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IMPACT OF GIARDIA ON HEMATOLOGICAL PARAMETERS OF DOGS IN THE CASE OF SPONTANEOUS INFECTION

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Summary. *Giardia duodenalis* is a globally distributed intestinal protozoan parasite that infects a variety of hosts, including humans and domestic and wild mammals. *G. duodenalis* is localized in the small intestine, mainly in the duodenum and jejunum, and causes gastrointestinal disease in infected hosts. This study aimed to determine the effect of giardia on the hematological parameters of infected dogs. The study was conducted in a private veterinary clinic 'ZooLux' (Kyiv, Ukraine). Four groups of dogs were formed, in which coproscopic and immunologic examinations confirmed spontaneous infection. During the experiment, it was found that regardless of the presence or absence of clinical manifestations of the disease, as well as the degree of parasite load in the body of the animal, the infection was accompanied by changes in hematological parameters. In dogs of the first experimental group, the hematological changes were characterized by a slight leukocytosis (by 16.8%). At the same time, in dogs of the second experimental group, when giardia was detected in the feces, but in the absence of disease manifestations, hematological changes were characterized by the appearance of anemia, where the number of erythrocytes decreased (by 17.5%), hemoglobin content (by 5.6%), hematocrit (by 9.2%), and the average concentration of hemoglobin in erythrocytes (by 6.3%). Leukocytosis was also more pronounced (by 23.2%). In dogs of the third experimental group, in which the disease was manifested by severe diarrhea, hematological changes were characterized by severe anemia, accompanied by a decrease in the number of erythrocytes (by 22.2%), hemoglobin content (by 13.3%), hematocrit (by 14.3%), average hemoglobin concentration in erythrocytes (by 16.1%), as well as a decrease in platelets (by 27.8%) and an even greater increase in the number of leukocytes (by 46.3%)

Keywords: protozoan infection, giardiasis, blood, *Giardia duodenalis*

Introduction. The fight against domestic animal invasions is becoming increasingly important due to climate change, urbanization, and the dynamics of parasite ecology (Bogach et al., 2022; Paliy et al., 2022). The widespread prevalence of animal parasitic pathogens requires constant monitoring and the development of modern strategies to combat them (Paliy et al., 2018; Kiptenko et al., 2024).

Giardiasis is a common protozoan infection affecting both humans and domestic animals worldwide. Scientific research indicates that *Giardia duodenalis* species is domestic dogs' most prevalent gastrointestinal parasite (Palmer et al., 2008; Epe et al., 2010; Halliez and Buret, 2013; Rumsey and Waseem, 2023). The infection caused by *G. duodenalisis* is responsible for diarrhea in approximately 280 million people globally. In the United States specifically, *G. duodenalis* causes enteritis in 15,000 to 17,000 children each year. Genetic studies have identified eight genotypes of *Giardia*, with genotypes A and B primarily found in humans and other animals, making them potentially zoonotic. In contrast, genotypes C and D are specialized for infecting dogs (Capelli et al., 2006; Paoletti et al., 2008; Popruk et al., 2023).

The life cycle of the causative agent of giardiasis consists of stages that include trophozoites and cysts. Trophozoites are the vegetative form of the parasite, which are binucleate, pear-shaped flagellate structures with bilateral symmetry that colonize the proximal parts of the small intestine, especially the duodenum and, less commonly, the jejunum and ileum. In contrast, cysts are an environmentally stable phase of the parasite's life cycle. They enter the environment with the host's feces and are subsequently transmitted by the fecal-oral route. Fecal excretion of cysts facilitates zoonotic transmission of *G. duodenalis* from one host through the environment to another host (Efstratiou, Ongerth and Karanis, 2017; Ryan et al., 2019; Zahedi et al., 2020; Rojas-López, Marques and Svärd, 2022).

The widespread occurrence of *Giardia* infection is evidenced by the scientific work of many authors. In particular, in Vietnam, *G. duodenalis* was diagnosed in 8.6% of dogs based on fecal smears, and in Thailand, in 7.9% of dogs (Traub et al., 2009; Li et al., 2012). In other regions where PCR or ELISA methods were used, the prevalence of *Giardia* infection in dogs was 11–16% in China (Yang et al., 2015), 25% in Trinidad and Tobago (Mark-Carew et al., 2013), 15% in the USA (Munoz and Mayer, 2016), 21% in the UK (Upjohn et al., 2010), and 57.9% in Italy (Simonato et al., 2015).

The role of giardia in causing a wide range of clinical manifestations, from asymptomatic to acute and chronic, remains a subject of debate among scientists. Despite the fact that giardia are often found in animals with diarrhea, especially in puppies, many hosts are asymptomatic,

where they are also a source of large numbers of environmentally resistant cysts (Tysnes, Skancke and Robertson, 2014). This is since the attachment of the parasite causes a loss of intestinal epithelial barrier function, facilitating the penetration of intestinal bacteria into the intestinal wall, resulting in permanent damage to the mucosal epithelium. In particular, pathological signs of the small intestine affected by giardia include villous atrophy, infiltration of granulocytes, lymphocytes, and plasma cells into the lamina propria, and mesenteric lymph node hyperplasia (Cotton, Beatty and Buret, 2011; Bartelt et al., 2013; Chen et al., 2013). Some authors suggest that alterations in the beneficial intestinal microflora are a factor contributing to the development of *Giardia* infection. However, the host-parasite interaction is not a one-way process, and changes in the host microbiome itself lead to negative effects of the parasite on the host organism as a whole (Singer and Nash, 2000).

Thus, the violation of the host-parasite equilibrium in giardiasis indicates the pathogenic effect of giardia, which may explain the variations in host symptoms, as well as changes in hematological and biochemical parameters of blood serum.

This study **aimed** to determine the effect of giardia on the hematological parameters of infected dogs.

Materials and methods. The work was carried out during 2024–2025 in a private veterinary clinic 'ZooLux' (Kyiv, Ukraine). To determine the effect of the agent of giardiasis on the hematological parameters of the infected animals, four groups of dogs (seven animals in each) were formed, including one control group, which included clinically healthy dogs with negative results of coproscopic and immunological examinations for giardiasis, as well as three experimental groups of dogs spontaneously infected with giardia. The first experimental group included dogs with a positive rapid test (VetExpert Rapid Giardia Ag, Poland), a negative coproscopic examination for giardiasis, and no clinical manifestations of infection; the second experimental group included dogs with a positive rapid test, a positive coproscopic examination for giardiasis, and no clinical manifestations of infection; the third experimental group included dogs with a positive rapid test, a positive coproscopic examination for giardiasis, and clinical manifestations of infection in the form of diarrhea.

Hematological parameters were determined using an automatic analyzer 'BC-30s' ('Mindray', China). The number of erythrocytes, leukocytes, and thrombocytes, mean concentration of hemoglobin in erythrocytes, hemoglobin content, and hematocrit were determined in the blood of control and experimental dogs.

Experiments performed on animals were conducted following the recommendations of 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'.

The mathematical analysis of the data was performed using the Microsoft Excel software package by determining the arithmetic mean (M), standard deviation (SD), and probability level (p) using the one-factor analysis of variance technique with Fisher's test.

Results and discussion. The experimental studies revealed only a slight increase in the number of leukocytes by 16.8% (11.1 ± 1.3 G/l, $p < 0.05$) in the blood of dogs of the first experimental group compared to the same indicator in dogs of the control group (Fig. 1). All other blood parameters in the experimental dogs did not have significant deviations from those in the blood of control animals.

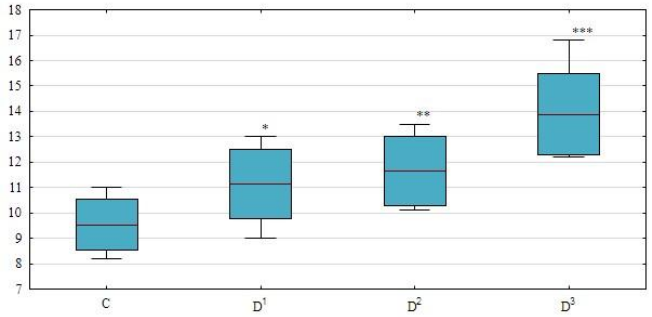

Figure 1. Indicators of the number of leukocytes (G/l) in the blood of dogs: C — control group; D^1, D^2, D^3 — 1st, 2nd, and 3rd experimental groups; * — $p < 0.05$, ** — $p < 0.01$, *** — $p < 0.01$ relative to the control group.

More significant changes were noted in the blood of dogs in the second experimental group. These changes were characterized by a 17.5% decrease in the number of erythrocytes (5.2 ± 0.4 T/l, $p < 0.05$) (Fig. 2), hemoglobin content decreased by 5.6% (135.4 ± 6.8 g/l, $p < 0.05$) (Fig. 3), the average hemoglobin concentration in erythrocytes decreased by 6.3% (309.9 ± 21.1 g/l, $p < 0.05$) (Fig. 4), and the hematocrit decreased by 9.2% (44.4 ± 3.2%, $p < 0.05$) (Fig. 5). Meanwhile, the number of leukocytes in the blood of the second experimental group increased by 23.2% (11.7 ± 1.4 G/l, $p < 0.01$) (Fig. 1), which was significantly higher than the control group. Thrombocyte counts did not differ significantly between the control group and the second experimental group.

Significant deviations from the indicators of healthy dogs were noted in the blood of dogs in the third experimental group. Specifically, the erythrocyte count decreased by 22.2% (4.9 ± 0.6 T/l, $p < 0.01$) (Fig. 2), and the hemoglobin content decreased by 13.3% (124.3 ± 11.7 g/l, $p < 0.01$) (Fig. 3), hemoglobin concentration in erythrocytes by 16.1% (277.3 ± 38.4 g/l, $p < 0.05$) (Fig. 4), hematocrit by 14.3% (41.9 ± 3.5%, $p < 0.01$) (Fig. 5), and platelet count by 27.8% (221.0 ± 40.6 G/l, $p < 0.05$) (Fig. 6).

Additionally, the number of leukocytes in the blood of the third experimental group increased significantly by 46.3% (13.9 ± 1.6 G/l, $p < 0.001$) compared to the control group and the first and second experimental groups (Fig. 1).

It is well-known that the relationship between parasites and their hosts is based on delicate molecular biology. In this regard, the pathogenic role of parasites extends beyond mechanical, toxic, and inoculatory effects on the host organism. The reactivity state of the host organism and its immunological and allergic reorganization are also important factors (Huang et al., 2020; Ruiz et al., 2024). Furthermore, the criterion for the pathogenic effect of parasites on the body includes not only a change in body weight, but also significant changes in the blood that nourishes the affected organs and tissues (Still and Konrád, 1985; Bai et al., 2017). Therefore, our research aimed to determine the effect of giardia on the hematological parameters of infected dogs.

During the experiment, it was found that the negative impact of the giardia pathogen on the hematological parameters of the experimental dogs depended on the pathogen load in their bodies and the presence or absence of clinical manifestations of the disease. In dogs confirmed to be parasitized by giardia only by rapid testing and exhibiting no clinical symptoms, hematological changes were characterized by a 16.8% increase in leukocytes.

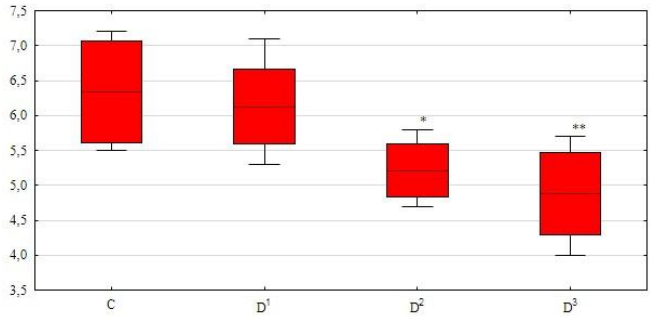

Figure 2. Indicators of the number of erythrocytes (T/l) in the blood of dogs: C — control group; D^1, D^2, D^3 — 1st, 2nd, and 3rd experimental groups; * — $p < 0.05$, ** — $p < 0.01$, *** — $p < 0.01$ relative to the control group.

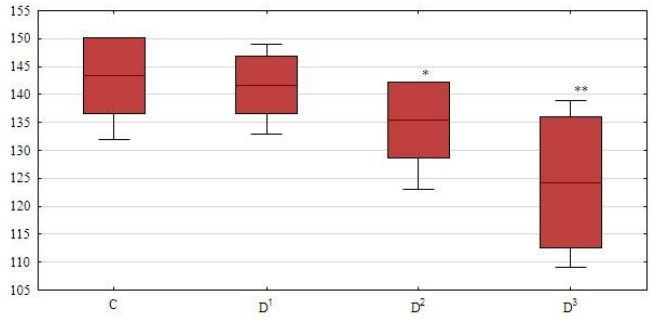

Figure 3. Indicators of hemoglobin content (g/l) in the blood of dogs: C — control group; D^1, D^2, D^3 — 1st, 2nd, and 3rd experimental groups; * — $p < 0.05$, ** — $p < 0.01$, *** — $p < 0.01$ relative to the control group.

