures 3.0×3.0×3.3 cm and has an irregularly shaped skin flap measuring 2.5×2.0 cm. The edges of the fragment were stained blue during specimen preparation. The tissue section is divided into two compartments throughout its entire thickness. The second fragment consists of an irregularly shaped nodular formation measuring 3.5×2.0×2.4 cm, which is partially covered by a smooth grey capsule. The tissue structure is similar to that of the formation in the first fragment. Microscopically, hairy skin is visible. An unencapsulated, infiltrative, densely cellular formation is present intradermally. The neoplastic cells are pleomorphic and polygonal with sparse or moderate cytoplasm, a central round nucleus, reticulated chromatin and distinct nucleoli. The cells form disorganized bundles and streams. Anisocytosis and anisokaryosis are pronounced, with mitotic activity. Multifocal areas resembling osteoid are present. Multinucleated giant cells are also visible. Histomorphological diagnosis: osteosarcoma of the breast.

On palpation, a neoplasm in the mammary gland of a cat named 'Plyusha' is determined to be a non-invasive, encapsulated mass with a heterogeneous structure and well-defined borders. The surrounding tissues are unchanged. A mastectomy was performed. Macroscopic description: 1k — a gray-colored skin flap, measuring 3.4×2.4 cm, with a hypodermis thickness of 1 cm. On the surface, a dome-shaped, gray-colored mass measuring 2.7×2.3×0.9 cm with a finely nodular surface is identified. On section: a nodular gray-colored formation, measuring 2.7×1.6×1.4 cm, with small cavities filled with mucus. The resection margins are marked with blue dye; 2k is a gray skin excision measuring 4.2×2.8 cm, with a

hypodermis thickness of 1.3 cm. A dome-shaped, gray-colored formation measuring 3×2.5×0.6 cm with a finely nodular surface is visible on the surface. On the section — a nodular gray-colored formation measuring 3×2.5×1 cm, with small cavities filled with mucus. The resection margins are marked with blue dye; 3k is a gray tissue fragment measuring 3×2.5×1.7 cm. A nodular formation measuring 1.5×1.4×1.2 cm is visible on the surface. Focal, moderately well-defined, multinodular, non-encapsulated, cell-rich neoplastic proliferation is observed in the dermis, consisting of epithelial cells with a large central, predominantly optically empty lumen. The neoplastic cells range in shape from cubic to oval, forming solid areas and often small tubular structures, some of which appear to be lined by a double layer of cells and resemble the Arabic letters. At the center of a smaller number of solid formations are multifocal areas of necrosis. The collagenous stroma is thin. Neoplastic cells show moderate variation among themselves, have relatively little eosinophilic cytoplasm, and contain an oval or rounded nucleus with fine, clumped chromatin and an absent or barely visible, small nucleolus. Anisocytosis and anisokaryosis are mild or moderate. Up to 7–8 mitoses are present in 10 fields at the highest magnification (×400, 2.37 mm^2). Multiple foci within and around the tumor contain a slight to moderate number of lymphocytes and plasma cells. A lymph node with signs of follicular hyperplasia (marked) is present. Histomorphological diagnosis: apocrine ductal adenoma, follicular hyperplasia of the lymph node.

The lesion in the mammary gland of the cat named 'Pusha' is encapsulated, homogeneous, and localized at the level of the 4th pair; hypertrophy of the axillary lymph node is palpable. Macroscopic description: 1k — container labeled No. 1, containing a fragment of irregularly shaped adipose tissue, 2.3×1.0×0.6 cm, with a lymph node 0.8 cm in diameter within it; 2k — an irregularly shaped skin fragment measuring 5.0×3.0 cm with a subcutaneous fragment of a firm, elastic, whitish nodule measuring 4.0×3.5×2.5 cm, with a homogeneous structure on sections. Microscopic description: lymph node in a state of follicular hyperplasia; microscopically, there is proliferation of the stroma and fibroblasts. Fibroblasts are spindle-shaped, tailed, with minimal or moderate anisokaryosis. Some ducts are dilated and lined with epithelial cells; others are lined with several layers of epithelial cells showing moderate anisocytosis and anisokaryosis. The lesion is covered by hyperplastic epidermis; orthokeratosis is noted. Subepidermally, there is infiltration by inflammatory cells — lymphocytes and plasma cells — with occasional neutrophils and macrophages. Histomorphological diagnosis: fibroadenomatous changes in the breast; a lymph node in a state of follicular hyperplasia.

Histological examination of breast tumor samples obtained from a cat named 'Helga' was labeled as follows: Sample 1 — left inguinal lymph node; Sample 2 — breast tumor. The lymph node is mobile and enlarged; the mammary gland neoplasm is encapsulated, round in shape, up to 2 cm in size, surrounded by an area of diffuse edema. Macroscopic description: 1k — lymph node measuring 1.5×1.0×0.5 cm, with a small amount of fatty tissue; 2k — tissue fragments with a total size of 3.3×3.0×1.2 cm, including fragments of skin. Marking is not possible due to fragmentation. On sectioning of the fragments, a dense, nodular, gray-colored formation is identified, with a maximum diameter of 0.4 to 1.0 cm; 3k — gray-colored skin flap measuring 1.5×1.0×0.3 cm. A dome-shaped, gray-colored formation measuring 1.0×0.6×0.2 cm with a finely nodular surface is observed on the surface. The resection margins are marked with black dye. Microscopically, the lymph node is infiltrated by carcinoma structures. The structure of the mammary gland is shown microscopically. Neoplastic cells are densely packed into irregular lobules and surrounded by a delicate fibro-vascular stroma. The cells are arranged in clusters, masses, and strands, without any clear spaces. The cells are polygonal or oval, with well-defined borders of eosinophilic cytoplasm (in some cases, the borders are poorly visualized). The nuclei are rounded or oval, often vacuolated, with deep chromatin and a central basophilic nucleolus. Anisocytosis and anisokaryosis are pronounced. Mitotic activity averages 3–4 mitotic figures per high-power field. Dense lymphocytic infiltrates are present at the periphery of the lesion. A skin specimen is presented for microscopic examination. The epidermis is diffusely hyperplastic to a moderate to marked degree, forming exophytic papillary projections, and is covered with a moderate amount of lamellar or orthokeratotic keratin, ranging in appearance from lamellar to braided. The formation of broad ridges of the epidermis is observed. There are ulcerated areas with blood and crusting. The cells show marked dysplastic features. Cells are large, with large hyperchromatic or normochromatic oval nuclei and prominent nucleoli. Anisocytosis and anisokaryosis range from moderate to marked; mitotic activity is observed. Several 'keratin pearls' are multifocally visible. The biopsy specimens also contain isolated areas in which the epidermis is hyperpigmented, with transepidermal hyperpigmentation. Multifocal, within the dermis, there is marked infiltration by inflammatory cells — lymphocytes, plasma cells, and neutrophils. Histomorphological diagnosis: solid breast carcinoma with metastatic spread to a regional lymph node.

Macroscopic examination of the left axillary lymph node, obtained during lymph node dissection, which had enlarged two months after unilateral mastectomy in a cat named 'Sonya', revealed focal areas of necrosis, labeled as 1k — a fragment of a firm-elastic whitish lymph node measuring 2.0×1.3×1.0 cm with dark-brown areas within its thickness. Microscopically, the lymph node contains large necrotic areas and carcinoma structures (metastatic growth). The carcinoma structures are tubular, lined with epithelial cells. The cells range in shape from rounded and oval to polygonal. They have well-defined borders and a small amount of eosinophilic cytoplasm. The nuclei are oval, hyperchromatic, with deep

chromatin and a central nucleolus. Anisocytosis and anisokaryosis range from moderate to marked. Mitotic activity is approximately 55–60 mitotic figures per 10 high-power fields. Histomorphological diagnosis: carcinoma with neoplastic invasion of a lymph node.

To assess the relationship between the clinical stage of mammary gland tumors (according to the TNM system) and their histological characteristics (benign or malignant neoplasms), a statistical analysis was performed using the χ^2 criterion and Fisher's exact test. Data were analyzed by constructing contingency tables.

All breast neoplasms in animals were divided into two groups: (i) benign (adenomas, mixed tumors, fibroadenomatous changes); (ii) malignant (carcinomas, sarcomas, osteosarcomas).

In addition, clinical stages were conditionally grouped into: (i) early stages: T_1–T_2, N_0, M_0; (ii) advanced stages: T_3 and/or the presence of N_1 and/or M_1.

The analysis revealed that malignant tumors were significantly more often associated with advanced stages of the disease (presence of lymph node metastases and/or distant metastases), whereas benign tumors predominantly corresponded to early stages ($T_1N_0M_0$).

Application of the χ^2 criterion showed a statistically significant association between TNM stage and tumor type ($p < 0.05$). Given the small sample size and the presence of expected frequencies of less than 5 in some cells of the table, Fisher's exact test was additionally applied, which confirmed the reliability of the results obtained ($p < 0.05$). The results demonstrate a consistent and biologically plausible trend toward a more aggressive course of the neoplastic process in animals of the second group. In particular, when assessing the distribution by primary tumor stage (T), it was found that early-stage neoplasms (T_1) predominate in animals of the first group, whereas more advanced stages (T_2–T_3) are more frequently recorded in the second group. The observed difference has a clear directionality and indicates possible differences in the rate of tumor progression or the time of their detection. The results of the analysis of metastatic involvement (N and M) proved to be the most revealing. A significant increase in the frequency of metastases was observed in the second group (50%) compared to the first (10%), accompanied by the lowest p-value among all analyses performed and reflecting a pronounced trend toward increased invasiveness and metastatic potential of the tumors.

Separately, it was established that:

1. The presence of metastases in regional lymph nodes (N_1) was observed exclusively in malignant tumors;
2. Distant metastases (M_1) were recorded only in animals with highly malignant neoplasms (in particular, carcinomas and osteosarcomas);
3. Benign neoplasms did not metastasize.

The results obtained are consistent with the current understanding of the biological behavior of breast tumors in animals and are supported by data from the literature. In particular, the statistically significant association established in our study between the clinical stage according to the TNM system and the histological malignancy of the neoplasms corresponds to the conclusions of a number of authors who emphasize the leading role of staging in predicting the course of the disease. It has been shown that as the stage of the tumor process increases, animal survival rates decrease significantly, especially in the presence of metastases (Petrucci et al., 2021; Vazquez et al., 2023).

The trend identified in our study toward the association of malignant tumors with advanced stages (T_3, N_1, M_1) is also confirmed by data from other studies. Thus, breast carcinomas are characterized by a high frequency of metastasis, particularly to regional lymph nodes and the lungs, which is a key prognostically unfavorable factor (Soultani et al., 2021; Klaengkaew et al., 2021). In contrast, benign tumors typically do not exhibit invasive growth or metastasis, which is consistent with our findings regarding their predominant localization in early stages ($T_1N_0M_0$).

Of particular importance is the fact that involvement of regional lymph nodes (N_1) and the presence of distant metastases (M_1) were observed exclusively in malignant tumors. This is fully consistent with the literature, where metastasis is considered one of the most important independent prognostic factors, significantly reducing the animals' lifespan (Soultani et al., 2021). Furthermore, it is emphasized that even in the absence of clinical signs of metastasis, their microscopic presence can influence the prognosis, which justifies the need for a thorough histological evaluation of the lymph nodes.

Our data on the distribution of tumors by T stage are also consistent with the results of other studies, which indicate the importance of tumor size as a prognostic criterion. In particular, an increase in the size of the primary tumor is associated with a higher probability of metastasis and a poorer prognosis (Rose and Worley, 2020; Sánchez et al., 2019). This explains the prevalence of higher stages (T_2–T_3) among malignant neoplasms in our sample.

At the same time, the results confirm that the use of univariate analysis alone may be insufficient for a comprehensive assessment of prognostic factors. The literature repeatedly emphasizes that many indicators lose statistical significance in multivariate analysis, which indicates the complex, multicomponent nature of the tumor process (Zhelavskyi and Dmytriv, 2023). In this context, the use of the χ^2 criterion in combination with Fisher's exact test in our study is methodologically sound, especially given the small sample size.

Furthermore, the results are consistent with current approaches to the assessment of prognostic factors in veterinary oncology, which emphasize the need for a comprehensive consideration of the clinical, histological, and molecular characteristics of tumors (Feliciano et al., 2023; Pastor et al., 2022). Although our study focused primarily on morphological and clinical parameters, the data obtained support the need for further research incorporating molecular markers.

It should be noted that the results of our study reflect the general trends described in the literature but have certain limitations. In particular, the small number of animals in the sample reflects a common problem in most veterinary studies, as highlighted by other authors (Greco et al., 2023). This limits the statistical power of the analysis and necessitates further studies with larger samples.

Thus, the obtained results not only align with the data in the current scientific literature but also complement them, confirming the important role of TNM clinical staging in predicting the course of mammary tumors in animals. The clear association identified between the malignancy of neoplasms and the extent of the tumor process underscores the need for early diagnosis and timely surgical intervention, and justifies the use of a comprehensive approach to the assessment of prognostic factors.

In summary, based on the results presented, the following conclusions can be drawn:

1. There is a statistically significant association between the clinical stage of breast tumors according to the TNM system and their histological characteristics;

2. Malignant tumors are significantly more often detected in the late stages of the disease and are accompanied by metastatic involvement of lymph nodes and distant organs;

3. Benign tumors predominantly correspond to early stages ($T_1N_0M_0$) and show no signs of metastatic growth;

4. The presence of N_1 and M_1 components is a poor prognostic sign and may be considered a marker of aggressive disease progression;

5. The use of the χ^2 criterion in combination with Fisher's exact test is appropriate for analyzing small samples in veterinary oncology studies and allows for increased reliability of the results obtained.

Overall, the data obtained confirm that clinical staging according to the TNM system has significant prognostic value and correlates with the histological malignancy of breast tumors in animals, a fact that should be taken into account when developing diagnostic and therapeutic approaches.

Conclusions. 1. The data obtained allow us to draw a fundamentally important conclusion: the differences between the examined groups are related not so much to the size of the primary tumor node as to the tumor's biological behavior, specifically its ability to invade surrounding tissues and metastasize to distant sites. This is consistent with current concepts in veterinary oncology, according to which metastatic potential is the key factor determining the course of the disease and prognosis.

2. The study results indicate that when assessing mammary gland neoplasia, a more informative indicator is not only the size of the primary tumor (T), but primarily the presence and extent of metastases (N and M), which reflect the aggressiveness of the neoplastic process and have higher prognostic value.

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CURRENT EPIZOOTOLOGICAL MANIFESTATIONS OF CANINE PARVOVIRUS INFECTION BASED ON CLINICAL AND PATHOLOGICAL CRITERIA AND SEASONALITY

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Summary. Canine parvovirus infection is a highly contagious viral disease caused by the canine parvovirus (CPV-2), characterized by acute hemorrhagic enteritis, leukopenia, and myocarditis. This study aimed to assess the current epizootiological manifestations of canine parvovirus infection based on clinical and pathological criteria and seasonality at the 'Zoolux' network of veterinary clinics in Kyiv. To this end, 3,760 rapid tests were conducted to detect parvovirus in dogs of various breeds at the 'Zoolux' veterinary clinic network in Kyiv between 2013 and 2025. Detection of the canine parvovirus antigen in dog feces was performed using immunochromatographic analysis with special reagent kits (the 'VeChek CPV Ag' and 'VetExpert CPV Ag' kits). Of the 3,760 rapid tests conducted, 679 (18.1%) returned positive results. Despite a pronounced summer-fall seasonality, which significantly exceeded the incidence in winter ($p < 0.05$) with peaks in October and June (12.5% and 10.8% of cases, respectively), parvovirus infection in dogs is observed throughout the year. The main clinical manifestations of viral enteritis in the dogs we examined were lethargy, vomiting, watery bloody feces with a sharp foul odor, refusal of food and water; signs of cardiovascular and respiratory system involvement were observed less frequently. Further research is needed to identify methods for the early and rapid diagnosis of the etiology of viral enteritis in dogs, which will allow for the precise identification of the causative agent and, consequently, the selection of effective treatment methods for the animal and the prevention of the spread of infection through the use of specific preventive measures

Keywords: dogs, viral enteritis, enteritis

Introduction. Despite significant advances in veterinary medicine, infectious diseases in animals, including dogs, remain a pressing issue. Most infectious and parasitic agents damage the gastrointestinal tract of dogs, causing symptoms ranging from mild diarrhea to severe enteritis, which can lead to dehydration, organ damage, and ultimately, death. One of the most common agents is the canine parvovirus (CPV-2), which causes parvovirus infection.

Canine parvovirus infection is a highly contagious viral disease caused by CPV-2 (canine parvovirus enteritis). It is characterized by acute gastroenteritis and is often accompanied by dehydration, myocarditis, and leukopenia. While the disease can occur at any age, puppies under six months old are most susceptible. It is believed that animals are more susceptible to parvovirus at this age due to the immaturity of their immune systems and the decline of maternal immune protection (Hasan et al., 2017; Ogbu et al., 2016; Tagorti, 2018).

Parvovirus enteritis in dogs is observed throughout the year, with periods of seasonal increases. However, data on the seasonality of parvovirus in the literature vary significantly, which may be primarily due to the climatic characteristics of a particular region. For example, Ngu Ngwa et al. (2020) noted a peak in parvovirus incidence in dogs in Cameroon in November, January, and December; whereas in Nigeria, according to data from Agada et al. (2022), the highest incidence occurred during the dry season in that region, specifically December–March, with a peak in January; in China, according to data from Dong et al. (2020), it occurred in the spring; and in India, according to data from Naveenkumar et al. (2025), it occurred during the winter and in July. At the same time, Naveenkumar et al. (2025) established a positive correlation between the increase in cases of canine parvovirus and maximum temperature (with a lag of 11 months), relative humidity in the morning (with a lag of 1 month), and precipitation (with a lag of 10 months); and negative correlations with maximum temperature (with a lag of 1 and 6 months), minimum temperature (with a lag of 4 months), relative humidity in the morning (with a lag of 7 months), relative humidity in the evening (with a lag of 7 months), precipitation (with a lag of 2 and 7 months), and wind speed (with a lag of 4 months) (Naveenkumar et al., 2025).

Canine parvovirus (CPV-2) is a member of the Parvoviridae family, which consists of the smallest spherical viruses; it contains DNA and lacks a lipoprotein envelope. This family includes more than 30 species belonging to three genera: *Parvovirus*, *Dependovirus*, and *Densovirus*. The genus *Parvovirus* consists of autonomous (complete, non-defective) viruses and can cause inflammation of the intestine (enteritis), myocardium (myocarditis), brain tissue (encephalitis, hemorrhagic encephalopathy), liver (hepatitis), embryonic and fetal tissues (leading to their death), suppression of hematopoiesis and lymphopoiesis (causing panleukopenia with a reduced immune response), and possible growth retardation in affected animals (Lisova et al., 2022; Radzykhovskyi, 2021).

In experimental studies, the incubation period of the disease was 4–5 days after infection of the animal; however, according to epidemiological data, it lasts

7–14 days. Active viral shedding by an infected animal begins as early as the asymptomatic incubation period (3–4 days after exposure) and lasts for 7–10 days. The production of virus-neutralizing, hemagglutinating, and complement-fixing antibodies begins as early as 5 days after the animal is infected. The viremic phase of the disease is characterized by initial viral replication in the tonsils and lymph nodes of the oropharynx, from where it spreads to other organs via lymphoid tissues such as the thymus, spleen, Peyer's patches, and lymph nodes. Canine parvovirus can be detected in the epithelium of the entire gastrointestinal tract, spleen, bone marrow, thymus, tonsils, lymph nodes, myocardium, kidneys, liver, and fetal tissues in a pregnant animal (Greene and Decaro, 2016). If a pregnant dog becomes ill, generalized intrauterine infection of the fetus or even termination of pregnancy may occur.