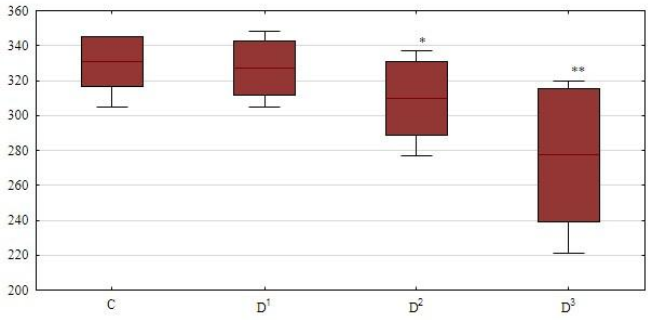

Figure 4. Indicators of the average concentration of hemoglobin in the erythrocyte (g/l) in the blood of dogs: C — control group; D^1, D^2, D^3 — 1st, 2nd, and 3rd experimental groups; * — $p < 0.05$, ** — $p < 0.01$, *** — $p < 0.01$ relative to the control group.

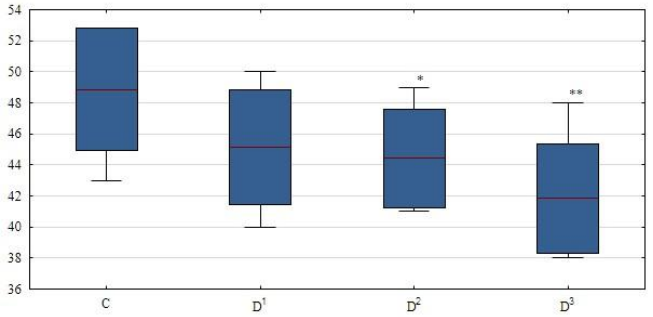

Figure 5. Hematocrit values (%) in the blood of dogs: C — control group; D^1, D^2, D^3 — 1st, 2nd, and 3rd experimental groups; * — $p < 0.05$, ** — $p < 0.01$, *** — $p < 0.01$ relative to the control group.

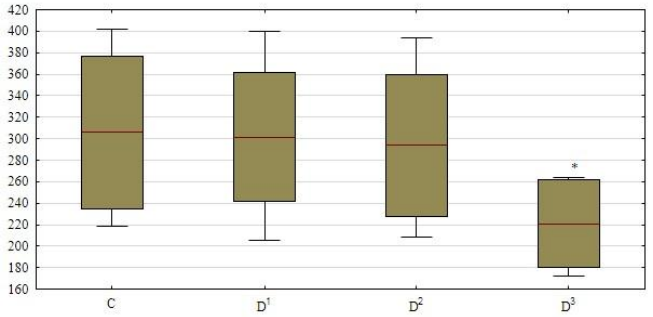

Figure 6. Thrombocyte counts (G/l) in the blood of dogs: C — control group; D^1, D^2, D^3 — 1st, 2nd, and 3rd experimental groups; * — $p < 0.05$, ** — $p < 0.01$, *** — $p < 0.01$ relative to the control group.

In dogs confirmed to be infected with giardia by a rapid test and a coproscopic examination without clinical manifestations of the disease, hematological changes were characterized by a 17.5% decrease in erythrocyte count, a 5.6% decrease in hemoglobin content, a 9.2% decrease in hematocrit, a 6.3% decrease in average hemoglobin concentration in erythrocytes, and a 23.2% increase in leukocyte count. In dogs confirmed to be parasitized by giardia by a rapid test and a coproscopic examination with severe diarrhea, the hematological changes were more significant, characterized by a

decrease in erythrocyte count by 22.2%, hemoglobin content by 13.3%, hematocrit by 14.3%, and average hemoglobin concentration in erythrocytes by 16.1%. There was also a decrease in thrombocytes by 27.8% and an even greater increase in leukocyte count by 46.3%.

Although there is little scientific data in the literature regarding the effects of giardiasis on hematological parameters in sick dogs, some reports do not reveal changes in hematological parameters in asymptomatic dogs (Peruzzo et al., 2023).

The results of these studies allow us to consider changes in dogs' blood depending on the parasite load and the characteristics of the clinical manifestation of the infection when prescribing complex treatment, thereby increasing its effectiveness.

Conclusions. It was found that the causative agent of giardiasis negatively affects the hematological parameters of infected dogs. The extent of the changes depends on the infection rate and manifestation of clinical signs in the dogs. Slight leukocytosis (16.8%, $p < 0.05$) was detected in the blood of dogs confirmed to have giardiasis by a rapid test, with no giardia found in their feces or clinical manifestations of infection. In dogs confirmed to have giardiasis by a rapid test and coproscopic examination, with no clinical signs of infection, signs of anemia were found due to a decrease in erythrocytes (17.5%, $p < 0.05$) and hemoglobin content (by 5.6%, $p < 0.05$). Hematocrit decreased by 9.2% ($p < 0.05$), and the average hemoglobin concentration in erythrocytes decreased by 6.3% ($p < 0.05$). A pronounced leukocytosis was also detected (by 23.2%, $p < 0.01$). In dogs confirmed to have giardiasis by a rapid test and coproscopic examination with severe diarrhea, significant changes were found in hematological parameters, characterized by a decrease in erythrocyte count (22.2%, $p < 0.01$), platelets (27.8%, $p < 0.05$), hemoglobin content (13.3%, $p < 0.01$), hematocrit (14.3%, $p < 0.01$), and average hemoglobin concentration in erythrocytes (16.1%, $p < 0.05$), as well as an increase in the number of leukocytes (46.3%, $p < 0.001$).

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MECHANISMS OF THE TOXIC EFFECTS OF *DRACAENA* COMPOUNDS ON CATS AND THE CONCEPT OF THERAPEUTIC MEASURES (LITERATURE REVIEW)

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Summary. Due to their external characteristics, ability to reduce bisphenol A, formaldehyde, toluene, and xylene levels in the air, and lack of special growing requirements, *Dracaena* plants are used for interior landscaping in residential and office spaces. The most common species are *D. fragrans*, *D. surculosa*, and *D. sanderiana*. *Dracaena* is placed indoors in bright areas where cats rest. The presence of a pleasant, specific odor when the leaves or flowers are damaged, due to the presence of multicomponent essential oils that irritate the senses, promotes the chewing of plant parts by companion animals. Consequently, veterinarians have recently reported an increase in cases of cat poisoning caused by *Dracaena* species. The study aims to analyze scientific studies of the content of toxic substances in *Dracaena* and their toxicodynamics in the organism of companion animals. Dhar, Maji and Ghosh (2013), Julsrigival, Julsrigival and Chansakaow (2020) and Ye et al. (2021) report on the spectrum of chemicals found in the flowers of *D. fragrans*. Julsrigival, Julsrigival and Chansakaow (2020) used solid-phase microextraction followed by gas chromatography-mass spectrometry identification to isolate 30 chemicals from *Dracaena* flowers overnight. Only eight of these chemicals (benzyl alcohol, phenylethyl alcohol, cinnamaldehyde, 3-hydroxyl-4-4-phenyl-2-2-butanone, methylene glycol, α-bergamotene, α-farnesene, and tetradecanal) were found in amounts greater than 4%. The amount of each substance varied depending on the time of day. The plant synthesized most of the substances from 8 p. m. to 10 a. m. During the day, however, α-farnesene was dominant at 23.1–50.8%. It has a green apple smell, and the LD_{50} for rats when ingested orally is 1.5 g/kg body weight, and for rabbits when applied dermally is > 5 g/kg body weight. In general, all the substances identified by scientists have a local irritating effect and are low-toxic. In 2010, Calderón et al. reported that *D. fragrans* contains substances with anticholinesterase activity that excite M- and H-cholinergic receptors in animals. Therefore, the specific antidotes are acetylcholinesterase reagents or a 1% atropine sulfate solution administered subcutaneously. In the scientific articles by Zheng et al. (2004) and Rezgui et al. (2015), it was published that all species of the genus *Dracaena* contain steroidal saponins. Xu et al. (2010) identified six new representatives of angudrakanosides A-F in the stems of *D. angustifolia*. Steroidal saponins are irritating and cause lacrimation, vomiting, and diarrhea. They form insoluble complexes with proteins and binders. Therefore, the goal of antidote therapy for suspected *Dracaena* poisoning is to reduce irritation caused by essential oils and steroidal saponins, as well as to restore the functional state of M- and H-cholinergic receptors

Keywords: *Dracaena fragrans*, α-farnesene, acetylcholesterase inhibitors, steroidal saponins, essential oils

Introduction. Modern life is comfortable, and for additional coziness, many people grow indoor flowers and keep companion animals. Different types of *Dracaena* are nowadays often used for interior landscaping. The book 'Encyclopedia of Garden and Indoor Plants' (Anufriieva, 2013) contains its botanical characteristics.

Additionally, Mabberley (2017) published that *Dracaena* is an evergreen, perennial with lanceolate or elongated-oval leaves. A special feature is the different colors and arrangement of the leaves depending on the species.

Anufriieva (2013) notes that 40 species of plants are used in the *Dracaena* genus for growing in greenhouses and only 10 — for indoor growing.

Dracaena fragrans is of great interest to scientists and florists alike. According to Julsrigival, Julsrigival and Chansakaow (2020), this species is a common ornamental plant used indoors in Thailand.

Bos et al. (1992) highlight the distribution regions of *Dracaena* species, their cultivation features, and the detailed botanical characteristics of over 15 species. The authors write in particular about *D. fragrans* Compacta, which has internodes from 5 to 10 mm. Its leaves are elongated or narrowly elongated and leathery. They gradually taper from the middle to a sharply pointed apex and are sometimes bent downward to varying degrees. The surface is glossy, moderately glossy, or matte. The surface may have shallow furrows and longitudinal stripes in shades of yellow, white, green, or grayish-green. The inflorescence is unbranched or has heavily crowded short branches. The authors note that the standard variety, *D. fragrans* Compacta, is also known as *D. deremensis* Compacta or *D. deremensis*.

It is interesting to note that *D. fragrans* got its name due to the pleasant and extremely strong smell of freshly cut grass released from the leaves (Roslynnyi Dim, 2025).

According to the 'Encyclopedia of Garden and Indoor Plants' (Anufriieva, 2013), indoor plants should be placed on windowsills that receive plenty of light. The book describes several species of *Dracaena*, including *D. fragrans*, *D. fragrans* Compacta (= *D. deremensis*), *D. surculosa*, *D. sanderiana*, and *D. reflexa var. angustifolia* (= *D. marginata*) (Fig. 1a–e).

Dracaena Tornado (Fig. 1f) is a hybrid species of *D. fragrans*. The species got its name because of the appearance of the plant's leaves, which are long, curved, and twisted into a spiral.

Figure 1. *Dracaena* species: a — *D. fragrans*; b — *D. deremensis* (photo from fleurplants.com.ua); c — *D. surculosa* (photo from eliteflowers.com.ua); d — *D. sanderiana* (photo from thursd.com); e — *D. reflexa* var. *angustifolia* (= *D. marginata*) (photo from frondlyplants.com); f — *D. fragrans* Tornado (photo from floren.com.ua).

The dark green color of the leaves with a bright light green edge, glossy surface, and low maintenance make the bush attractive to people and used for interior design. This, in turn, increases the likelihood of cats contacting this type of dracaena.

Julsrigival, Julsrigival and Chansakaow (2020) report that *Dracaena* flowers very rarely. Thus, the Thais believe that the plant's production of an inflorescence is a harbinger of good luck and fortune for the owner. This belief led to the plant being called the 'plant of happiness' by the people.

The botanical characteristics, unpretentious growth, and presence of essential oils make *Dracaena* interesting to florists, interior designers, and scientists. Due to the plant's biological characteristics, owners of *Dracaena* species typically place them on windowsills or in sunny areas of their homes. These locations are attractive to cats, who like to relax there. This causes cats to damage the leaves, leading to varying degrees of poisoning, especially in cities. Therefore, on the internet and official clinic websites, veterinarians publish informative articles highlighting the possibility of *Dracaena* poisoning in cats. Considering the industrial need for scientific evidence of the toxicological significance of *Dracaena* species for cats, we conducted a literature analysis on the presence of toxic substances in the plant.

This study **aims** to analyze scientific research on the presence of toxic substances in *Dracaena* and their toxicodynamics in companion animal organisms.

Materials for the study were published articles and books reporting the results of scientific research on the chemical content of various parts of *Dracaena* plants.

Results and discussion. Julsrigival, Julsrigival and Chansakaow (2020) and Ye et al. (2021) inform about the chemical composition of *Dracaena* parts (inflorescences, leaves), which can be used as plant material for the development of new and improvement of existing cosmetic and medical products.

Lacroix et al. (2011) and Moshi, Otieno and Weisheit (2012) report that *D. fragrans* is a plant used in folk medicine, and Kamatenesi-Mugisha and Oryem-Origa (2007) and Lacroix et al. (2011) inform about its antimalarial effect.

Wolverton (1997) and Saiyood et al. (2010) reported that *D. fragrans* can reduce the content of bisphenol A, formaldehyde, toluene, and xylene in the air.

Considering the pharmacological effects of *D. fragrans* and its value in medicine and other industries, Lu and Morden (2014) performed a phylogenetic analysis of 95 *Dracaena*, *Pleomele*, and *Sansevieria* species. Their results, published in the paper 'Phylogenetic Relationships among Dracaenoid Genera (Asparagaceae: Nolinoideae) Inferred from Chloroplast DNA Loci', suggest that *Sansevieria* should be recognized as a species of *Dracaena*.