Many people assume that the sudden onset of bloody, foul-smelling diarrhea in a young dog is always caused by a CPV infection. However, this is far from the case. First, not all dogs with bloody diarrhea (regardless of whether vomiting is present) are infected with parvovirus. Second, parvovirus can also cause non-hemorrhagic diarrhea. Furthermore, it is rare for all typical clinical signs of parvovirus to be observed simultaneously. Acute hemorrhagic diarrhea syndrome in dogs can result from parvovirus infection or the action of other infectious agents. These pathogens include canine coronavirus, canine distemper virus, and *Clostridium perfringens* type A.

Most animals (about 80% of dogs), primarily older ones, experience a subclinical disease course; 10% have a moderate-to-severe course, and only 10% have a severe course (Sato-Takada et al., 2022).

A characteristic feature of parvovirus is its active replication in rapidly dividing cells, i. e., those with high mitotic activity. This fact explains its tropism specifically for intestinal epithelium and lymphoid tissue cells. The virus's entry into the intestine and its replication there lead to the death of embryonic epithelial cells of the intestinal glands, followed by epithelial desquamation and shortening of the villi, causing vomiting, hemorrhagic diarrhea, dehydration, fever, central nervous system depression, shock, and even death (Lisova et al., 2022). The decrease in intestinal immune reactivity is associated with the development of secondary Gram-negative and anaerobic bacterial infections, which cause additional complications such as bacteremia, endotoxemia, disseminated intravascular coagulation, systemic inflammatory response, and cardiorenal syndrome (Zamoshnikov, 2024a; Greene and Decaro, 2016).

Currently, myocarditis (i. e., inflammation of the myocardium) is a relatively rare clinical syndrome associated with parvovirus infection and occurs primarily in puppies born to unvaccinated females or those that are malnourished (Ford et al., 2017; Lixandru and Ursachi, 2020). In addition, it is more common in young puppies under 1–2 months of age, whereas the enteritic form predominates in older animals. Myocarditis may often be completely asymptomatic, or it may present with mild diarrhea, cyanosis of the mucous membranes, wheezing, labored breathing, and a rapid, weak pulse (Lisova et al., 2022). The development of myocarditis is characterized by the onset of myocardial necrosis and inflammation, leading to acute heart failure, which may clinically manifest as pulmonary edema and/or hepatic venous congestion. Quite often, the animal dies precisely because of these complications. If the animal survives this acute phase, it most often develops replacement or interstitial fibrosis and develops chronic heart failure.

The pathomorphological changes that are typically characteristic of parvovirus enteritis include hemorrhagic-necrotic inflammation of the small intestinal mucosa and hyperplasia with atrophy of the lymphoid tissue of the intestinal tract. Often, there is also eosinophilic alterative myocarditis. Histological changes in dogs that died from parvovirus correspond to intestinal and/or cardiac forms of the disease. In the intestinal form, the main changes are observed in the intestine and include hemorrhagic enteritis, hepatitis with dystrophic changes in hepatocytes, serous-hemorrhagic inflammation of the mesenteric lymph nodes, splenomegaly, and dystrophic and necrotic changes in the pancreas. In the cardiac form, acute alterative myocarditis is often accompanied by hemorrhages in the lungs and atelectatic foci (Radzykhovskyi, 2021).

According to data from the experimental reproduction of parvovirus infection in dogs, the morphological criteria for the disease include hemorrhagic inflammation of the jejunum, ileum, and mesenteric lymph nodes; necrosis of intestinal lymphoid tissue; and hemorrhages in the mucous and serous membranes of the small intestine. Lesions of the large intestine are segmental and predominantly localize in the cecum and colon (Radzykhovskyi, 2021).

Due to the variability in clinical presentation, the prevalence and contagiousness of the infection, and the economic and psychological impact on pet owners, early diagnosis and personalized etiological and pathophysiological treatment are critical for enterovirus infections in dogs, including parvovirus. A comprehensive diagnosis is necessary, taking into account the dog's medical history (onset and progression of clinical signs, condition before disease onset, contact with potentially infected animals, living conditions, age, acquisition source, administered preventive vaccinations, and prior medication use), physical examination findings, vital signs, and hematological and biochemical analysis results. Although virological testing is the most accurate method for determining the cause of a viral infection, it is not widely used due to the time-consuming nature of the procedure, the need for expensive equipment, technical difficulties, and the high cost. Therefore, rapid diagnostic methods are more commonly used in practice. These methods allow one to

'circumvent' the limitations and complexities of virological testing while offering high sensitivity and specificity for detecting the pathogen's antigens. These methods primarily include the immunochromatographic assay (ICA) and the polymerase chain reaction (PCR) (Ilchenko and Tsarenko, 2020; Prosolenko, 2023).

This study **aims** to assess the current epizootological manifestations of canine parvovirus infection based on clinical and pathological criteria and seasonality at the 'Zoolux' network of veterinary clinics in Kyiv (Ukraine).

Materials and methods. To achieve the stated objective, an analysis and synthesis of current scientific and methodological literature was conducted, with searches performed in Google Scholar, Scopus, PubMed, Web of Science, and EuroPub databases.

Additionally, an analysis was conducted of the veterinary records of dogs of various breeds that presented with symptoms of enterovirus infection at the 'Zoolux' network of veterinary clinics in Kyiv between 2013 and 2025.

The etiological factor of the infection was determined using rapid methods to detect the pathogen's antigen in the feces or vomit of affected animals via immunochromatographic analysis. The canine parvovirus antigen was detected using the 'VeChek CPV Ag' (Quicking Biotech, China) and 'VetExpert CPV Ag' (VetExpert, Republic of Korea) test kits. Testing and interpretation of the results were performed according to the instructions for these kits (BIOVET.UA, 2026).

The clinical manifestations of parvovirus infection in the examined dogs, the results of additional diagnostic methods, and the seasonality of the disease were analyzed and systematized.

Under the current procedure, the research program was reviewed and approved by the Bioethics Committee of the Institute of Veterinary Medicine of the National Academy of Agrarian Sciences of Ukraine.

The results were processed by methods of variation statistics using Excel 2010 and StatSoft Statistica 10.0. To compare mean values Student's *t*-test was used (Van Emden, 2019).

Results and discussion. A test to detect CPV antigen was performed on 3,760 dogs, 679 of which (18.1%) tested positive. In the remaining cases, a different cause of enteritis was identified, medical care was sought too late, or the test was performed too early when the viral load in the samples was below the diagnostic sensitivity of the test system.

According to the literature, three clinical forms of parvovirus infection are recognized: enteritic (intestinal), myocardial (cardiac), and mixed.

The enteritic (intestinal) form predominantly occurs between 2 and 6 months of age. The disease begins suddenly with loss of appetite, sometimes accompanied by mild fever and abdominal tenderness on palpation; abdominal muscle rigidity may also be present, and vomiting occurs, often containing mucus and bile; Diarrhea usually begins within a day, with stools that are watery, foul-smelling, yellowish, and mixed with blood or completely saturated with it. Vomiting and diarrhea contribute to dehydration, the development of toxicosis, and often the onset of shock. In about one-third of cases, leukopenia is observed during the first 4–5 days of the disease, often accompanied by a fever. Older dogs may experience a subclinical form of the disease for 2–3 weeks, often manifesting as a sharp loss of appetite and lethargy, but rarely accompanied by other gastrointestinal disorders (Mazzaferro, 2020). After recovering from the disease, dogs may experience some growth retardation; in males, hypospermia or azoospermia (complete absence of sperm) may occur. If a pregnant female dog is infected, pregnancy loss, stillbirth, or fetal developmental abnormalities may occur (persistent cleft palate, nasal and lip abnormalities, brain hypoplasia) (Streletskyi, 2022).

The myocardial (cardiac) form of parvovirus infection in dogs occurs much less frequently than the enteritis form, in puppies without active or passive antibodies under 2 months of age, most often by 4 weeks of age, which is primarily associated with the high mitotic activity of cardiomyocytes during this period of life. More than 50% of puppies die from acute heart failure, which occurs suddenly in previously healthy puppies and is accompanied by a weak, rapid pulse, labored breathing, cyanosis of the mucous membranes, collapse, and convulsions. In puppies older than 8 weeks, subacute heart failure is more common; it can last several months and is characterized by weakness, shortness of breath, prostration, cardiac arrhythmias, and the development of congestive changes in the lungs and liver (Streletskyi, 2022). In addition, some dogs may develop acute kidney injury against a background of acute heart failure, i. e., cardiorenal syndrome (Zamoshnikov, 2024b; Ford et al., 2017; Lixandru and Ursachi, 2020).

The mixed form of the disease is rare and is characterized by combined involvement of the gastrointestinal tract and the heart.

Macroscopic changes observed during autopsies of dogs that have died from the enteritic form of parvovirus infection are characterized by external signs of dehydration, while the abdominal cavity may contain a small amount of straw-colored fluid; the intestines may be empty or contain bile, sometimes hemorrhagic fluid; their mucous membrane is bright red with signs of severe inflammation; intussusception with hyperperistalsis is often observed; the mesenteric lymph nodes are edematous, enlarged, and hemorrhagic; Peyer's patches are often hemorrhagic as well; erosions and vascular inflammation may be observed in the internal organs (Goralskii, Radsikhovskii and Zaika, 2018; Prosolenko, 2023).

Changes characteristic of the myocardial form of the disease include: in acute heart failure, the lungs are edematous, with localized reddish-gray areas, predominantly located in the middle and cranial lobes; the bronchi contain mucous exudate; hemorrhages in the spleen, often with infarcts, and frequently an

enlargement of the spleen; in subacute heart failure, signs of congestion in the liver and fluid accumulation in the serous membranes (hydrothorax, ascites, hydropericardium) are observed (Sato-Takada et al., 2022).