In other words, modern genetic research methods make it possible to establish the species affiliation of related plant genera, thus increasing the number of *Dracaena* species.

A distinctive biological trait of *D. fragrans* is its nocturnal blooming pattern, where its fragrant flowers only open during the nighttime hours. Dhar, Maji and Ghosh (2013) and Julsrigival, Julsrigival and Chansakaow (2020) report that the chemical composition of the flower fragrance varies throughout the day. Julsrigival, Julsrigival and Chansakaow (2020) studied the change in the amount of volatile chemicals from the flowers of fragrant dracaena during the day. The flowers were sampled every two hours. The researchers employed a method known as solid-phase microextraction, followed by identification through gas chromatography-mass spectrometry. A total of 30 volatile compounds were identified, grouped into eight categories: aldehydes (maximum accumulation in flowers at 6–8 a. m.), alcohols (maximum amount 6 p. m.–4 a. m.), esters (10 a. m.–4 p. m.), ketones (6 p. m.–4 a. m.), monoterpenes (12 p. m.–2 p. m.), sesquiterpenes (6–8 a. m.), phenylpropenes (12–6 p. m.), and other substances (10–8 p. m.). All groups of substances were detected in the samples during the day, with the maximum amount occurring in the hours indicated in parentheses.

Regardless of the time of day, the authors found that the flowers and buds of *D. fragrans* contained the following compounds: benzyl alcohol, phenylethyl alcohol, cinnamon alcohol, 3-hydroxyl-4-4-phenyl-2-2-butanone, methyleugenol, α-bergamotene, α-farnesene, and tetradecanal. Alpha-farnesene was the predominant compound in all sampling periods (23.1%–50.8%). During the flowering period from 6 p. m. to 10 a. m., the predominant compounds were 2-pentylfuran, β-ocimene, benzene aldehyde, linalool oxide, linalool, 2,6-nonadienal, 2-nonenol, and 2,4-decadienal. These compounds increased significantly at night, including 2-pentylfuran, linalool oxide, linalool, and 2-nonenal.

The maximum synthesis is from 6 to 10 a. m. (maximum 8 a. m.), with linalool oxide (4.8%) and linalool from 2 a. m. to 10 a. m. (maximum 10 a. m.) (3.1%). From the data presented in the article by Julsrigival, Julsrigival and Chansakaow (2020), some volatile chemicals (pentyl furan; 2,6-nanodienal, (E, Z)-; nonet-1-al; 2-nonenol, (E)-) were not registered in the samples from 12 a. m. to 6 p. m., at 6 p. m. a small amount of linalool was detected, at 8 p. m. — a twofold increase in linalool and traces of linalool oxide and 2,6-nanodiunal, (E, Z)-, at 10 p. m. — all these substances were synthesized with an increase in the amount of linalool and its maximum at 10 a. m.

Cinnamyl acetate was synthesized from 10 a. m. to 4 p. m., and 1-dodecanol was synthesized from 12 p. m. to 4 p. m.

Based on the results of scientific studies by Julsrigival, Julsrigival and Chansakaow (2020) regarding the presence of volatile substances in *D. fragrans* flowers and the chemical characteristics of available sources, we compiled a table (Table 1) on the degree of toxicity of some isolated substances whose content exceeded 4% at certain times of day. We also determined and substantiated their toxicodynamics.

α-Farnesene is the dominant substance in the flowers of *Actinidia deliciosa* (Nieuwenhuizen et al., 2009), *Lonicera japonica* (Schlotzhauer et al., 1996) and *Murraya exotica* (Raina et al., 2005).

Published data on the constituents of *D. fragrans* buds and flowers suggest the presence of synergistic effects among the substances present in the highest concentrations. After all, the published characteristics of the chemically pure compounds listed in the table for each substance indicate irritating properties. However, toxicological studies have only been conducted to determine the LD_{50} for oral administration to rats for a few of the dominant substances. It should be noted that all substances in the table in this article are marked with GHS07 (irritants) and are low-toxicity.

Calderón et al. (2010) reported that substances from *D. fragrans* may act as an acetylcholinesterase inhibitor. The toxicodynamics consist of blocking acetylcholinesterase in the intersynaptic clefts of the parasympathetic division of the peripheral nervous system. This is clinically manifested as nicotine and muscarinic effects. These findings can explain general depression, bradycardia, epigastric pain, and abdominal wall tension upon palpation.

Taking into account the toxicological characteristics of *D. fragrans* components, it is advisable to include medicines in the treatment regimen:

— enterosorbents form insoluble complexes with toxic substances and are effective at any stage;

— enveloping (mucous) or astringent agents, such as a decoction of oak bark, gray alder cones, or plantain, which reduce the irritating effect of substances identified by Julsrigival, Julsrigival and Chansakaow (2020) and slow the absorption rate of substances with anticholinesterase activity. These preparations can be prescribed if the animal is experiencing initial stages of poisoning (e. g., recent gnawing, depression, or vomiting, with a deteriorating but not critical condition). In cases

Table 1 — Description of the significant constituents of *Dracaena fragrans* essential oils from the flowers and saponins from the bark and leaves

Constituent	Localization in nature	Use	Toxicological effect	LD_{50}, oral
Benzyl alcohol	A component of the essential oils of jasmine, hyacinth, etc.	Flavoring agent for cosmetics, detergents	Pure — causes corneal necrosis (Kulkarni and Mehendale, 2005). Toxic doses cause respiratory arrest, vasodilation, hypotension, convulsions, and paralysis (Brühne and Wright, 2000)	For rats — 1.25 g/kg
Phenylethyl alcohol, $C_8H_{10}O$	Contained in rose and geranium essential oils. An important component of perfumes	Preservative in dosage forms for the eyes, nose and ears. It has bactericidal properties (Rybachuk, 2016)	—	—
Cinnamon alcohol, $C_9H_{10}O$	Only in combination with essential oils of hyacinth, Peruvian balsam	Flavoring agent for cosmetics, soaps, food products to create strawberry, lemon, peach, apricot, cognac flavors, intermediate in the synthesis of streptomycin, plasticizer for plastics	Skin and/or eye irritant; mutagenicity: DNA repair test: *Bacillus subtilis* 10 mg/disc (BDMAEE, 2024)	For rats — 2 g/kg; for rabbits — ≥ 5 g/kg; for mice — 2,675 mg/kg (BDMAEE, 2024)
3-hydroxyl-4-4phenyl-2-butanone, C_4H_8O	—	—	—	—
Eugenol	A component of essential oils of clove (80–90%), nutmeg, cinnamon, bay leaves, basil. It attracts males of the Euglossini species (NCBI, 2021)	It has a clove-like odor and is a flavoring agent in perfumes, cosmetics, and cooking. Vanillin is produced from eugenol. Dentistry. It has anesthetic and analgesic properties. It is eliminated from the body within a day	It is hepatotoxic (Eugenol (Clove Oil), 2019). Eugenol increases histamine release (NCBI, 2021). In case of poisoning: hematuria, nausea, diarrhea, dizziness, tachycardia, renal dysfunction (Heller and Zieve, 2010). Antidote — N-acetylcysteine	—
α-berga-motene	Component of essential oils of plants	Flavoring agent	—	—
α-farnesene	In the peel of apples, giving the aroma of green apples, in essential oils of orange, rose	Flavoring for perfumes, household chemicals	—	For rats — 1.5 g/kg; for rabbits dermally — > 5 g/kg
Tetra-decanal (myristyl alcohol)	Component of nutmeg	Component of cold cosmetic creams due to its emollient properties	Used on the skin — a consequence of a tumor. Symptoms are not described, only the LD_{50} is given	For rats — > 5 g/kg (Noweck and Grafahrend, 2006)
Steroid saponins	Components of foxglove purpurea leaves, less in lily of the valley (saponin conalarin), snowdrop, narcissus, snowflakes, Orobanche cumana, tobacco, jimsonweed, Mandragora, etc.	They have fungicidal and antitumor activity. The drug polysponin (made from *Dioscorea*) is used to treat atherosclerosis due to its cholesterol-lowering effect	Locally — irritation, after absorption, a hemolytic effect is possible	The minimum lethal dose of digitalis is 2.25 g. The actual LD of steroid saponins for laboratory animals was not found in the literature

of severe clinical conditions and depression, however, they will not be effective;

— intravenous blood substitutes and crystalloids can be used to have a diuretic effect because essential oils are mainly eliminated from the body by the urinary and respiratory systems and, to a lesser extent, the digestive system;

— in cases of anticholinesterase action, acetylcholinesterase inhibitors are effective. If they are unavailable, it is advisable to use atropine sulfate solution at therapeutic doses for the animal species. The active substance's pharmacodynamics will provide M-cholinolytic action and restore the physiological function of M-cholinergic receptors in the body.

In the scientific articles by Zheng et al. (2004) it is reported that all species of the genus *Dracaena* belonging to the family Asparagaceae contain steroid saponins. Xu et al. (2010) published data that new steroid saponins were isolated in the stem of *D. angustifolia*: angudrakanosides A–F (six in total).

Rezgui et al. (2015) reported that 15 steroid saponins were isolated in the study of chemical components of the bark and roots of *D. angustifolia*.

Rybachuk and Halatiuk (2022) report that consuming plants containing steroid saponins orally can cause vomiting and diarrhea due to irritation of the gastric and intestinal mucosa. Therefore, if such clinical signs appear after eating or gnawing on the leaves of various *Dracaena* species, it is necessary to administer antiemetics (such as ondansetron solution for injection in cases of debilitating vomiting) followed by protein solutions or astringents. Note that saponins form complexes with proteins, lipids, sterols, and tannins. If there is contact with the mucous membranes of the upper respiratory tract (e. g., coughing or sneezing) or the eyes (e. g., lacrimation), rinse with isotonic crystalloid solutions (e. g., sodium chloride solution at 0.9% or glucose solution at 5%).

If hemolytic effects occur, administer intravenous neohemodesis, detox, rheosorbilact, etc., as there are no specific antidotes.

Using dosage forms and drugs in the proposed complex therapy will quickly eliminate the toxic effects of *Dracaena* substances in an animal's body.

Conclusions. The flowers and buds of *Dracaena fragrans* contain low-toxic, locally irritating substances: benzyl alcohol, phenylethyl alcohol, cinnamon alcohol, 3-hydroxyl-4-4-phenyl-2-2-butanone, methyleugenol, α-bergamotene, α-farnesene, and tetradecanal.

The leaves and bark contain steroid saponins, which cause local irritation, as well as anticholinesterase substances that lead to overstimulation of parasympathetic synapses.

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SEASONAL DYNAMICS OF HISTOMONIASIS AND TRICHOMONIASIS IN TURKEYS ON FARMS IN ODESA REGION, UKRAINE

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Summary. The article focuses on the seasonal dynamics of histomoniasis and trichomoniasis in turkeys on farms in Odesa Region. Studies have shown that pathological changes in turkeys have a pronounced seasonal dependence. In the spring and summer, an increase in the intensity of histomoniasis and trichomoniasis lesions was observed, which is associated with optimal conditions for the development of pathogens. The study found that when turkeys are infected, pathological changes are primarily observed in the cecum, and necrotic processes are noted in the liver. In the acute form of histomoniasis, macroscopic changes in the liver are observed. The autopsy revealed significant lesions in the small intestine and liver, accompanied by characteristic changes including hemorrhagic typhlitis, fibrinous ulcerative inflammation, and fibrinous masses. A histological examination confirmed the presence of hyperemia, diffuse infiltrates, fibrinous changes, and necrosis in the intestinal mucosa and liver. A pathological examination of the turkey corpses revealed a pattern similar to that of histomoniasis and trichomoniasis, with hemorrhagic or fibrinous inflammatory changes in the intestines and liver changes. The data suggest that the combination of these infections complicated the disease

Keywords: *Histomonas meleagridis*, pathological changes, acute form

Introduction. The development of industrial poultry farming in Ukraine is rapid. The latest data show a slight lag in gross poultry production in rural areas compared to industrial poultry farms (Bogach, 2011; Daş et al., 2021; McDougald and Hu, 2001).

One poultry industry in Ukraine that has begun to develop rapidly in both private households and industrial farms is turkey farming. This can be interpreted as the importation of early-maturing, highly productive meat breeds of turkeys from abroad. The latest statistics show a slight lag in gross poultry production in households compared to industrial farms (Chadwick et al., 2020; Klodnicki, McDougald and Beckstead, 2013; Abdelhamid et al., 2021).

Poultry farms are often adjacent to private households that use different poultry-keeping technologies. Breeding new turkey breeds makes it possible to produce a significant amount of meat in a short time with minimal labor and feed costs per unit, which was not possible with traditional breeds. However, the breeding of highly productive turkey breeds, especially in private households, has faced the problem of extreme turkey sensitivity to bacterial and, especially, parasitic contaminants, which caused minor damage in native breeds (Abdelhamid et al., 2020; Bilic and Hess, 2020).

Ensuring food safety is a critical challenge for the international agricultural sector, and the poultry industry is one of the key sectors contributing to this goal. In particular, poultry farming — the production of chickens, turkeys, and other poultry species — plays an important role in providing the population with meat, meat products, eggs, and other egg products. The development of this industry is closely linked to improving the diagnosis, treatment, and prevention of diseases, which helps ensure product safety and quality. One of the most pressing issues is combating parasitic diseases, which are becoming increasingly prevalent among poultry, particularly in small-scale poultry operations. The spread of these diseases requires new approaches to diagnosis and prevention, as traditional methods used for large-scale poultry enterprises are often inapplicable to smaller farms (McDougald et al., 2020; Landman et al., 2015; Fudge et al., 2024; Bogach et al., 2016; Jones et al., 2020).

Histomoniasis is a disease that requires special attention. Caused by the parasite *Histomonas meleagridis*, this disease can lead to significant economic losses. Studying its epizootiology and developing effective treatment and prevention methods are important for improving poultry production, especially given the increased interest in farm production and small farms (Jones et al., 2020; Liebhart, Windisch and Hess, 2020).

Poultry histomoniasis is more prevalent during the warm, humid seasons because high temperatures and humidity create ideal conditions for oocyst maturation and preservation in the environment. Under these conditions, oocysts can remain viable for extended periods, thereby increasing the risk of infection in poultry. The high humidity characteristic of summer months, in particular, contributes to infection development by creating favorable conditions for parasite reproduction and survival (Liebhart and Hess, 2009; Daugschies, Bangoura and Lendner, 2013).

On farms where proper veterinary, sanitary, and zoohygienic conditions are not maintained, seasonal fluctuations in disease incidence can be more pronounced. Poor poultry housing conditions increase the risk of infection, especially during periods of high humidity and temperature. Overcrowding, poor ventilation, poor hygiene, and improper feeding also have a negative impact. In industrial poultry farming, seasonality is not always clear. On such farms, the risk of

enzootics depends more on production technology and sanitary conditions. If poultry rearing technology includes proper hygiene, ventilation, and environmental control, infection may be less pronounced, even during periods of high humidity (Badparva and Kheirandish, 2017; Huber et al., 2006).

Turkeys appear to be more susceptible to histomoniasis due to their behavior and greater tendency to congregate. This ensures the parasites' survival, even in the absence of vectors, leading to higher transmission. Outbreaks can be significant in cases with high mortality, such as on organic farms where turkeys and broilers are raised, due to co-infection with trichomoniasis (Hu and McDougald, 2003).

Correlation between age, genetics, and severity of infection: It has been determined that the dynamics of age and genetic differences in birds can affect the frequency and severity of histomoniasis. For instance, chickens are less susceptible to the disease than turkeys. However, despite being previously considered symptom-free carriers, outbreaks have recently been reported among broilers, even under free-range conditions. This suggests that chickens may be infected without showing obvious symptoms, but are not resistant to infection. Different strains of *H. meleagridis* can have different levels of virulence, which affects disease severity in birds. Therefore, the degree of infection depends not only on the species and age of the birds but also on the dose of parasites to which they are exposed. Traditionally, laboratory detection of *H. meleagridis* in poultry has relied on microscopy, clinical symptoms, and culture. However, numerous studies have shown that the morphology of this protozoan is very similar to that of other protozoa, such as the pseudocysts *Tetratrichomonas gallinarum* and *Blastocystis* sp., which can be found in the ceca of poultry. Conversely, an early diagnosis may be ambiguous due to the similarity of symptoms to those of other avian diseases, such as coccidiosis. Additionally, culturing protozoa for diagnostic purposes has proven to be challenging due to the presence of non-pathogenic organisms in poultry feces and ceca that can inhibit *H. meleagridis* growth (Purple et al., 2015).

Further research in this area will enable the development of more effective and cost-efficient strategies to combat histomoniasis, trichomoniasis, and other diseases. This will ultimately lead to increased production stability and improved product quality on an international scale.

The study **aimed** to investigate seasonal dynamics and pathomorphological changes in turkeys with histomoniasis and trichomoniasis, considering the influence of climate in northern and southern districts of Odesa Region.

Materials and methods. The spread of histomoniasis and trichomoniasis in turkeys was studied in the northern and southern districts of Odesa Region (the Podilsk and Bolhrad districts). The research was conducted in several private households and in the Laboratory of Epizootology, Parasitology, Animal Disease Monitoring, and Providing at the Odesa Research Station of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine'.

The study examined the corpses of turkeys that died from histomonosis. Autopsies were performed no later than five hours after death or slaughter. A total of 18 turkeys were examined: 10 aged 45–90 days and 8 aged 5–6 months. During autopsy, pathological tissue samples were collected from the gastrointestinal tract, including the glandular and muscular gizzards, liver, and various parts of the small and large intestines. The samples were fixed in a 10% aqueous solution of neutral formalin. After fixation, the pieces were washed in running water and embedded in paraffin using the conventional method. Histologic sections 5–8 μm thick were made from the paraffin blocks using a sled microtome.

The obtained sections were stained using hematoxylin and eosin, as well as the Van Gieson and Mallory methods. Micrographs were taken with an Olympus 2000 microscope visualization center. Morphometric studies were performed using the VideoTest-Master 4.0 software. Histomoniasis was diagnosed through a parasitological examination of the contents of the small and large intestines. To rule out bacterial diseases, microbiological studies were performed on samples from the cecum, liver, bone marrow, and heart of dead birds, which were cultivated on Levine and Endo media. To exclude fungal infections, the samples were inoculated on Sabouraud's medium. For the bacterioscopic examination, smears and prints were prepared from the mucous membranes of the small intestine and cecum and the affected areas of the liver. These samples were stained using Romanowski–Giemsa, Ziehl–Neelsen, and Gram stains. To exclude eimeriosis, the Fülleborn method was employed.

Results and discussion. We conducted a study on the seasonal dynamics of histomoniasis and trichomoniasis in Odesa Region (Table 1).

The seasonal dynamics of these diseases in turkeys depend significantly on climatic conditions, the type of bird farming, and the regional characteristics of Odesa Region.

Our analysis revealed that during the warm season (spring and summer), the prevalence of infection increases, particularly in regions with high humidity. The relatively high rates of histomoniasis infection in Podilsk District are due to more stable humidity conditions, which create a favorable environment for parasite preservation and reproduction. Conversely, the spread of the disease is less pronounced in Bolhrad District due to its drier climate.

The highest prevalence of histomoniasis infection was recorded in the summer, due to high temperatures and high humidity. These factors create a favorable environment for the maturation of *H. meleagridis* oocysts and ensure their survival for a long time in the environment.

Table 1 — Seasonal dynamics of histomoniasis and trichomoniasis in turkeys on the farms in Odesa Region

Season	District	Examined turkeys	Infected turkeys	Prevalence, %	Histomoniasis		Trichomoniasis	
					Infected turkeys	Prevalence, %	Infected turkeys	Prevalence, %
Winter	Podil	10	4	40.0	3	30.0	1	2.5
	Bolhrad	10	0	0.0	0	0.0	0	0.0
Spring	Podil	15	7	46.7	5	33.3	2	4.3
	Bolhrad	15	3	20.0	3	20.0	0	0.0
Summer	Podil	25	13	52.0	11	44.0	2	3.8
	Bolhrad	25	8	32.0	7	28.0	1	3.1
Autumn	Podil	15	6	40.0	5	33.3	1	2.5
	Bolhrad	15	4	26.7	3	20.0	1	3.8
Total		130	45	34.6	36	27.7	9	6.9

The highest prevalence of histomoniasis infection was recorded in the summer, due to high temperatures and high humidity. These factors create a favorable environment for the maturation of *H. meleagridis* oocysts and ensure their survival for a long time in the environment.

In Podilsk District, where moderate humidity prevails, the peak of infection occurs in the summer (prevalence — 44%). In Bolhrad District, which has a drier climate, prevalence of histomoniasis is slightly lower (28% in the summer), though infection rates remain stable in the spring and summer.

Trichomoniasis, unlike histomoniasis, shows a relatively low prevalence of infection throughout the year. The maximum level of infection (4.3%) was detected in spring, which may be due to the exacerbation of immune processes in birds after the winter period, when the conditions of keeping could be unfavorable. In other seasons, the prevalence of trichomoniasis varies from 2.5% to 3.8%, which confirms a less pronounced seasonal dependence.

The autopsy of turkeys that died from spontaneous histomoniasis showed lesions mainly in the cecum and liver. The cecum was significantly enlarged, containing a dark red semi-liquid mass with gas bubbles, indicating the presence of hemorrhagic typhlitis. With a prolonged course of the disease, fibrinous-hemorrhagic changes were observed in the cecum with the formation of dense grayish-white masses, partially saturated with blood. Fibrinous and ulcerative inflammation was recorded in the blind intestines, which led to an increase in intestinal volume and the accumulation of dense grayish-white masses that were easily removed from the lumen (Fig. 1).

The thickness of the intestinal wall was variable, with areas of varying color ranging from dark red to grayish pink. There were areas with a thin wall that was white or gray and very thin. In 2.5% of cases, necrosis of the walls of the small intestine with fibrinous peritiflite characterized by white-gray films on the serous membrane of the intestine was detected. When the disease progressed to a chronic form, connective tissue overgrowths were observed at the site of the films, and in some cases, perforated ulcers of the cecum, complicated by fibrinous inflammation of the thoracic-abdominal cavity. Significant fibrin accumulations were found between the intestinal loops and on the surface of organs such as the liver and spleen, confirming the complicated course of the disease.

Histological studies revealed the following pathological changes in the gastrointestinal tract of turkeys with histomoniasis: mucocutaneous inflammation of the glandular gizzard and a heterogeneous color of the mucous membrane ranging from grayish pink to dark red. The small intestine exhibited hyperemia of the mesenteric blood vessels, and its lumen contained a yellowish-brown mucous mass. The mucous membrane was swollen and had a heterogeneous color, mostly pink with a gray or red tint.

Microscopic examination revealed hyperemia of the arteries and capillaries. Thickening and homogenization of the arterial vessel walls, partial endothelial desquamation, and focal endothelial cell proliferation were observed in the submucosal layer of the large intestine. Focal infiltrates of macrophages, histiocytes, lymphocytes, and erythrocytes were present around the arteries in the submucosa of the cecum. The venules and capillaries of the mucous membrane were brightly hyperemic with large accumulations of fluid and erythrocytes. Diffuse proliferation of macrophages, histiocytes, and lymphocytes was observed in the mucosa. The glands located closer to the mucosa were compressed by fluid and cell proliferation; some decreased in size, while others completely atrophied. The epithelial cells were in a state of mucosal dystrophy, and most of them had desquamated. Mucus, exfoliated epithelial cells, and macrophages accumulated on the mucosal surface. In longer cases of the disease, coagulation necrosis of the epithelium and of the mucosal layer of the cecum was observed. In some cases, necrosis covered the entire intestinal mucosa and extended to the submucosal and muscular layers.

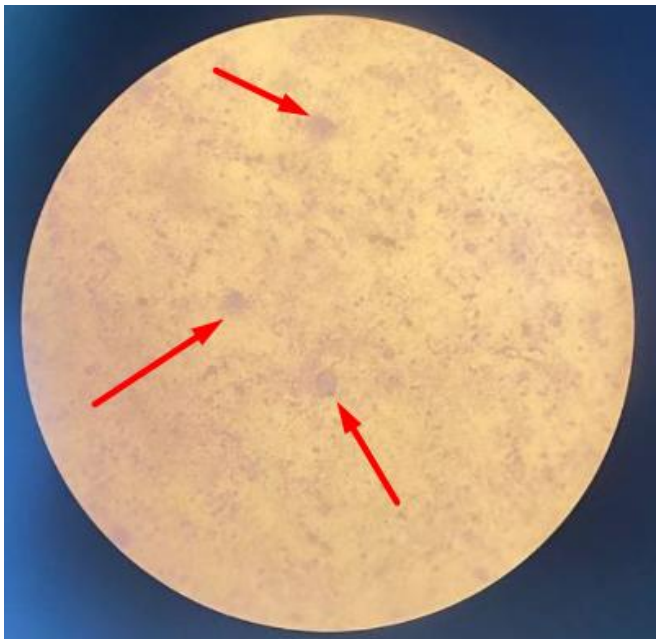

Figure 1. Histomonads in the cecum.

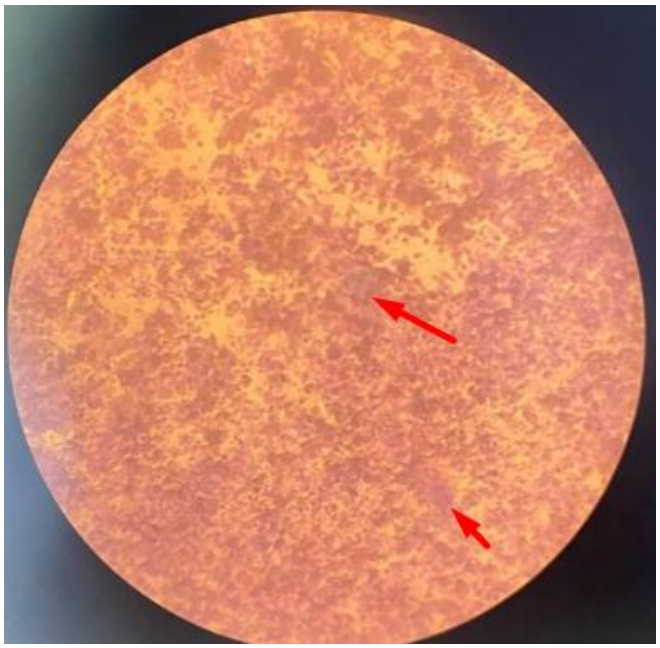

Figure 2. Histological changes in the liver of turkeys with histomoniasis.