Morphological changes in the small intestine include focal lesions of the epithelial crypts with destruction of the epithelial villi; eosinophilic inclusions are found in the crypt nuclei. In the mesenteric lymph nodes, Peyer's patches, thymus, and spleen, there is destruction of lymphocytes and necrosis of lymphoid tissue. Morphological changes in myocarditis are characterized by interstitial inflammation of the heart muscle, its edema, and the accumulation of lymphocytes, histiocytes, plasma cells, and intranuclear inclusions in varying amounts. It is generally believed that necrosis of lymphoid tissue and its depletion in the lymph nodes, Peyer's patches, thymus, and spleen are the main pathomorphological features of parvovirus infection (Prosolenko, 2023; Mazzaferro, 2020).

In the thymus, the blood vessels dilate, leading to organ swelling and disruption of its cellular structure; lymphocyte differentiation in the cortical and medullary regions of the lobules is impaired. As a result of red blood cell breakdown, hemosiderin accumulates in the red pulp of the spleen, causing it to become edematous. Renal involvement is characterized by hemorrhages in the stroma of both the cortex and medulla, progressive edema of the renal glomeruli with their subsequent breakdown and necrosis, and dystrophic changes in the epithelium of all segments of the tubules. The liver is edematous; venous stasis, granular degeneration of hepatocytes, and their disintegration may be observed. Granular and hydropic degeneration may occur in the pancreas (Radzykhovskyi, 2021).

The clinical manifestations of viral enteritis in the dogs we examined included lethargy, vomiting, watery bloody feces with a sharp foul odor, refusal of food and water, and, less frequently, signs of cardiovascular and respiratory system involvement (Fig. 1).

Examinations and treatments at clinics often had to be performed in a veterinary hospital with intensive care units during infusion therapy to rehydrate the animal, using an infusion stand with an infusion pump (for precise control of the dose and rate of drug administration) or an IV drip (for the intravenous administration of drugs or fluids) (Fig. 2).

In general, Fig. 2 shows a typical layout of an intensive care unit in a veterinary clinic for the treatment of animals with parvovirus enteritis. The core of the unit consists of equipment for infusion therapy (infusion pumps, volumetric infusion pumps, infusion stands), patient support (an oxygen concentrator or oxygen source for animal oxygen therapy), and a stationary enclosed patient box (an isolated area for intensive monitoring, with temperature control and stress minimization).

When conducting additional diagnostic tests, anemia of varying severity was predominantly detected; occasionally, hemoglobin and red blood cell levels were

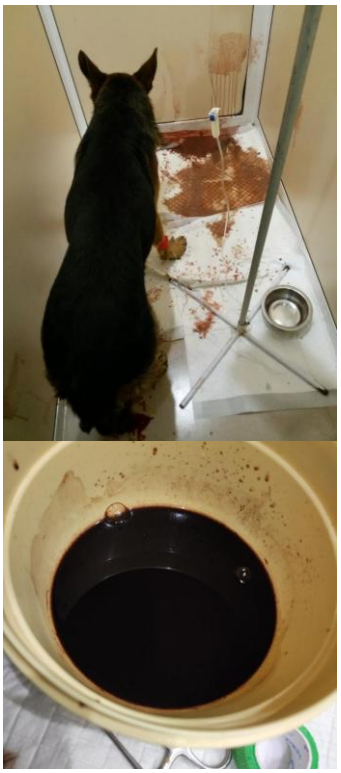

Figure 1. Predominant clinical signs of parvovirus infection in the dogs examined.

Figure 2. Examination and treatment procedures, and a view of the hospital with an intensive care unit at the 'Zoolux' veterinary clinic network in Kyiv.

within normal limits, which was due to hemoconcentration caused by the animal's dehydration. Less frequently, leukopenia, elevated erythrocyte sedimentation rate (ESR), and decreased total blood protein were observed. Instrumental diagnostic methods sometimes included ultrasound examination of the abdominal organs, chest X-rays, contrast-enhanced gastrointestinal tract X-rays, echocardiography when necessary, and so on.

An example of a contrast-enhanced X-ray of the gastrointestinal tract in an affected dog is shown in Fig. 3. The X-ray contrast medium is visible in the stomach with uneven distribution and delayed evacuation, indicating impaired motor and evacuation function. The loops of the small intestine have an uneven diameter, appear tortuous in places, with no distinct fluid levels and no sharp break in contrast. No signs of mechanical obstruction, intussusception, or foreign bodies were detected. The radiographic picture is characterized by functional (paralytic) ileus, presumably caused by inflammatory and necrotic changes in the intestinal mucosa against the background of a parvovirus infection. No signs of free gas in the abdominal cavity were detected (Fig. 3). Impaired motility and delayed passage of the contrast agent may be a consequence of damage to the intestinal crypt cells, characteristic of parvovirus infection, leading to wall edema, hypomotility, and the development of paralytic ileus. The obtained radiographic data confirm the appropriateness of conservative therapy and rule out the need for surgical intervention at the time of the examination.

Parvovirus enteritis in dogs exhibits marked seasonality, which has been identified as a risk factor in Colorado, USA (summer–fall), in Brazil, Canada, and New Zealand (spring–summer), and in Australia (spring–fall) (Kelman et al., 2020; Oliveira et al., 2018; Jiang, 2018). In our study, the disease was significantly more common in the summer-fall period than in winter ($p < 0.05$) (Fig. 4).

An analysis of the incidence by month revealed two peaks during the year, specifically in October and June (12.5% and 10.8% of cases, respectively). The disease was diagnosed slightly less frequently in September and May (9.9% and 9.0%, respectively). The fewest cases were observed in January and February (4.7% and 5.7%, respectively) (Fig. 5).

According to the literature, the prevalence of parvovirus enteritis throughout the year depends on the climatic conditions of the country where the study was conducted, the breeding season, and the movement of animals to shows and breeding facilities (Lisova et al., 2022). In Ukraine, an increase in the incidence of parvovirus enteritis is generally observed during the spring-summer and summer-fall periods, with a slight decrease in the number of cases in winter. For example, in Poltava, the highest incidence of the disease in 2016–2018 occurred in the fall (36.2% of cases) and the lowest in the winter (16.3%) (Titarenko, Pokhylets and Karasenko, 2021). In Brovary (Kyiv Region), parvovirus enteritis exhibits a clearly defined seasonal pattern, with dogs being more susceptible during the summer–fall season, and the peak incidence of infected animals was recorded in July at 23.1% (Hrebinichenko et al., 2025). In private and state veterinary clinics in Kyiv, Zhytomyr, Vinnytsia, and Berdychiv, according to data from Radzykhovskyi (2021), parvovirus enteritis was most frequently recorded in the fall (29%), less frequently in the summer (25.7%) and in the spring (24.2%), and the lowest number of affected dogs was recorded in the winter (21.1%). Other authors also noted a similar seasonal pattern in their studies (Kone and Romanova, 2017).

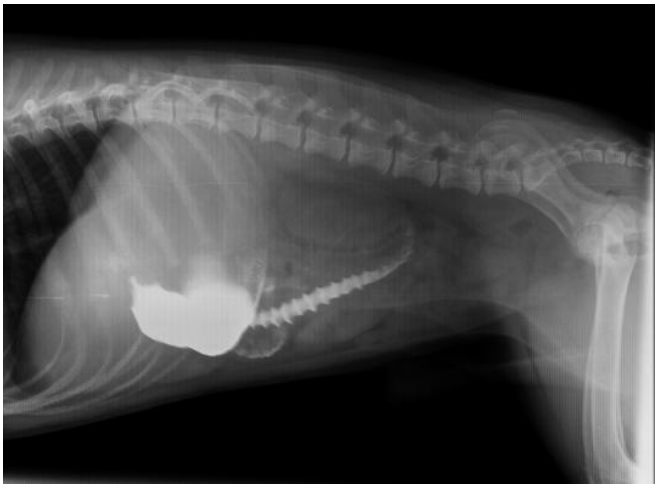

Figure 3. Contrast-enhanced X-ray of the abdominal organs in the examined dog.

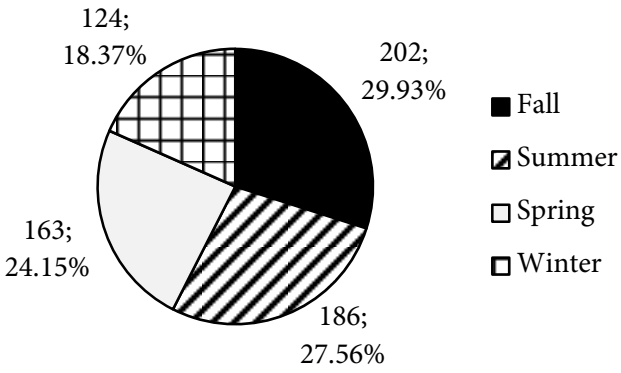

Figure 4. Distribution of positive CPV-2 antigen test results among the dogs examined, by season.

Conclusions. Parvovirus infection is a fairly common condition among dogs seen at the veterinary clinics of the 'Zoolux' network in Kyiv, accounting for 18.1% of examinations.

Despite pronounced seasonality in the summer and fall, which significantly exceeded winter incidence ($p < 0.05$), parvovirus infection in dogs is observed year-round.

In our study, peaks in canine parvovirus incidence were observed in October and June (12.5% and 10.8% of cases, respectively), which is typical for Ukraine.