These pathomorphological changes confirm that the acute and prolonged course of histomoniasis causes severe dystrophic and inflammatory changes localized in different parts of the turkeys' gastrointestinal tracts.

A study of the pathological and morphological changes in the livers and immunocompetent organs of turkeys with histomoniasis revealed the following characteristic features: necrotizing hepatitis and lymphoid granulomas around histomonads. Diffuse and focal proliferations of lymphoid and histiocytic tissues were also common. These proliferations caused compression, atrophy, and hepatocyte death. They also caused pericholangitis and perivascularitis, which resulted in the formation of bile cylinders and blood clots. In turkeys aged 60–90 days, liver necrosis was observed in only 40% of cases and was mainly represented by miliary and submiliary necrosis up to 2 mm in diameter.

The main macroscopic changes included protein dystrophy and congestive hyperemia. These changes were accompanied by an increase in liver volume and a heterogeneous dark red, brown, or clay color. The liver also had a flaccid consistency.

In 50% of cases, particularly when fibrinous or fibrinous-hemorrhagic typhlitis was present, fibrinous changes and connective tissue proliferation with grayish-white, dense formations were observed on the liver serosa.

Histologic examination revealed congestive hyperemia, protein dystrophy, and the accumulation of cellular infiltrates consisting of macrophages, lymphocytes, and histiocytes surrounding the bile ducts and hepatocytes. The presence of granulomas in some areas resulted in hepatocyte necrosis (Fig. 2).

As the disease progressed, proliferative inflammation characteristic of catarrhal cholangitis formed around the bile ducts.

Conclusions. The highest prevalence of histomoniasis, at 44%, was recorded in summer in Podilsk District, while in Bolhrad District, a maximum of 28% was recorded. Trichomoniasis infection remained relatively low throughout the year, peaking at 4.3% in spring.

The warmer climate in the southern districts (Bolhrad) contributes to the disease spreading more actively in the spring and summer. In the northern districts (Podilsk), higher humidity allows pathogens to survive longer, increasing the risk of infection in the summer and autumn.

Analysis of pathomorphological changes confirmed that histomoniasis causes severe lesions of the cecum, liver, and immunocompetent organs.

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SUSCEPTIBILITY OF RABBITS, AS HETEROLOGOUS ANIMALS, TO BOVINE LEUKEMIA VIRUS

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Summary. Given the ability of the bovine leukemia virus (BLV) to overcome the interspecies barrier under experimental conditions — leading to the development of an infectious process in pigs, monkeys, rats, capybaras, and other animal species — the study of the susceptibility of various animal species to the pathogen and the determination of their potential role in the epizootic process is relevant and requires further research. Therefore, the investigation of the possible use of laboratory animals, particularly rabbits, for studying the infectious process in leukemia is of scientific interest and may contribute fundamental knowledge about the ability of BLV to cross the species barrier. The possibility of infecting rabbits was studied by subcutaneous inoculation of stabilized blood, followed by assessment of hematological, serological, and molecular-genetic indicators in animals from both the experimental and control groups at distant time points after inoculation. Every 15 days, hematological parameters (ESR, hemoglobin level, and leukocyte differential count) were examined in both groups. Seroconversion in the infected animals was determined using the agar gel immunodiffusion test. At the same time, the presence of the virus's genetic material was detected by polymerase chain reaction (PCR) using a specific primer pair. Analysis of hematological results from the experimental and control rabbit groups at later stages after infection revealed that 60 days after inoculation, there was an increase in leukocyte count due to a rise in band neutrophils and lymphocytes. Most hematological parameters (hemoglobin, neutrophils, basophils, ESR) in the experimental group returned to baseline levels, except for lymphocyte count. Seroconversion in the experimental group animals was observed starting from day 60 post-infection, with peak levels recorded between days 105–120. The presence of the leukemia virus in the animals during this period was confirmed by molecular-genetic studies, which correlated with the hematological findings, particularly the development of lymphocytosis starting on day 60, which is characteristic of the infectious process typical of BLV infection. Thus, the study experimentally confirmed the ability of the bovine leukemia virus to cross the species barrier and induce an infectious process in heterologous animal species, namely rabbits

Keywords: infectious process, hematology, lymphocytosis, seroconversion, molecular-genetic research

Introduction. Among viral cattle diseases, leukemia is considered the most significant neoplastic disease, caused by Bovine leukemia virus (BLV). The pathogen belongs to the Retroviridae family, *Deltaretrovirus* genus, which also includes human T-cell leukemia viruses (HTLV-1, HTLV-2, HTLV-3), simian T-lymphotropic viruses (STLV), and bovine leukemia viruses (BLV) (Hossain, Tan and Satou, 2025). The disease is classified as a slow or minor infection due to its long incubation period and chronic manifestation. A recent comparative analysis of HTLV-1/BLV provirus integration sites in host genomes from primary tumors and asymptomatic infection stages was conducted using high-throughput sequencing, mapping, and RNAseq. This research demonstrated that HTLV/BLV proviruses integrate near cancer driver genes, contributing to malignancy development via polyclonal expansion of infected cells (Forlani et al., 2021).

In cattle, the disease manifests in two stages after the incubation period: the seroconversion stage and the clinical-hematological stage. The seroconversion stage has no pronounced clinical symptoms, as the virus remains in a latent state; thus, infected animals are classified as carriers. After a prolonged latent infection lasting 3 to 8 years, approximately 30% of BLV-infected cattle develop persistent lymphocytosis, and fewer than 5% eventually develop malignant B-cell lymphoma (Marawan et al., 2021; Lv et al., 2024). The clinical-hematological stage is characterized not only by significant lymphocytosis but also by immunosuppression, along with a decline in productivity and product quality. BLV infects the epithelial cells of the mammary gland in dairy cows, reducing their antimicrobial capacity. BLV-encoded microRNAs (BLV-miRNAs) can modify host genes and promote viral replication. According to recent data (Lian et al., 2023), BLV-miR-B1-5p suppresses the expression of the *mucin 1* (MUC1) gene in bovine mammary epithelial cells, significantly enhancing the adhesion of *Staphylococcus aureus* — one of the most common mastitis pathogens.

According to Buehring, Choi and Jensen (2001), immunoblotting of sera from 257 people who had contact with BLV-infected animals or consumed unsterilized/raw milk and untreated beef products containing proviral BLV DNA fragments revealed antibodies to the BLV capsid antigen in 74% of cases. Although the presence of BLV genomic fragments and the p24 protein in bovine and human mammary tissue and cell cultures is not directly linked to disease manifestation, this demonstrates the virus's tropism to these tissues. Furthermore, research indicates a significantly higher BLV detection rate in breast cancer patients compared to healthy control groups (Lv et al., 2024). The *tax* gene in BLV is responsible for oncogenic

activity, suppressing DNA excision repair mechanisms and causing oxidative cellular damage. This may be associated with various cancers, including breast and lung cancer. A literature review confirms that BLV infection is statistically associated with breast cancer (Saeedi-Moghaddam, Mohammaditabar and Mozhgani, 2024; Khatami et al., 2020). This raises significant socio-biological concerns regarding public health and safety, as milk, dairy products, and beef, are key sources of human nutrition, and consuming them raw may be a route of BLV transmission to humans.

Transmission of BLV to susceptible cattle occurs via blood, as well as through all bodily secretions and excretions containing infected lymphocytes. Two transmission pathways are distinguished: vertical (transplacental) and horizontal, including iatrogenic transmission (via human involvement). The alimentary route concerns calf infections during suckling, with an increased risk when milk is contaminated with blood lymphocytes, especially in mastitis-afflicted carrier cows. Other horizontal transmission cases involve mass veterinary procedures using inadequately disinfected tools, and surgical or obstetric interventions performed without proper aseptic precautions. Biting flies, such as the stable fly (*Stomoxys calcitrans*), pose a significant risk for horizontal spread, transferring the virus from infected to healthy animals. The critical role of biting flies in BLV epidemiology was highlighted in epidemiological studies conducted in the USA and Japan, where stringent fly control eliminated new BLV cases in beef cattle herds (Marawan et al., 2021).

Studies have shown that BLV can infect human mammary and lung cell lines, as well as HeLa cell cultures. Literature also confirms that various cell lines derived from primates (chimpanzees, rhesus macaques), dogs, pigs, sheep, goats, and bats can be infected with BLV through inoculation with cell-free viral preparations. In all cases, viral replication was observed (Graves and Ferrer, 1976; Bai et al., 2020).

Under natural conditions, BLV is transmitted among domestic cattle (*Bos taurus*), zebu (*Bos indicus*), and water buffalo (*Bos bubalis*). Besides cattle, BLV can infect sheep and goats, causing leukemia and lymphoma. Sheep experimentally infected with BLV are considered the best model for studying leukemia/lymphoma, as the disease develops in about 20 months (Forlani et al., 2021). The ability of BLV to cross species barriers has been confirmed experimentally. Successful infections have been established in pigs, monkeys, rats (Buehring, Choi and Jensen, 2001), capybaras, and rabbits (De Oliveira et al., 2016). When rabbits and rats were inoculated with material derived from FLK cells, about 30% of animals became seropositive to BLV and developed symptoms of lymphoid leukemia, including immunosuppression, increased lymphocyte and lymphoblast counts, and preneoplastic lymphoid cell clusters in the liver, lungs, kidneys, and lymph nodes. BLV genetic material was detected in sick animals by PCR, confirming the virus's role as the etiological agent of the observed lymphoid leukemia (Dimitrov et al., 2012). Thus, the investigation of the use of laboratory animals — particularly rabbits — for studying the infectious process of leukemia is of scientific interest and may provide fundamental insights into the ability of BLV to overcome interspecies barriers (Dimitrov et al., 2013).

The **research objective** is to determine the feasibility of using laboratory animals, particularly rabbits, for studying the infectious process of leukemia.

Materials and methods. The study was conducted on 10 rabbits weighing 2–2.5 kg. The animals were divided into two groups — experimental and control. The rabbits in the experimental group (n = 5) were subcutaneously inoculated with 1.0 cm^3 of EDTA-stabilized blood from cattle infected with the bovine leukemia virus (BLV). The animals in the control group received an equivalent volume of phosphate-buffered saline.

Experiments on rabbits were conducted following the recommendations of 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'.

To study the effect of inoculating biological material from a BLV-infected bovine donor on the rabbits, serum and EDTA-stabilized blood samples were collected from animals in both the experimental and control groups every 15 days. At the same time, clinical observation of the rabbits was conducted.

Hematological examinations of EDTA-stabilized blood were performed using light microscopy by counting the cellular elements of the leukocyte fraction (lymphocytes, basophils, band and segmented neutrophils, atypical and immature forms of the mentioned cells) and determining their proportions. Blood smears were stained using the Romanowsky-Giemsa method (Wittekind and Gehring, 1985). Additionally, at each stage, the erythrocyte sedimentation rate (ESR) and hemoglobin levels were measured (Dudchenko et al., 2019).

Serological testing for the presence of specific antibodies in the blood serum of experimental and control rabbits to BLV was conducted using the agar gel immunodiffusion (AGID) test, employing a 'Set of liquid stabilized components for serological diagnosis of bovine leukemia by immunodiffusion (AGID)' produced by LLC 'Scientific Research Enterprise 'Veterinary Medicine' (Kharkiv, Ukraine).

Detection of proviral BLV DNA was carried out using a pair of BLV-env 3–4 primers according to WOAH recommendations (Fechner et al., 1996), flanking a 444 bp fragment of the *env* gene of BLV. Reverse transcription and DNA synthesis were performed using

MMLV reverse transcriptase following the manufacturer's guidelines. Amplification was carried out using a Biometra thermocycler (USA). Visualization of PCR results was performed by horizontal gel electrophoresis in 1.5% agarose gel.

Results and discussion. Immediately before the inoculation of blood from BLV-infected cattle, baseline hematological parameters were determined in rabbits of both the experimental and control groups. It was established that hemoglobin levels, the number of erythrocytes and leukocytes, as well as the ratio of leukocyte fraction elements, were within normal ranges: hemoglobin — 116.0 ± 14.0 g/l, erythrocytes — 6.8 ± 0.4×10^6/ml, leukocytes — 5.8 ± 2.2×10^3/ml, erythrocyte sedimentation rate (ESR) — 2.0 ± 1.2 mm/h. It should be noted that the ESR in animals of the experimental group increased significantly by day 30 after experimental infection of the rabbits, with a trend toward decreasing values starting from day 45 after the inoculation of the biological material and continuing until the end of the experiment. From day 60 onward, the ESR returned to within the normal range. Between days 30 and 45 post-infection, concentration was increased by 20–25 units in rabbits of the experimental group compared to the control group, where no changes in hematological parameters were recorded. By day 60 of the study, the hemoglobin concentrations of the experimental and control animals were nearly equal and remained stable until the end of the observation period.

Analysis of the leukocyte formula results in both groups at later stages after infection showed that, by day 60 post-inoculation of the biological material, there was an increase in the number of leukocyte fraction cells (up to 9.1 ± 0.7×10^9/l), due to elevated numbers of band neutrophils and lymphocytes (Table 1).

Table 1 — Dynamics of leukocyte fraction cell concentration at later stages after rabbit infection

Observation period, days	Leukocyte fraction cells, ×10^9/l							
	Segmented neutrophils		Band neutrophils		Basophiles		Lymphocytes	
	Experiment	Control	Experiment	Control	Experiment	Control	Experiment	Control
15	29.4 ± 2.6	25.4 ± 2.6	4.5 ± 1.2	5.6 ± 1.2	1.4 ± 0.5	1.3 ± 0.2	48.3 ± 4.0	47.6 ± 4.0
30	26.6 ± 3.3	23.4 ± 3.3	3.6 ± 1.4	3.8 ± 1.5	0.8 ± 0.4	2.2 ± 0.3	59.1 ± 3.0	52.2 ± 3.0
45	19.4 ± 2.6	27.1 ± 1.9	4.5 ± 1.1	4.1 ± 0.7	0.5 ± 0.3	3.2 ± 0.6	67.3 ± 4.0	49.4 ± 5.0
60	18.7 ± 2.2	24.6 ± 2.4	5.8 ± 1.4	4.3 ± 1.1	0.8 ± 0.4	2.6 ± 0.5	85.2 ± 5.0	51.3 ± 4.0
75	21.2 ± 3.5	26.4 ± 3.7	3.8 ± 1.5	3.2 ± 0.2	0.9 ± 0.4	1.8 ± 0.3	80.6 ± 3.0	47.6 ± 3.0
90	23.7 ± 2.3	30.3 ± 2.1	3.3 ± 1.2	4.4 ± 1.2	1.6 ± 0.2	1.6 ± 0.2	81.8 ± 4.0	49.1 ± 5.0
105	21.9 ± 3.9	32.5 ± 2.9	3.9 ± 0.7	5.5 ± 1.3	0.8 ± 0.3	1.8 ± 0.3	88.4 ± 3.0	50.6 ± 2.0
120	20.8 ± 2.7	33.4 ± 2.7	4.1 ± 1.1	5.9 ± 1.3	1.2 ± 0.4	1.2 ± 0.1	67.2 ± 5.0	49.4 ± 6.0
135	20.8 ± 2.7	32.4 ± 2.7	4.0 ± 1.1	5.7 ± 1.3	1.1 ± 0.4	1.3 ± 0.1	68.2 ± 5.0	49.4 ± 6.0

As shown in Table 1, the number of lymphocytes began to increase as early as 30 days after inoculation of the biological material, reaching a peak value of 88.4 ± 3.0×10^9/l, and gradually decreased by day 135 (the end of the observation period) to 68.2 ± 5.0×10^9/l. It should be noted that by day 30 after the experimental infection of rabbits, most hematological parameters in the blood of the experimental group (leukocytes, neutrophils, basophils, ESR) returned to their baseline levels. This indicates that the presence of BLV in the rabbits' bodies does not significantly affect their hematological parameters. However, a notable shift in the leukocyte fraction composition — toward a marked increase (up to 80–88%) in the proportion of lymphocytes — suggests the development of an immunosuppressive state in the experimental animals. Additionally, during this period, the erythrocyte sedimentation rate (ESR) increased to 3–4 mm/hour.

Serological and molecular genetic analyses revealed that the genetic material of BLV was detected in two rabbits from the experimental group as early as 15 days after the start of the experiment. Later, at 30–45 days, BLV genetic material was detected in four experimental animals, and it continued to be present throughout the 120-day observation period. On day 135, a negative result was obtained in one of the four previously infected rabbits, in which BLV genetic material had previously been identified in blood samples. The results of serological and molecular-genetic tests of biological material (serum and stabilized blood) from animals in the experimental and control groups are presented in Table 2.

Table 2 — Results of serological and molecular-genetic studies of biological material from rabbits

Group	Method	Observation period after infection, days								
		15	30	45	60	75	90	105	120	135
Experimental n = 5	AGID	0/5	0/5	0/5	1/5	2/5	3/5	3/5	4/5	3/5
	PCR	2/5	3/5	4/5	4/5	4/5	4/5	4/5	4/5	3/5
Control n = 5	AGID	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
	PCR	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

As shown in Table 2, the presence of antibodies to BLV was detected in one experimental animal 60 days after the start of the study. Subsequently, antibodies to

bovine leukemia virus (BLV) were identified in two animals (on day 75), three animals (on days 90–105), and four animals (on day 120). These findings correlate with the hematological results, particularly the development of lymphocytosis beginning on day 60 of observation, which is characteristic of the typical infectious process associated with bovine leukemia.

Thus, it has been experimentally proven that the bovine leukemia virus is capable of crossing the species barrier and inducing an infectious process in heterologous animal species, specifically in rabbits. This opens the possibility of using rabbits as a model for studying the leukemia virus *in vivo*. Furthermore, considering the susceptibility of other animal species to BLV and the virus's potential to overcome interspecies barriers (even under artificial conditions), their possible role in the epizootic process of leukemia should also be considered.

Conclusions. 1. Inoculating rabbits with biological material containing the bovine leukemia virus (BLV) results in the persistence of the pathogen in 60% of experimental animals, as confirmed by molecular-genetic and serological studies.

2. BLV persistence does not cause significant hematological changes in rabbits. However, it leads to a redistribution of leukocyte subpopulations toward marked lymphocytosis. These changes correlate with serological findings, indicating the development of an infectious process and an immunosuppressive state in the rabbits.

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STUDY OF BIOLOGICAL PROPERTIES OF SOME SPECIES OF ATYPICAL MYCOBACTERIA IN GUINEA PIGS

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Summary. As the eradication of tuberculosis in farm animals progresses, the importance of atypical mycobacteria (AM) and various types of mycobacteriosis is becoming more significant. These mycobacteria can sensitize animals to tuberculin and, in some cases, cause tuberculosis-like lesions, complicating the implementation of anti-tuberculosis measures. The study aimed to assess the persistence of *M. scrofulaceum*, *M. avium*, and *M. phlei* in guinea pigs after single and three oral administrations, in comparison to *M. bovis*. It also examined their ability to cause sensitization to allergens and the duration of this effect based on bacterial load and elimination rates. Results indicated that the persistence of *M. avium*, *M. scrofulaceum*, and *M. phlei* in guinea pigs was temporary following oral administration. These bacteria caused sensitization but did not lead to the development of an infectious pathological process. After three administrations, compared to a single administration, the excretion time of *M. avium* and *M. scrofulaceum* in feces increased from 15 days to 30 days (*M. phlei* remained 15 days). Additionally, the allergic response to the allergens from atypical mycobacteria extended from 60 days to 90 days (for *M. phlei*, it increased from 30 days to 60 days). The persistence of *M. bovis* was a permanent colonization, the excretion of the pathogen in the feces occurred after the dissemination of the pathological process, i. e., in the later stages of the disease, the allergic state persisted for up to 90 days. The duration of the allergic state, persistence, and elimination depended on the bacterial load and the type of mycobacteria

Keywords: persistence, sensitizing, allergic state, pathogenicity

Introduction. The main method of *in vivo* diagnosis of tuberculosis in farm animals is the intradermal tuberculin test (PPD) for mammals, which is the most informative test in the system of early diagnosis of tuberculosis. It is known that not only tuberculosis pathogens but also certain types of atypical mycobacteria (AM), which have antigens closely related to pathogenic species, are capable of sensitizing a macroorganism to tuberculin (Jenkins et al., 2018). Detection of animals with non-specific reactions to tuberculin leads to unwarranted slaughter of healthy animals, causing economic losses to livestock farms. The phenomenon of para-allergies in human and veterinary medicine is widespread and has a steady tendency to increase, as evidenced by the literature (Zavgorodnii et al., 2018, 2021; Zavgorodniy et al., 2021, 2023; Pavlik, Ulmann and Falkinham, 2022), and Ukraine is no exception in this regard. Thus, in tuberculosis-free livestock farms, 0.02–0.03% of animals reacting to tuberculin are detected annually.

According to official data, in 2024, allergic tests identified reactive animals on 14 farms in Vinnytsia, 3 — in Zhytomyr, 5 — in Kyiv, 7 — in Khmelnytskyi, 16 — in Cherkasy, 1 — in Sumy, 1 — in Volyn, and 1 — in Chernihiv regions. However, none of these animals were confirmed to have tuberculosis through necropsy or bacteriological methods.

At present, there are more than 160 species of AM recognized. AM are classified as saprophytes, symbionts, and commensals. They are isolated from the environment, water, soil, and biofilm, meaning that sensitized animals may potentially be exposed to high concentrations of bacteria (up to 10^6/mg CFU) over an extended period, which can result in the development of infection or the induction of an immune response (Falkinham, 2021). In the field of humane medicine, there has been a notable rise in the isolation and diagnosis of mycobacteriosis cases worldwide, though the reasons for this trend are not fully understood. However, the development of new methods, particularly in the areas of molecular biology and diagnostics, has played a significant role in the detection and identification of new AM species. Furthermore, it has been observed that as the number of tuberculosis cases decreases, the number of AM infections increases (Falkinham, 2021; Pavlik et al., 2022). The most common clinical manifestations of mycobacteriosis in humans include lung lesions caused by *Mycobacterium avium* complex (MAC) (Pavlik, Ulmann and Falkinham, 2022; Zhurilo, Barbova and Sladkova, 2020; Heifets, 2004; Field and Cowie, 2006; Mourad, Baker and Stout, 2021; Kim et al., 2021; Park, Kang and Choi, 2021), lymphadenitis (*M. scrofulaceum*), and skin diseases. In addition, there are cases of soft tissue and bone infections, as well as disseminated diseases caused by *M. ulcerans, M. marinum, M. fortuitum, M. abscessus*, and *M. chelonae* (Griffith, 2007; Goldstein et al., 2019; Lobo and Lun, 2021; Trčko, Plaznik and Miljković, 2021; Hendrikx et al., 2022).

In veterinary medicine, AM are also of great clinical importance (Weese and Gomez-Nieto, 2016; Silva et al., 2019; Mönki et al., 2016; Li et al., 2023). The majority of mycobacteria pathogenic in farm animals (artiodactyls, ungulates, pigs, poultry, rabbits), as in human medicine, belong to MAC (Harris and Barletta, 2001; Hewes et al., 2005; Agdestein et al., 2012; Thorel, Huchzermeyer and Michel, 2001; Manning and Collins, 2001), and

M. genavenze is most commonly detected in exotic birds, especially parrots.

The detection of AM in biological samples from animals cannot be the basis for the diagnosis of tuberculosis. To determine the clinical significance of a particular type of AM, it is necessary to study its biological properties when repeatedly introduced into the body of animals. It is known that the development of tuberculosis is based on the intracellular persistence of mycobacteria. Long-term persistence in a macroorganism is of epidemiological importance as a mechanism of the infectious process. However, the temporary presence of mycobacteria in the body should be distinguished from their permanent colonization. The latter may indicate a possible invasion of tissues with the possibility of further development of the pathological process (Griffith et al., 2007).

Based on the foregoing, the study of the terms of persistence of AM in the macroorganism and their ability to cause sensitization to allergens, the rate of excretion, and the duration of the allergic state depending on the bacterial load is of great practical importance in determining the epizootic situation in livestock farming.

The study **aimed** to assess the persistence of *M. scrofulaceum*, *M. avium*, and *M. phlei* in guinea pigs after single and three oral administrations, in comparison to *M. bovis*. It also examined their ability to cause sensitization to allergens and the duration of this effect based on bacterial load and elimination rates.

Materials and methods. For infection of laboratory animals (guinea pigs), the most common species isolated from cattle, poultry, and environmental objects were used, namely atypical cultures of groups II and III according to the Runyon classification — *M. scrofulaceum* and *M. avium* and a fast-growing culture of group IV — *M. phlei*. In addition, *M. b*ovis was also used in the experiments for comparison. The mycobacterial cultures belong to the collection of mycobacterial cultures of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Kharkiv, Ukraine). Suspensions of AM and *M. bovis* were administered to guinea pigs *per os*, by single and triple administration.

From healthy, previously non-reactive to allergens (PPD tuberculin for mammals and AAM) guinea pigs, seven experimental groups were formed (10 animals per group). Group I animals received a single dose of a suspension of *M. bovis* at a concentration of 1.0 mg/cm^3 of sterile isotonic solution. Animals in groups II–IV received suspensions of *M. scrofulaceum, M. avium, M. phlei* cultures at a concentration of 5.0 mg of bacterial mass in 1.0 cm^3 of sterile isotonic solution. Guinea pigs in groups V–VII were given the same AM cultures, but three times at a dose of 5.0 mg/cm^3 with an interval of two days. Thus, guinea pigs in groups V–VII received a total of 15.0 mg of bacterial mass from each AM culture. Animals in the control group (n = 3) received saline (*per os*).