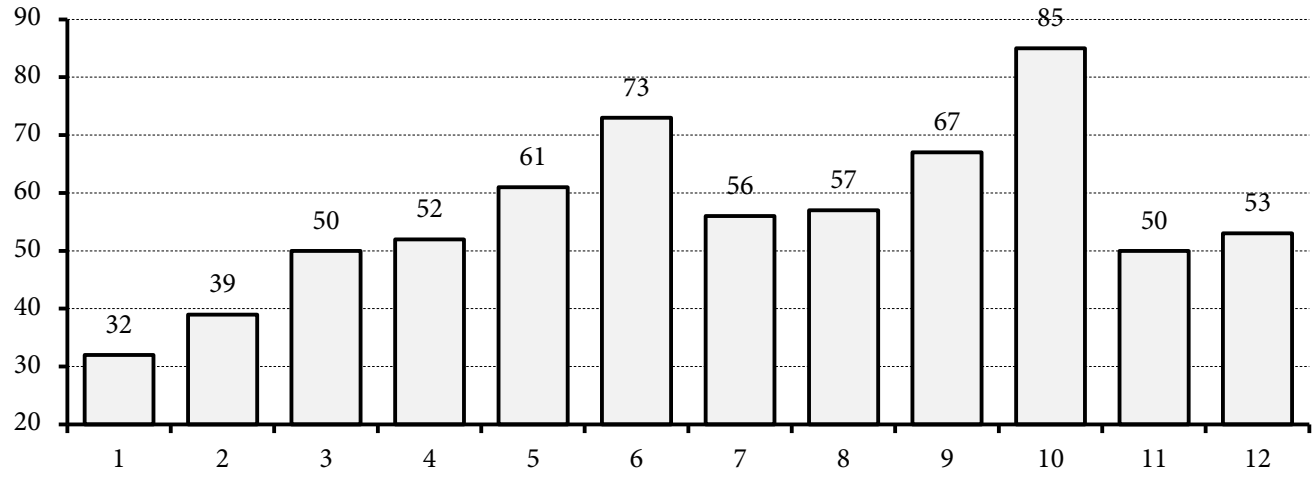

Figure 5. Distribution of positive CPV-2 antigen test results among the dogs examined, by month.

The most common clinical signs of viral enteritis in the dogs examined were lethargy, vomiting, bloody, watery stools with a foul odor, and a refusal to eat or drink. Less frequent signs included cardiovascular and respiratory system involvement.

In clinics, examinations and therapeutic procedures often had to be performed during infusion therapy aimed at rehydrating the animal. This therapy used an infusion stand with an infusion pump or an IV drip.

Diagnostic procedures performed on sick dogs sometimes included ultrasounds of the abdominal organs, chest X-rays, contrast-enhanced X-rays of the gastrointestinal tract, and echocardiography when necessary.

Prospects for further research. Viral gastroenteritis is a significant challenge in modern veterinary medicine. Further research is needed to develop methods for early and rapid diagnosis of its etiology in dogs. In our opinion, the polymerase chain reaction is the most promising method because it allows for precise identification of the causative agent of the disease. This enables the selection of effective treatment methods for the animal and the prevention of infection spread through the use of specific preventive measures.

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Part 2. Biosafety

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STUDY OF THE BACTERICIDAL PROPERTIES OF AN OXYGEN-CONTAINING DISINFECTANT AGAINST MYCOBACTERIA

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Summary. This study aimed to determine the bactericidal properties of an oxygen-based disinfectant against mycobacteria and evaluate its effectiveness in decontaminating production surfaces. The study material was the universal, oxygen-containing disinfectant 'Famidez Sanoksil 100', which contains hydrogen peroxide, silver nitrate, and phosphoric acid. The preparation's bactericidal activity was assessed against a *Mycobacterium phlei* test culture using the suspension method at concentrations of 0.5%, 1.0%, 2.0%, and 3.0%, under various exposure conditions. Additionally, tuberculocidal properties were determined on test objects (batiste, wood, and tile) contaminated with *M. bovis* culture. The decontamination effectiveness of the test objects was confirmed by a biological study on laboratory animals (guinea pigs), which included an intradermal tuberculin test as well as pathological and bacteriological examinations. The bactericidal activity of the disinfectant was found to be directly dependent on the concentration of the working solution and the exposure time. Destruction of the *M. phlei* test culture was observed after 48 h at a concentration of 1.0%, whereas a 2.0% solution of the disinfectant provided a tuberculocidal effect only after 24–48 h. The 3.0% solution was the most effective, ensuring complete inactivation of mycobacteria after 5 h, 24 h, and 48 h of contact. After treatment with a 3.0% solution of the preparation, no mycobacterial growth was detected on test objects contaminated with the *M. bovis* culture (after exposure for 5 h, 24 h, and 48 h). In the biological experiment, laboratory animals in the experimental groups did not react to the tuberculin injection, and no mycobacterial cultures were isolated from their biomaterial. These results indicate the disinfectant's pronounced tuberculocidal properties and confirm its effectiveness in decontaminating mycobacterium-contaminated objects

Keywords: disinfection, *Mycobacterium bovis*, atypical mecobacteria, test objects, guinea pigs

Introduction. Structural transformations in Ukraine's economy have exacerbated the agricultural sector crisis and significantly altered domestic and international operating conditions for livestock enterprises. Currently, livestock farming is one of the key components of Ukraine's national economy and positions the country among the leading exporters of certain types of animal products. However, the sector is currently in a difficult state due to prolonged military hostilities, the consequences of the pandemic, and general economic instability (Ushkalov, 2023; Bozhyday and Kralia, 2026).

Under these conditions, food producers must ensure product quality, increase export potential, and promptly identify and resolve issues related to maintaining facilities and equipment in accordance with established hygiene requirements (Betoret, Betoret and Glicerina, 2024).

The approach to ensuring proper sanitary conditions at facilities — including those involved in primary production — has changed significantly: it is no longer limited to measures taken after the fact to mitigate the consequences of adverse impacts. Instead, it involves a systematic set of preventive measures regulated by production control programs that enable the timely resolution of hygiene and anti-epizootic issues (Awuchi, 2023; Ushkalov et al., 2025).

Within the framework of veterinary and sanitary measures aimed at maintaining proper sanitary conditions in livestock facilities and improving the safety of raw materials, feed, and animal-derived products, disinfection plays a leading role (Zavgorodniy et al., 2013; Paliy, 2018).

The rational selection of disinfectants is of fundamental importance in practical veterinary medicine, as such products must combine high biological efficacy, environmental safety, and cost-effectiveness (Rodionova, Paliy and Khimych, 2021).

The main goal of disinfection is to interrupt the epizootic process by targeting the key link in pathogen transmission. This factor facilitates its movement from the source of infection to a susceptible organism and to contaminated environmental objects, raw materials, or food products. Mycobacteria, particularly the causative agents of animal tuberculosis, require special attention in this context, as they are characterized by increased resistance to physical and chemical factors due to the specific structure of their cell wall, enriched with mycolic acids. Their ability to persist for long periods in the environment and on production infrastructure surfaces

necessitates the use of highly effective disinfectants with proven mycobactericidal activity, which is a crucial component of the veterinary and sanitary measures system (Garkavenko and Kovalenko, 2017; Wales et al., 2021; Pedreira, Taşkın and García, 2021).

The problem of tuberculosis in cattle remains a pressing issue for many countries around the world, as the pathogens of the *Mycobacterium tuberculosis* complex, particularly *M. bovis*, are highly resistant to environmental factors and can persist for long periods in soil, water, and on surfaces in production facilities. Under current conditions, disease control requires a comprehensive approach that combines diagnosis, isolation of infected animals, and the implementation of systematic veterinary and sanitary measures, including cleaning and disinfection (Paliy et al., 2024; Korniienko et al., 2021).

An analysis of the epizootic situation regarding bovine tuberculosis in Ukraine indicates that the effectiveness of anti-epizootic measures largely depends on strict adherence to sanitary requirements on farms. Proper organization of cleaning and disinfecting premises, equipment, and vehicles, alongside allergic testing and diagnostic slaughter of reactive animals, plays a crucial role in reducing the risk of pathogen persistence and circulation within herds (Korniienko et al., 2021).

International studies also confirm the need for an integrated approach to tuberculosis control at the farm level. Thus, the development and implementation of protocols to minimize the risk of tuberculosis spread on farms involves not only testing animals but also a set of biosecurity measures, including the disinfection of equipment, animal housing areas, and zones of potential contact with wild reservoirs of infection (Wales et al., 2021). The authors emphasize that underestimating the role of environmental sanitation can contribute to the reintroduction or continued circulation of the pathogen.

The importance of controlling environmental factors in the spread of mycobacteria is also confirmed within the framework of the 'One Health' concept. A review by Zhang et al. (2022) emphasizes that environmental contamination is a significant link in the transmission of the *Mycobacterium tuberculosis* complex between animals, humans, and the environment. Systematic cleaning and disinfection of premises, instruments, vehicles, and water supply systems are considered critical measures aimed at breaking the epizootic chain.

Additionally, it has been established that farms with substandard veterinary, sanitary, and hygiene control measures are at a higher risk of introducing *M. bovis* into their herds. A study by Weldegebriel et al. (2025) identified several key risk factors, including noncompliance with biosecurity requirements, inadequate sanitation of facilities, and the uncontrolled introduction of new animals into the herd. These findings suggest that disinfection should be considered a strategic component, rather than an auxiliary measure, in tuberculosis prevention and control programs for livestock operations.

Therefore, current scientific data confirm that effectively controlling tuberculosis in livestock farming is impossible without systematically implementing cleaning and disinfection measures that inactivate mycobacteria in the production environment and minimize the risk of further transmission.

The current market offers a wide variety of disinfectants for use on livestock farms. These disinfectants come in various combinations of active ingredients, dosage forms, and from different manufacturers. However, not all of them have proven mycobactericidal properties or meet their claimed specifications under real-world production conditions. This creates a need for targeted research, the results of which would provide a well-founded assessment of the bactericidal activity of individual agents, particularly oxygen-based disinfectants, against mycobacteria. Furthermore, the use of the same disinfectants may lead to the emergence of resistant mycobacterial forms, which would contribute to their prolonged persistence in the environment.

The study **aimed** to determine the optimal concentrations of working solutions and exposure times for the bactericidal activity of the oxygen-based disinfectant 'Famidez Sanoksil 100' against *M. bovis* and atypical mycobacteria.

Materials and methods. The study utilized cultures of *M. phlei* (accession No. 23) and *M. bovis* (Vallee strain) from the collection housed at the Laboratory of Tuberculosis of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Kharkiv, Ukraine).