Preparation of suspensions of mycobacterial cultures for inoculation. For the preparation of suspensions, mycobacterial cultures in the logarithmic growth phase grown on Pavlovskiy potato medium were used: *M. bovis* strain Vallee, *M. scrofulaceum* and *M. avium* after 30 days of cultivation, and the fast-growing group IV culture *M. phlei* after 5 days of cultivation. The suspension for infection was prepared as follows: the bacterial mass of each mycobacterial culture was added to a pre-weighed sterile vial containing beads and weighed. The difference between the first and second weighs determined the amount of bacterial mass. Sterile saline was then added to the vials containing atypical cultures at a rate of 1.0 cm^3 of solution per 5.0 mg of bacterial cells, and a concentration of 1.0 mg/cm^3 was prepared for *M. bovis*. The vials containing the bacterial mass were vortexed to a homogeneous suspension.

Allergic study. Mycobacterial allergens were used to determine the state of delayed-type hypersensitivity to the injected mycobacterial cultures in guinea pigs: PPD tuberculin for mammals in a standard solution and AAM, according to the 'Guidelines for the Diagnosis of Animal and Poultry Tuberculosis' (MDVMSVIMAPFU, 1994). Animals were examined 30, 60, and 90 days after administration of the cultures. Allergens were injected intradermally, tuberculin (PPD) for mammals at a dose of 25 IU/0.1 cm^3, AAM — 10 U/0.1 cm^3. The reactions were recorded after 24 h by measuring two diameters of erythema and the area (mm^2) was determined by the formula: $S = \pi r^2$.

After 30, 60, and 90 days of recording allergic reactions to the intradermal tuberculin test, three animals from each group were euthanized for pathological and bacteriological examination.

Bacterioscopic examination of feces. After 15, 30, 60, and 90 days, fecal samples were collected from each group of animals in sterile plastic containers. Sterile distilled water was added to the feces and stirred, large particles were allowed to settle, and the top layer was removed and applied to three slides. After the drops dried, the smears were stained using the Ziehl–Nielsen method.

Cultural examination of feces and pathological material. Fecal decontamination was performed using a 0.9% solution of cetylpyridinium chloride (CPC) with an exposure time of 20 h. Fecal samples were poured with distilled water, stirred, and allowed to settle for 15–20 min. From the supernatant, 10.0 cm^3 of liquid was collected into centrifuge tubes and centrifuged at 3,000 rpm for 15 min. To the precipitate, 10.0 cm^3 of 0.9% CPC solution was added, stirred, and kept at room temperature for 20 h. After exposure to the decontamination solution, the fecal samples were washed with distilled water by centrifugation at 3,000 rpm. The supernatant was discarded, and the precipitate was resuspended in a small amount of 0.85% sodium chloride solution and inoculated into 10 test tubes containing egg nutrient medium for mycobacterial culture.

The organs (liver, spleen) removed after necropsy were treated with 5.0% sulfuric acid. For this purpose, the crushed organs were rubbed with sterile sand, poured

with sterile distilled water, stirred, and allowed to settle for 15–20 min. From the supernatant, 10.0 cm^3 of liquid was taken into centrifuge tubes and centrifuged at 3,000 rpm for 15 min. To the precipitate, 10.0 cm^3 of 5.0% sulfuric acid was added, stirred, kept at room temperature for 10 min, and centrifuged at 3,000 rpm for 15 min. The precipitate was washed by centrifugation, resuspended, and inoculated into 10 tubes with nutrient medium. The cultures were incubated in a thermostat at a temperature 37.5 ± 0.5°C.

Animals that died during the experiment and euthanized after 90 days were pathologically examined for tuberculosis.

Experiments on guinea pigs were conducted following the recommendations of 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'.

Results and discussion. ***Bacterioscopic and cultural examination of feces.*** According to the results of microscopy (Table 1), it was found that 15 days after a single inoculation of AM (dose 5.0 mg), single (2 ± 1 per 100 fields of view) acid-fast bacilli (AFB) were detected in the feces of animals. A large number of clusters and single AFB (12 ± 3) and (20 ± 4), (13 ± 4) per 100 fields of view, respectively) were detected in animals that received a triple dose of *M. scrofulaceum, M. avium, M. phlei*). In guinea pigs infected with *M. bovis*, no mycobacteria were detected in feces collected during this period (Fig. 1).

After 30 days, no mycobacteria were detected by microscopy of feces from animals inoculated with AM at a dose of 5.0 mg. However, in the feces of guinea pigs receiving a triple dose (15 mg total), acid-fast rods *M. scrofulaceum* (5 ± 2) and *M. avium* (15 ± 3) were revealed.

The data obtained indicate that in the first two weeks, there is an active excretion of atypical mycobacteria from the macroorganism in the feces, and with an increase in the multiplicity (3 times) of the same dose (5 mg) in the body of animals, it was expected to increase the amount of excretion of AFB in the feces, which was confirmed by microscopy. It should be noted that the excretion and thus the detection of AFB in the feces decreased over time. Thus, after 30 days in the feces of guinea pigs that received 5 mg of bacterial cells once, no AFB was detected by microscopy, but with 3 times the same dose, AFBs were observed only in slow-growing cultures of *M. scrofulaceum* and *M. avium*), the saprophyte *M. phlei* was not detected by microscopy during this period.

On the 60th and 90th days after AM inoculation, no acid-fast bacilli were observed in any group of guinea pigs by fecal microscopy, but single AFB (2 ± 1/100 fields of view) were detected in the feces of some animals infected with *M. bovis*. Thus, after administration of a triple dose of *M. scrofulaceum* and *M. avium* to guinea pigs, their temporary presence in the body was observed for 30 days. In the case of *M. bovis*, to which guinea pigs are very sensitive, persistence was a permanent colonization with bacterial growth at the site of adhesion to a critical concentration that can cause pathological effects.

Differences in the timing of the excretion of AM and *M. bovis* in the feces indicate that the biological activity of mycobacteria of different species in the body of animals, particularly guinea pigs, is not the same. The results of the bacterioscopic study show that in guinea pigs infected by the alimentary route, the excretion of *M. bovis* pathogen in the feces occurred after the dissemination and spread of the pathological process, i. e., at later stages of the disease. At the same time, AM was excreted from the body in the first weeks.

All fecal samples collected were treated with cetylpyridinium chloride and inoculated onto a dense nutrient medium for mycobacterial cultivation. According to the results of the culture study (Table 2), initial cultures of AM were isolated from feces collected 15 days after inoculation from animals that received a triple dose (15 mg), with inoculated tubes accounting for 10–20% of the samples. Only *M. avium* was isolated from feces collected 30 days after triple inoculation of animals (10% of tubes) (Fig. 2). That is, a sufficiently high dose of AM infection, resulting in active shedding of mycobacteria in the feces during the first two weeks and for *M. avium* during 30 days, led to a positive cultural test result.

After 60 and 90 days of AM inoculation, no growth of cultures from fecal samples was observed in animals from any group of guinea pigs.

In the *M. bovis*-infected guinea pigs, the initial culture was isolated only from the fecal sample collected after 90 days (Table 2).

Allergic study. The results of the allergy study are presented in Table 3. Thus, in the allergy study, 30 days after a single feeding of mycobacterial cultures, all animals except those in the *M. phlei* group reacted to AAM and PPD, but some guinea pigs reacted to both allergens. In the *M. scrofulaceum* sensitized group (n = 10), one of three animals reacting to AAM reacted to PPD, in the *M. avium* group (n = 10) five animals reacted to AAM, two of them to both allergens, in the *M. phlei* group only one animal reacted to AAM. No non-specific reactions to PPD were detected after 60 and 90 days.

It should be noted that the intensity of reactions (area of erythema) in the groups of animals sensitized with *M. avium* and *M. scrofulaceum* was significantly higher for AAM (8.9 and 6.3 times, respectively) than for mammalian PPD. At the same time, in animals infected with *M. bovis*, allergic reactions were more intense to the administration of PPD (8.7 times).

Table 1 — Results of microscopy of guinea pig feces

Mycobacteria species	Fecal swabs (n = 3) after one time infection of animals, in days				Fecal swabs (n = 3) after three times infection of animals, in days			
	15	30	60	90	15	30	60	90
M. scrofulaceum	+/–/+	–/–/–	–/–/–	–/–/–	+/+/+	+/–/+	–/–/–	–/–/–
M. avium	–/+/+	–/–/–	–/–	–/–/–	+/+/+	+/+/+	–/–/–	–/–/–
M. phlei	+/+/–	–/–/–	–/–/–	–/–/–	+/+/+	–/–/–	–/–/–	–/–/–
M. bovis	–/–/–	–/–/–	+/–/+	+/+/+	Not infected			

Notes: '–' — AFB not detected; '+' — AFB detected.

Table 2 — Results of the fecal cultural examination

Mycobacteria species	Growth of initial cultures (one time infection of animals) from feces collected, in days				Growth of initial cultures (three times infection of animals) from feces collected, in days			
	15	30	60	90	15	30	60	90
M. scrofulaceum	–	–	–	–	+	–	–	–
M. avium	–	–	–	–	+	+	–	–
M. phlei	–	–	–	–	+	–	–	–
M. bovis	–	–	–	+	Not infected			

Notes: '–' — culture is not isolated; '+' — culture is isolated.

Table 3 — Results of the guinea pig allergy study

Mycobacteria species	Animals reacted, in days					
	30		60		90	
	10 animals in the group		6 animals in the group		3 animals in the group	
	PPD	AAM	PPD	AAM	PPD	AAM
	Average erythema area, mm^2/number of reacting animals					
Single sensitization						
M. scrofulaceum	3.7/1*	23.5/3	–	19.6/2	–	–
M. avium	4.2/2*	37.7/5	–	28.2/3	–	–
M. phlei	–	9.2/1	–	–	–	–
M. bovis	24.4/4	2.8/2*	185.6/6	3.4/1*	219.6/2	–
Triple sensitization						
M. scrofulaceum	5.8/5*	92.4/9	8.8/2*	153.2/6	–	10.2/2
M. avium	6.2/8*	108.8/9	10.8/5*	197.8/6	–	24.0/3
M. phlei	4.7/3*	76.6/9	3.6/2*	37.7/5	–	–
M. bovis	Not infected					

Note: * — reacted to both allergens.

After 60 days, in guinea pigs sensitized with *M. avium* (n = 6) and *M. scrofulaceum* (n = 6), the state of delayed-type hypersensitivity (DTH) to AAM persisted in three and two animals, respectively. On day 90, none of the animals sensitized with AM showed DTH.

In guinea pigs infected with *M. bovis*, responses to PPD were observed on days 30, 60, and 90, and the intensity of the immune response increased with time. For example, the average erythema area was 219.6 mm^2 on day 90 after infection, which was 9 times larger than the erythema area 30 days after infection (24.4 mm^2). In addition, some animals (1–2 individuals) reacted to AAM in the allergy studies at 30 and 60 days.

According to the results of the allergy study, it was found that three times AM feeding led to allergization of all experimental animals. This was manifested by an increase in the intensity of reactions to both allergens. Thus, after 30 days, in animals sensitized three times, the average area of erythema to AAM administration increased 3.9 times (*M. scrofulaceum*) (Fig. 3), 2.9 times (*M. avium*) (Fig. 4), 8.3 times (*M. phlei*) compared to the reactions observed with a single administration. After 60 days, reactions to AAM in animals sensitized once and three times with *M. avium* and *M. scrofulaceum* differed by almost seven times. It should be noted that the peak intensity of reactions to AAM after a single injection of *M. avium*, *M. scrofulaceum,* and *M. phlei* occurred on day 30, after three times sensitization of animals with *M. avium, M. scrofulaceum* — on day 60, in animals infected with *M. bovis* — on day 90. The lowest

intensity of reactions to allergens was observed in guinea pigs sensitized with *M. phlei* during the whole period of the experiment. In addition, it was found that the number of animals with non-specific mammalian PPD tuberculin reactions increased as the bacterial load increased. Thus, after 30 days, the number of animals reacting to PPD triple-sensitized with *M. scrofulaceum, M. avium*, and *M. phlei* increased to 5, 8, and 3, respectively, and after 60 days, their numbers decreased to 2, 5, and 2, respectively. After 90 days, no mammalian tuberculin (MTB) reaction was observed in the animals.

No reactions were observed in guinea pigs in the negative control group throughout the experiment. It should be noted that two guinea pigs infected with *M. bovis* culture died after 58 and 74 days, and another guinea pig infected with *M. avium* once died on day 63.

Pathologic examination and cultural examination of biomaterial. After 30, 60, and 90 days, three animals of each group were examined pathologically for macroscopic pathological tuberculosis changes, and the biomaterial collected from them was tested for tuberculosis by the cultural method.

There is conflicting data in the scientific literature regarding the pathogenicity of AM in guinea pigs, particularly *M. avium*. For example, Gomez-Buendia et al. (2024) found granulomatous lesions similar to tuberculosis in a postmortem examination of a guinea pig experimentally infected with *M. avium*. The lesions were observed in the pre-lobar and mediastinal lymph nodes. After seeding the pathologic material, they isolated the initial culture (Gomez-Buendia et al., 2024). In another study of three cultures of *M. avium* isolated from cattle, it was found that all three strains were pathogenic to guinea pigs and caused local lesions in the spleen and lungs with varying degrees of edema and hemorrhage. Microscopy of lung and spleen tissue sections from these infected guinea pigs showed scattered infiltration of red mycobacteria (Xin et al., 2022). In the above studies, guinea pigs were infected parenterally by intramuscular injection of *M. avium*. In our study, we considered the situation closest to the natural one, i. e., the ability of AM to cause pathological lesions in the body of guinea pigs under the alimentary method of infection with different bacterial loads.