The bacterial cultures of *M. phlei* and *M. bovis* were grown on Pavlovsky's nutrient medium for 14–21 days, and *M. bovis* for 30–45 days at a temperature of 37.5 ± 0.5 °C.

The preparation 'Famidez Sanoksil 100' (DezoMark LLC, Ukraine), a disinfectant widely used in human medicine, was used as a model oxygen-containing disinfectant in the study. 'Famidez Sanoksil 100' contains hydrogen peroxide (50.0 g/100 g), silver nitrate, phosphoric acid, and water; it was studied at concentrations of 0.5%, 1.0%, 2.0%, and 3.0%.

The bactericidal properties of the disinfectant 'Famidez Sanoksil 100' were determined using the suspension method, test objects, and biological testing in accordance with the methodological guidelines 'Determination of the Bactericidal Properties of Disinfectants, Disinfection Procedures, and Quality Control in the Context of Tuberculosis in Farm Animals' (Zavgorodniy et al., 2007).

The biological study was conducted on six clinically healthy guinea pigs (three experimental and three control) weighing 300–350 g, none of which had reacted to mammalian tuberculin (MTB) before the start of the experiment. The experimental group received a subcutaneous injection of *M. bovis* suspension obtained after treating the test objects with a 3.0% solution of 'Famidez Sanoksil 100' (24 h exposure), while the control

group received an untreated bacterial mass at a dose of 1 cm^3. The animals were observed for 90 days and underwent tuberculin skin testing during this period. Upon completion of the experiment or in the event of an animal's death, postmortem and bacteriological examinations were performed.

All manipulations with experimental animals were carried out in accordance with the 'European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes' (CE, 1986) and Council Directive 2010/63/EU (CEC, 2010), and under Art. 26 of the Law of Ukraine No. 3447-IV of 21.02.2006 'About protection of animals from cruel treatment' (VRU, 2006) and basic bioethical principles (Simmonds, 2017). Under the current procedure, the research program was reviewed and approved by the Bioethics Committee of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Kharkiv, Ukraine).

Results and discussion. The results of determining the bactericidal activity of the disinfectant 'Famidez Sanoksil 100' on a test culture of the atypical mycobacterium *M. phlei* using the suspension method are presented in Table 1.

Table 1 — Bactericidal properties of the drug 'Famidez Sanoksil 100' by the suspension method regarding *M. phlei*

Culture	Concentration of disinfectant solution, %	Exposure, h	Experiment	Control	
				Negative	Positive
M. phlei	0.5	5	+++	–	++++
		24	++	–	++++
		48	+	–	++++
	1.0	5	++	–	++++
		24	+	–	++++
		48	–	–	++++
	2.0	5	+	–	++++
		24	–	–	++++
		48	–	–	++++
	3.0	5	–	–	++++
		24	–	–	++++
		48	–	–	++++

Notes: '++++' — intense colony growth; '+++' — growth of 50 colonies; '++' — growth of 20 colonies; '+' — growth of up to 10 colonies; '–' — no growth.

As shown in Table 1, the bactericidal activity of the 'Famidez Sanoksil 100' preparation against the *M. phlei* test culture was directly dependent on the concentration of the working solution and exposure time. At a 0.5% concentration of the disinfectant solution and exposure times of 5 h, 24 h, and 48 h, the drug exhibited only bacteriostatic properties against *M. phlei*.

Increasing the disinfectant solution concentration to 1.0% with exposure of 5 h and 24 h resulted in the growth of isolated *M. phlei* colonies. However, with a 48 h exposure, no colony growth was observed on the culture medium, indicating the culture's inactivation. No growth of *M. phlei* colonies was observed at a disinfectant solution concentration of 2.0% with exposure times of 24 h and 48 h or at a concentration of 3.0% with exposure times of 5 h, 24 h, and 48 h, indicating the bactericidal effect of the preparation.

No mycobacterial growth was observed in the negative control samples, whereas colony growth was recorded in the positive control, confirming the correctness of the experimental setup and the reliability of the results obtained.

The next stage involved conducting studies with a culture of the tuberculosis pathogen *M. bovis* on test objects (batiste, wood, tile), taking into account the biological load. The results of determining the bactericidal activity of the drug 'Famidez Sanoksil 100' against *M. bovis* on the test objects are presented in Table 2.

As shown in Table 2, the disinfectant 'Famidez Sanoksil 100' at a concentration of 3.0% exhibits pronounced bactericidal properties against *M. bovis* cultures on various test objects after 5 h, 24 h, and 48 h in the presence of a biological load. Meanwhile, the positive control exhibited mycobacterial colony growth (*M. bovis*), while the negative control showed no growth, confirming the experiment's validity. These results indicate the tested preparation's high tuberculocidal activity against the tuberculosis pathogen on various types of surfaces that may be contaminated under industrial conditions.

To confirm the results of the cultural study on the bactericidal activity of the disinfectant under investigation, a biological study was conducted on laboratory animals.

According to the results of the intradermal tuberculin test using a mammalian PPD, a positive reaction was recorded only in animals of the control group, while a postmortem examination of guinea pigs infected with swabs from test objects (positive control) revealed lesions characteristic of tuberculosis in their internal organs.

Table 2 — Bactericidal properties of the drug 'Famidez Sanoksil 100' against *M. bovis* on test objects

Culture	Concentration, %	Exposure, h	Test object	Experiment	Control	
					Negative	Positive
M. bovis	3.0	5	Batiste	–	–	++++
			Wood	–	–	++++
			Tile	–	–	++++
		24	Batiste	–	–	++++
			Wood	–	–	++++
			Tile	–	–	++++
		48	Batiste	–	–	++++
			Wood	–	–	++++
			Tile	–	–	++++

Notes: '++++' — intense colony growth; '+++' — growth of 50 colonies; '++' — growth of 20 colonies; '+' — growth of up to 10 colonies; '–' — no growth.

Cultural studies of pathological material collected from experimental and control animals allowed for the isolation of *M. bovis* cultures exclusively from animals in the control group. In the experimental animals, no reactions to intradermal tuberculin injections or pathological lesions were observed, and after the completion of the experiment, no mycobacterial cultures were isolated from the biological material.

Conclusions. It has been established that the bactericidal effect of an oxygen-containing disinfectant depends on the concentration of the working solution and the duration of exposure.

Complete inactivation of the *M. phlei* test culture was achieved with a 1.0% solution after 48 h, while a 2.0% solution provided a tuberculocidal effect as early as 24 h and 48 h.

The 3.0% solution was the most effective, ensuring a complete absence of mycobacterial growth after 5 h, 24 h, and 48 h of contact, as well as the effective decontamination of objects contaminated with the *M. bovis* culture.

Biological tests on laboratory animals confirmed the absence of viable mycobacteria after treatment with the disinfectant, indicating its high tuberculocidal activity.

Based on the results of the study on the bactericidal efficacy of the disinfectant 'Famidez Sanoksil 100', its use is justified for preventive and emergency disinfection of agricultural enterprise premises, including tuberculosis foci in cattle and poultry. The optimal application method is a 2.0% aqueous solution with a 24 h exposure time, or a 3.0% solution with exposure times of 5 h, 24 h, and 48 h. The consumption rate is 1,000.0 cm^3 per 1 m^2 of treated surface area.

Declaration of competing interest. The authors declare that they have no conflict of interest.

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JUSTIFICATION FOR THE EFFICACY OF THE BIOCIDE 'KREZONID' IN CONTROLLING BACTERIAL INFECTIONS

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Summary. The study aimed to experimentally substantiate the bactericidal activity of the biocidal agent 'Krezonid' (based on meta-cresol, lactic acid, and a quaternary ammonium compound) and to determine the minimum effective regimens for its use to completely inactivate Gram-positive and Gram-negative pathogenic bacteria, which are important in veterinary medicine and poultry farming, while simultaneously verifying the absence of a bacteriostatic effect. The study was conducted using the in vitro suspension method in accordance with the requirements of European standards (EN 1040:2005, EN 1656:2019, EN 12353:2021) and methodological recommendations for veterinary disinfectants. Standard strains of *Staphylococcus aureus* ATCC 6538 and *Pseudomonas aeruginosa* ATCC 15442 were used as test microorganisms. Working concentrations of the drug of 0.1%, 0.3%, 0.5%, and 1.0% were tested at exposure times of 10, 20, and 30 minutes. After contact, the samples were rinsed three times with saline, and cultures were inoculated onto tryptone-soy agar (to assess bactericidal activity) and into tryptone-soy broth with repeated re-inoculations over 72 hours (to rule out bacteriostasis). The results showed a clear dependence of efficacy on concentration and exposure time. A concentration of 0.1% did not ensure complete inactivation even after 30 minutes. At 0.3%, complete inactivation was achieved inconsistently, with occasional residual growth. A stable and reproducible bactericidal effect against both test cultures was observed at a concentration of 0.5% after just 20 minutes of exposure, and at 30 minutes in 100% of replicates. The maximum rate of action was noted at 1.0% — destruction of microorganisms after 10 minutes of contact. No bacteriostatic effect was observed under effective conditions: growth did not resume after repeated inoculations. The data obtained confirm the pronounced bactericidal (rather than bacteriostatic) activity of 'Krezonid' against Gram-positive and Gram-negative bacteria. The recommended minimum concentration is 0.5% for a 30-minute exposure (or 20 minutes under stable conditions); a 1.0% concentration for 10 minutes is optimal for rapid disinfection. The results allow us to recommend the product for use in veterinary and sanitary measures at industrial livestock and poultry facilities, provided that appropriate protocols are followed

Keywords: bactericidal activity, bacteriostatic effect, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, disinfection

Introduction. The rapid expansion of industrial livestock and poultry farming is accompanied by an increase in the biological load on the production environment, which creates favorable conditions for the circulation and accumulation of bacterial pathogens. Under such conditions, the effectiveness of veterinary and sanitary measures directly determines the level of epizootic stability of farms and the safety of animal-derived products (Melo et al., 2020; Wales et al., 2021).

One of the key elements of the biosecurity system is the use of disinfectants capable of ensuring rapid and complete inactivation of pathogenic and opportunistic microorganisms. At the same time, practical experience shows that a decrease in their effectiveness may accompany prolonged use of products with the same mechanism of action. Therefore, there is a need for a scientifically sound selection and periodic replacement of disinfectants within a given area (Ponomarenko et al., 2021; Paliy et al., 2020). At the same time, the costs of disinfection measures directly affect the production cost of livestock products.

In recent years, a decline in the sensitivity of microorganisms to certain disinfectants has been increasingly observed in production conditions due to their prolonged and repetitive use. The development of pathogenic bacteria's resistance to disinfectants negatively impacts the epizootic stability of farms and necessitates the regular rotation of products with different biocidal mechanisms of action (Rutala and Weber, 2019; Scicchitano et al., 2024).

The market for veterinary disinfectants features several dozen products from various chemical groups, among which those based on quaternary ammonium compounds predominate (Kovalenko and Nedosiekov, 2011; Ponomarenko et al., 2020). However, objective data on their actual effectiveness under livestock production conditions are often limited or promotional in nature, which complicates making an informed choice (Tarka and Nitsch-Osuch, 2021; Koti et al., 2024).

The development and implementation of effective, domestically produced biocidal agents with high antimicrobial activity, an acceptable toxicological profile, and affordability is particularly relevant (Kovalenko

et al., 2013; Paliy et al., 2018). In this context, the biocide 'Krezonid' shows promise; however, its efficacy requires scientific validation using standardized laboratory methods (Kovalenko et al., 2018).

The objective of this study is to experimentally investigate the bactericidal activity of 'Krezonid' at various concentrations and exposure times. Additionally, we aim to determine the minimum effective application conditions for completely inactivating Gram-positive and Gram-negative pathogenic and opportunistic bacteria that are significant in animal husbandry.

Materials and methods. Experimental studies investigating the bactericidal activity of the new biocide 'Krezonid', which is based on meta-cresol, lactic acid, and a quaternary ammonium compound, were conducted in the laboratory at the Institute of Veterinary Medicine of the National Academy of Agrarian Sciences of Ukraine. These studies were performed according to the most recent methodological recommendations and regulatory documents for determining bactericidal activity and verifying the absence of bacteriostatic effects in veterinary disinfectants (Harkavenko et al., 2020; SE 'UkrNDNC', 2022a, 2022b, 2022c).

In the first stage of the study, the drug's primary bactericidal activity was evaluated *in vitro* using the suspension method. Standard strains from the institute's collection were used as test microorganisms: the Gram-positive culture *Staphylococcus aureus* ATCC 6538 and the Gram-negative culture *Pseudomonas aeruginosa* ATCC 15442 (Kovalenko and Nedosiekov, 2011; SE 'UkrNDNC', 2022a, 2022b, 2022c).

To prepare the inoculum, colonies from daily cultures were washed off the surface of tryptone soy agar (TSA) with sterile saline under aseptic conditions. The turbidity of the resulting bacterial suspensions was adjusted to a standard of 0.5–1.0 on the McFarland scale, corresponding to a microorganism concentration of $1.35–3.0\times10^8$ CFU/cm³.

Working solutions of the biocidal product 'Krezonid' were prepared immediately before the experiments according to the manufacturer's recommendations. The working concentrations used in the experiments were 0.1%, 0.3%, 0.5%, and 1.0%. The amount of disinfectant and water used was calculated based on preparing 100 ml of each working solution.

Suspension tests were conducted in triplicate for each concentration and each test culture. Sterile test tubes were filled with 4.5 cm³ of the appropriate working solution of the biocide, and 0.5 cm³ of bacterial suspension was added. The mixtures were thoroughly mixed and incubated for 10, 20, and 30 minutes.

After the microorganisms had been in contact with the biocide, the test cultures were washed three times with sterile saline solution to completely remove any remaining product. The washed cultures were brought to the original volume (4.5 cm³) by adding tryptone-soy broth, after which they were inoculated.

To determine bactericidal activity, 0.1 cm³ of the suspension was inoculated onto the surface of tryptone-soy agar, using three Petri dishes for each concentration and exposure. The plates were incubated in a thermostat at 37 ± 1 °C for 48 h, with preliminary results recorded after 24 h. To identify or rule out a bacteriostatic effect of the drug, parallel cultures were prepared by inoculating 0.1 cm³ of the suspension into test tubes containing tryptone-soy broth (TSB). Incubation was carried out for 72 h at a temperature of 37 ± 1 °C with daily reinoculations on fresh liquid nutrient medium every 24 h.

The results of the bactericidal activity of the test biocide 'Krezonid' were recorded after 24–48 h on TSA plates; to detect the bacteriostatic effect of the preparation, results were recorded after 24–72 h in TSB tubes. Growth of the test cultures was monitored by performing similar inoculations of bacterial suspensions that were not exposed to the biocide.

Results. Experimental studies have shown that the biocide 'Krezonid' exhibits pronounced bactericidal activity against the test strains *Staphylococcus aureus* ATCC 6538 and *Pseudomonas aeruginosa* ATCC 15442; however, the level of efficacy depended significantly on the concentration of the working solution and the duration of exposure.

According to the test results, when the preparation was applied at a concentration of 0.1%, a significant reduction in the viability of both test cultures was observed after just 10 minutes of exposure, as confirmed by a decrease in the number of colonies when plated on solid nutrient medium (Tables 1 and 2). However, complete inactivation of microorganisms under these conditions was not achieved, as isolated growth of colonies of both staphylococci and pseudomonads was observed after incubation.

Extending the exposure time to 20 and 30 min at a concentration of 0.1% led to a further reduction in the infectious load; however, even at the maximum exposure time, residual growth of the test cultures was observed, indicating that this treatment regimen was not sufficiently bactericidal.

When using a 0.3% working solution of the drug 'Krezonid', a significant increase in the antimicrobial activity of the drug was observed. Thus, after 20 min of exposure in cultures on solid medium, only isolated colonies of *Staphylococcus aureus* were detected, while the growth of *Pseudomonas aeruginosa* was significantly inhibited. As the contact time was increased to 30 min, the number of viable cells in both cultures decreased to minimal levels.

The maximum bactericidal effect at a concentration of 0.3% was achieved after 30 minutes of exposure, during which no growth of the test cultures on solid medium was observed in most replicates. At the same time, in isolated cases, when samples were inoculated after contact with the drug, weak growth was observed, which did not allow us to conclude unequivocally that this regimen was fully bactericidal for all microorganisms studied.

Table 1 — Study of the bactericidal activity and bacteriostatic effect of the biocide 'Krezonid' on test cultures of *Staphylococcus aureus* ATCC 6538

<table>
<tr><td rowspan="5">Working concentrations of 'Krezonid', %</td><td colspan="6">Assessment of the growth of test cultures on nutrient media following treatment with various concentrations of the biocide 'Krezonid' at different exposure times</td></tr>
<tr><td colspan="6">Cultivation of test microbial cultures on solid and liquid media in a thermostat at a temperature of 37 ± 1°C for a period of:</td></tr>
<tr><td colspan="4">48 hours</td><td colspan="2">72 hours</td></tr>
<tr><td colspan="3">Bactericidal activity of 'Krezonid'</td><td colspan="2">Bacteriostatic effect following treatment with 'Krezonid'</td></tr>
<tr><td colspan="2">Tryptone-soy agar (solid culture medium)</td><td>Growth control</td><td>Tryptone-soy broth (liquid culture medium)</td><td>Growth control</td></tr>
<tr><td colspan="7">Multiplicity of studies, number of Staphylococcus aureus ATCC 6538 culture tubes</td></tr>
<tr><td colspan="7">Exposure 10 minutes</td></tr>
<tr><td></td><td colspan="3">TSA plates' numbers</td><td rowspan="5">Continuous growth</td><td colspan="2">TSB plates' numbers</td><td rowspan="5">Severe turbidity, sediment</td></tr>
<tr><td></td><td>1</td><td>2</td><td>3</td><td>1</td><td>2</td><td>3</td></tr>
<tr><td>0.1</td><td>Continuous growth on the surface of the TSA</td><td>Growth of colonies across the entire surface of the TSA plate</td><td>Continuous growth on the surface of the TSA</td><td colspan="3" rowspan="2">Medium turbidity, growth (+)</td></tr>
<tr><td>0.3</td><td colspan="3">Growth of colonies across the entire surface of the TSA plate</td></tr>
<tr><td>0.5</td><td>11 isolated colonies on the TSA surface</td><td>9 isolated colonies on the TSA surface</td><td>5 isolated colonies on the TSA surface</td><td colspan="3">No growth</td></tr>
<tr><td>1.0</td><td colspan="3">No colony growth</td><td colspan="3">No growth</td></tr>
<tr><td colspan="7">Exposure 20 minutes</td></tr>
<tr><td>0.1</td><td colspan="3">Growth of colonies across the entire surface of the TSA plate</td><td rowspan="3">Continuous growth</td><td colspan="2">Medium turbidity, growth (+)</td><td rowspan="3">Severe turbidity, sediment</td></tr>
<tr><td>0.3</td><td>3 isolated colonies on the TSA surface</td><td>5 isolated colonies on the TSA surface</td><td>7 isolated colonies on the TSA surface</td><td colspan="2">No growth</td></tr>
<tr><td>0.5
1.0</td><td colspan="3">No colony growth</td><td colspan="2">No growth</td></tr>
<tr><td colspan="7">Exposure 30 minutes</td></tr>
<tr><td>0.1</td><td colspan="3">Growth of individual colonies on the surface of TSA</td><td rowspan="3">Continuous growth</td><td colspan="2">Medium turbidity, growth (+)</td><td rowspan="3">Severe turbidity, sediment</td></tr>
<tr><td>0.3
0.5
1.0</td><td colspan="3">No colony growth</td><td colspan="2">No growth</td></tr>
</table>

The results of the evaluation of the bactericidal activity of the drug 'Krezonid' against a culture of *Staphylococcus aureus* ATCC 6538 indicate that complete inactivation of the staphylococcus is achieved when the drug is used at concentrations of 0.5% or higher for an exposure time of at least 20 min. At lower concentrations, partial growth inhibition was observed, which did not guarantee complete elimination of the pathogen.

The use of the biocide at a concentration of 0.5% provided a stable and reproducible bactericidal effect. After 20 min of exposure across all replicates, there was no growth of *Staphylococcus aureus* or *Pseudomonas aeruginosa*. Further increasing the contact time to 30 minutes did not result in the appearance of viable cells, confirming the complete inactivation of the test cultures.

'Krezonid' demonstrated the highest antimicrobial activity at a concentration of 1.0%. For all exposure times studied (10, 20, and 30 min), no growth of the test microorganisms was detected in either solid or liquid culture media, indicating the rapid and intense bactericidal action of the product.

Analysis of the results presented in Table 2 showed that *Pseudomonas aeruginosa* exhibits slightly higher resistance to the tested biocide compared to *Staphylococcus aureus*. At the same time, the use of 'Krezonid' at a concentration of 0.5% ensured the complete absence of *Pseudomonas aeruginosa* growth after 20 and 30 min of exposure, whereas at a concentration of 1.0%, the bactericidal effect was achieved after just 10 min of contact.

An important aspect of the study was to determine the product's potential bacteriostatic effect. Based on the results of repeated inoculations into a liquid culture medium after prior contact with 'Krezonid' at effective concentrations, no resumption of growth was observed in the test cultures.

Table 2 — Study of the bactericidal activity and bacteriostatic effect of the biocide 'Krezonid' on test cultures of *Pseudomonas aeruginosa* ATCC 15442

<table>
<tr><td rowspan="6">Working concentrations of 'Krezonid', %</td><td colspan="6">Assessment of the growth of test cultures on nutrient media following treatment with various concentrations of the biocide 'Krezonid' at different exposure times</td></tr>
<tr><td colspan="6">Cultivation of test microbial cultures on solid and liquid media in a thermostat at a temperature of 37±1°C for a period of:</td></tr>
<tr><td colspan="4">48 hours</td><td colspan="2">72 hours</td></tr>
<tr><td colspan="3">Bactericidal activity of 'Krezonid'</td><td colspan="2">Bacteriostatic effect following treatment with 'Krezonid'</td></tr>
<tr><td colspan="2">Tryptone-soy agar (solid culture medium)</td><td>Growth control</td><td>Tryptone-soy broth (liquid culture medium)</td><td>Growth control</td></tr>
<tr><td colspan="5">Multiplicity of studies, number of Pseudomonas aeruginosa ATCC 15442 culture tubes</td></tr>
<tr><td colspan="7">Exposure 10 minutes</td></tr>
<tr><td></td><td colspan="3">TSA plates' numbers</td><td rowspan="2">Continuous growth</td><td colspan="2">TSB plates' numbers</td><td rowspan="2">Severe turbidity, sediment</td></tr>
<tr><td>1</td><td>2</td><td>3</td><td>1</td><td>2</td><td>3</td></tr>
<tr><td>0.1</td><td colspan="3" rowspan="2">Continuous growth on the surface of the TSA</td><td rowspan="3">Continuous growth</td><td colspan="2" rowspan="2">Medium turbidity, growth (+)</td></tr>
<tr><td>0.3</td></tr>
<tr><td>0.5</td><td colspan="3">Growth of individual colonies on the surface of TSA</td><td colspan="2" rowspan="2">No growth</td></tr>
<tr><td>1.0</td><td colspan="3">No colony growth</td><td rowspan="3">Severe turbidity, sediment</td></tr>
<tr><td colspan="7">Exposure 20 minutes</td></tr>
<tr><td>0.1</td><td colspan="3">Continuous growth on the surface of the TSA</td><td rowspan="3">Continuous growth</td><td colspan="2">Medium turbidity, growth (+)</td></tr>
<tr><td>0.3</td><td>9 isolated colonies on the TSA surface</td><td>Growth of individual colonies on the surface of TSA</td><td>No colony growth</td><td>Medium turbidity, growth (+)</td><td>No growth</td></tr>
<tr><td>0.5</td><td colspan="3" rowspan="2">No colony growth</td><td colspan="2" rowspan="2">No growth</td></tr>
<tr><td>1.0</td></tr>
<tr><td colspan="7">Exposure 30 minutes</td></tr>
<tr><td>0.1</td><td colspan="3">Growth of individual colonies on the surface of TSA</td><td rowspan="3">Continuous growth</td><td colspan="2" rowspan="2">Medium turbidity, growth (+)</td><td rowspan="3">Severe turbidity, sediment</td></tr>
<tr><td>0.3</td><td>Growth of individual colonies on the surface of TSA</td><td>8 isolated colonies on the TSA surface</td><td>6 isolated colonies on the TSA surface</td></tr>
<tr><td>0.5</td><td colspan="3" rowspan="2">No colony growth</td><td colspan="2" rowspan="2">No growth</td></tr>
<tr><td>1.0</td></tr>
</table>

This indicates an absence of bacteriostatic activity and confirms the bactericidal mechanism of the drug's antimicrobial action.

These results are consistent with the current understanding of how combined-type biocidal compounds act, and confirm that the biocidal agent 'Krezonid' is appropriate for sanitising poultry facilities, provided the recommended concentrations and exposure times are adhered to.

Discussion. The experimental data obtained demonstrate the high bactericidal activity of the biocide 'Krezonid' against the standard test strains *Staphylococcus aureus* ATCC 6538 and *Pseudomonas aeruginosa* ATCC 15442, which are of key epizootic importance in livestock and poultry farming. The observed dependence of the product's efficacy on the concentration and duration of exposure is consistent with general patterns of action for chemical disinfectants and the requirements of current European standards for evaluating bactericidal activity.

The partial inhibition of growth in test cultures at a concentration of 0.1%, even with prolonged exposure, indicates that this treatment regimen is insufficient for the complete inactivation of microorganisms. Similar results are reported in the works of other authors, who note that reducing the concentration of biocidal agents below the minimum effective level may lead to the survival of part of the bacterial population and create conditions for the development of adaptive resistance (Koti et al., 2024).

A significant increase in bactericidal activity accompanied an increase in the concentration of 'Krezonid' to 0.3%. However, the residual growth of test cultures observed in isolated replicates following a 30-minute exposure indicates that this regimen cannot be considered fully reliable for practical use under conditions of high microbial load. This is particularly important given the known higher natural resistance of *Pseudomonas aeruginosa* to many disinfectants, as

confirmed by the results of numerous studies (Wlazlo et al., 2020).

It has been established that using the preparation at a concentration of 0.5%, with an exposure time of at least 20 minutes, provides a stable and reproducible bactericidal effect against both test microorganisms. It is this regimen that can be considered the minimum effective and technologically feasible for implementation in veterinary and sanitary practices. The results obtained are consistent with the literature, which emphasizes that to ensure the complete elimination of both Gram-positive and Gram-negative bacteria, it is necessary to use disinfectant concentrations capable of causing irreversible damage to cellular structures (Van Haute et al., 2015).

'Krezonid' exhibited its highest antimicrobial activity at a concentration of 1.0%, ensuring complete inactivation of the test cultures with minimal exposure. This rapid action is a significant advantage of the product, particularly in production settings where the length of sanitation breaks is limited. However, the feasibility of using the maximum concentration must be evaluated in terms of economic factors, the potential impact of organic contamination, and biosafety requirements.

The absence of a bacteriostatic effect following repeated inoculations confirms that 'Krezonid' acts through a bactericidal mechanism rather than by temporarily inhibiting microbial growth. This is of fundamental importance in preventing the formation of resistant bacterial populations, and is consistent with current recommendations for selecting disinfectants for livestock facilities (Curran et al., 2019).

Thus, the results of the studies confirm the potential of the biocide 'Krezonid' as an effective disinfectant for preventing bacterial infections in poultry and livestock farming. At the same time, the data obtained highlight the importance of adhering to scientifically sound concentration and exposure regimens and the need for further studies under production conditions.

Conclusions. Laboratory tests have shown that the biocide 'Krezonid' exhibits significant bactericidal activity against Gram-positive and Gram-negative microorganisms, in particular the standard test strains *Staphylococcus aureus* ATCC 6538 and *Pseudomonas aeruginosa* ATCC 15442.

The effectiveness of the product's bactericidal action directly depends on the concentration of the working solution and the duration of exposure. The minimum effective regimen, which ensures complete inactivation of both test cultures without residual growth, is the use of 'Krezonid' at a concentration of 0.5% for a 30-minute exposure.

Use of the biocide at a concentration of 1.0% provides a rapid and stable bactericidal effect against the microorganisms under study after just 10 min of exposure, indicating the high intensity of the product's biocidal action.

Based on the results of the bacteriostatic effect tests, it was concluded that, upon coming into contact with effective concentrations of 'Krezonid', the microorganisms did not resume growth in the liquid culture medium. This confirms the absence of a bacteriostatic effect and the presence of a bactericidal inactivation mechanism.

The experimental data obtained allow the biocide 'Krezonid' to be recommended for use in preventive veterinary and sanitary measures at poultry facilities, provided scientifically sound concentration and exposure regimens are followed.

Prospects for further research. Further scientific research should focus on studying the efficacy of the biocide 'Krezonid' in industrial settings, considering various types of organic contamination and technological surfaces. Particular attention should be paid to evaluating the product's virucidal and fungicidal activity, as well as determining its effectiveness against bacterial pathogens circulating on farms with varying levels of biosecurity.

Additionally, investigating the possibility of using 'Krezonid' for the aerosol disinfection of premises in the presence of animals and poultry is promising, as is evaluating the product's toxicological parameters and its impact on the microbiocenoses of the production environment during prolonged use.

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