According to the results of pathological examinations 30, 60, and 90 days after infection, no macroscopic tuberculous changes in the organs were detected in any animal, regardless of the frequency of AM administration by the dietary route. Culture examination of organs from all animals receiving AM did not isolate the initial cultures.

It should be noted that one guinea pig that received *M. avium* only once died on day 63. According to the necropsy results, no macroscopic lesions characteristic of tuberculosis were found, but hyperemia and exudate were noted in the lungs. The lungs were enlarged, and some areas were dark purple-red. Bacteriologic examination of biomaterial (spleen, liver, lungs) did not reveal mycobacteria. The exact cause of death was not determined, but the animal likely died of pneumonia.

Necropsy results of guinea pigs infected with *M. bovis* showed that pathologic changes in organs were observed in animals that died during the experiment and in those that were euthanized after 60 and 90 days. The largest tuberculous lesions were observed in the liver, spleen, and inguinal lymph nodes 90 days after infection. These organs were enlarged 1.5–2 times, the liver had areas of hyperemia and multiple gray-yellowish nodules of various sizes (Fig. 5). The presence of tuberculous granulomas was also observed in the inguinal lymph node, from which a caseous-necrotic mass protruded at the incision (Fig. 5). A large granuloma with caseous-necrotic contents was found on the surface of the spleen (Fig. 6). In the intestine and abdominal cavity, the presence of fluid, thickening of the mucous membrane with single small gray nodules was observed. After decontamination and seeding of pathological material on dense mycobacterial culture medium for 16–18 days, the growth of the first colonies characteristic of *M. bovis* was detected (Fig. 7).

Thus, with a single intake of *M. scrofulaceum, M. avium, M. phlei* into the body of guinea pigs by the alimentary route, their excretion in the feces was most active in the first 15 days, which was recorded by microscopy. When the same dose was administered three times, the period of slow-growing *M. scrofulaceum* and *M. avium* excretion in the feces increased to 30 days, and the amount of mycobacteria in the feces was sufficient to isolate them on the nutrient medium. The saprophyte *M. phlei* was not detected by microscopy during this period, regardless of the dose. On the contrary, *M. bovis* was excreted in feces at later stages of generalization of the infectious process.

Single and triple sensitization with *M. scrofulaceum, M. avium, M. phlei* induced a delayed hypersensitivity state in guinea pigs, the duration of which depended on the bacterial load and the type of mycobacteria. The saprophytic fast-growing culture of *M. phlei* caused a short-organism increased the intensity of reactions to AAM by 2.9–8.3 times in 100% of animals. The duration of the allergic state in animals sensitized with *M. scrofulaceum* and *M. avium* cultures three times (separately) lasted up to 90 days, in some animals sensitized with *M. phlei* — not more than 60 days. In addition, the percentage of animals responding to the mammalian PPD increased.

Animals infected with *M. bovis* remained positive to mammalian tuberculin for 90 days.

Conclusions. The issues of para-allergy, epizootic, and clinical significance of atypical mycobacteria, and their ability to cause mycobacteriosis can be resolved with an integrated approach, using a simultaneous test, bacteriological examination with identification of isolated mycobacteria and biological test, taking into account the bacterial load and duration of exposure.

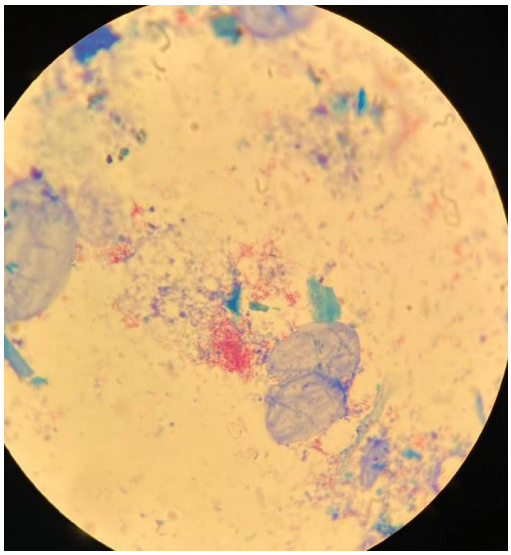

Figure 1. Acid-fast bacilli *M. avium* in feces.

Figure 2. Growth of *M. avium* from feces.

Figure 7. Growth of *M. bovis.*

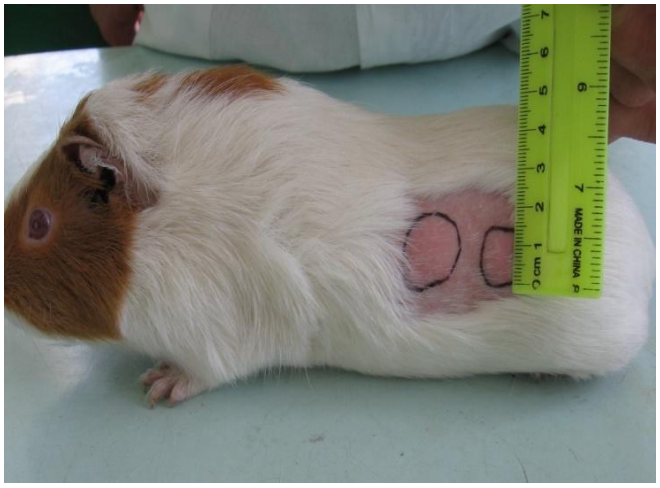

Figure 3. Triple sensitization (*M. scrofulaceum*): left erythema — reaction to AAM, right — to PPD.

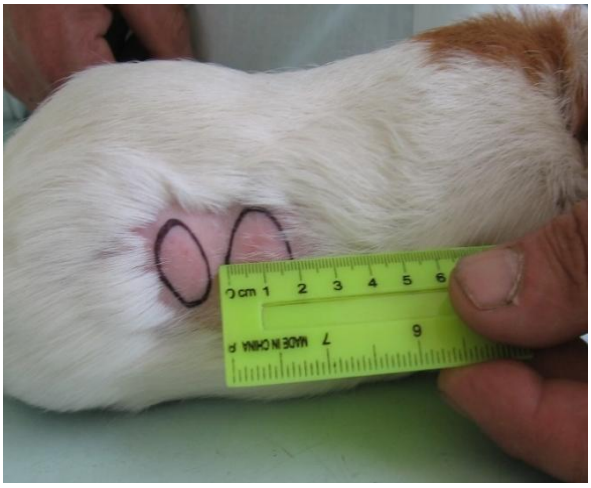

Figure 4. Triple sensitization (*M. avium*): left erythema — reaction to PPD, right — to AAM.

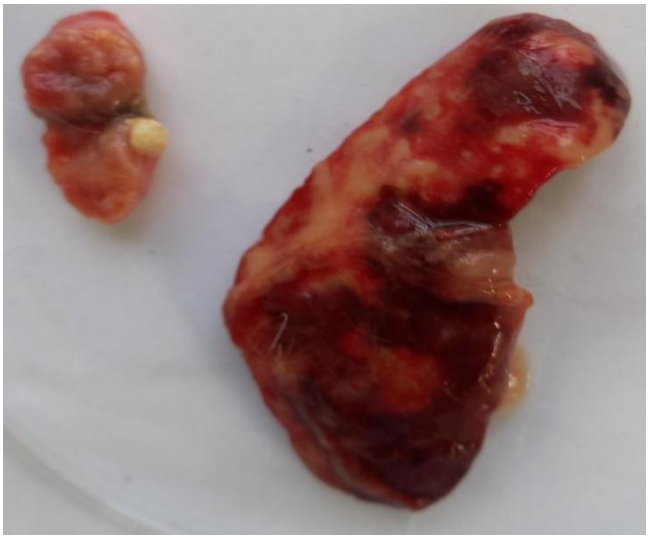

Figure 5. Lymph node and a piece of liver.

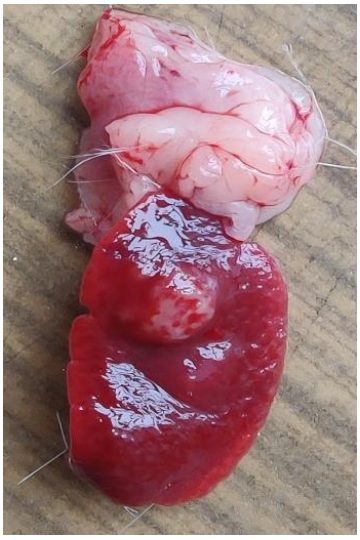

Figure 6. Spleen.

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Part 2. Biosafety

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BIOSAFETY IN THE HOUSING ENVIRONMENT AS A FACTOR FOR COMPREHENSIVE PREVENTION OF METABOLIC SYNDROME IN HORSES

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Summary. The aim of this study was to evaluate the effectiveness of a comprehensive biosecurity program in the prevention of equine metabolic syndrome, specifically focusing on the use of biocide complexes in horse housing environments and their impact on both the microclimate and the horses' health. Materials used included biocides such as 'DZPT-2', based on glutaraldehyde, and 'Yodesol' (iodine-based), 'Geocid' (benzalkonium chloride and deltamethrin-based), which were applied during the disinfection and decontamination processes in horse stables. Methods involved a combination of biosecurity measures, including disinfection procedures and the monitoring of microbial and parasitic loads in the stables. Additionally, the effects of these treatments on horses' immune systems were assessed. The results indicated that the application of the mentioned biocides significantly improved the microclimate within the horse stables, reducing microbial load without negatively impacting the horses' non-specific immunity. The study confirmed that these biocides do not have adverse effects on the general health of the horses, and their use was associated with a noticeable improvement in the overall environmental conditions. The findings support the notion that maintaining proper hygiene and implementing comprehensive biosecurity measures can effectively reduce the risk of infections and contribute to the prevention of metabolic disorders such as metabolic syndrome in horses. In conclusion, the study demonstrates the importance of biosecurity practices, including regular disinfection and the use of effective biocides, in ensuring the health and well-being of horses and preventing metabolic complications associated with poor environmental conditions

Keywords: disinfection, microbiological load, immunity, metabolic disorders

Introduction. Disinfection is an important part of preventing and controlling infectious diseases in horses. It reduces the risk of spreading pathogens that cause diseases such as ringworm, streptococcal, and other infections. Disinfection includes cleaning equipment, facilities, and animals, which reduces the risk of pathogen transmission (Halatiuk, 2009; Kryvoshyia, 2013).

Disinfection is quite widespread in horse breeding. For instance, equine dentistry requires adherence to hygiene standards because equipment can be difficult to disinfect. After procedures, the number of bacteria on equipment remains high, indicating the need for effective disinfection methods. Studies have detected bacteria in varying amounts on dental equipment after cleaning or disinfection, indicating a risk of spreading infections during use (Alsing-Johansson et al., 2021; Verwilghen and Weese, 2021).

Eye antiseptics: Studies have shown that 0.1% polyhexamethylene guanidine is more effective than 0.2% povidone iodine in reducing bacterial load during ophthalmic procedures in horses. When disinfecting hooves, colloidal iron is added to chlorhexidine, which significantly increases the effectiveness of hoof disinfection by reducing the bacterial load (Isola et al., 2021).

General recommendations are especially important: keeping clean, hand hygiene, avoiding stress, regular deworming, and vaccination are the main preventive measures in stables. All new or returning animals should be quarantined as an important preventive measure. Repeated hand washing and disinfection can prevent the spread of infectious agents to humans and horses (Hopka, Khomenko and Pavlenko, 2004; Sinitsyn et al., 2013).

When keeping horses, disinfection measures are aimed at preventing and controlling infectious diseases such as equine influenza, which is highly contagious and transmitted by airborne droplets. Glanders is a bacterial disease that can also affect humans. Leptospirosis is a zoonotic infection that can be transmitted through water and wet bedding. Salmonellosis is a bacterial gastrointestinal disease. Pasteurellosis often occurs in poor housing conditions. Purulent skin and hoof infections can be caused by staphylococci and streptococci. Mycoses, or fungal diseases, include trichophytosis, also known as ringworm (Halatiuk, 2009; Nedosiekov et al., 2021; Ponomarenko et al., 2021).

Disinfecting the premises helps reduce the risk of infection, especially in stables with a large number of animals. Disinfection is carried out both preventively

and after the disease is detected (Kovalenko, 2017; Paliy et al., 2024a).

The impact of disinfection on the development of internal non-contagious pathologies, such as laminitis, is also known. Laminitis is a multifactorial disease of the hoof plate that develops against the background of systemic disorders, including metabolic syndrome, endotoxemia, hormonal imbalance, and disruption of the body's microbial homeostasis (Tuniyazi et al., 2021). Environmental stressors, such as high concentrations of ammonia, hydrogen sulfide, fungal toxins, and pathogenic bacteria, are known to hurt homeostasis and contribute to the development of chronic hoof inflammation (Peters, Nawrot and Baccarelli, 2021).

Equine metabolic syndrome is a pathological condition characterized by obesity, insulin resistance, and a high risk of laminitis. It is most often recorded in animals kept in conditions of limited physical activity, with excessive or irrational nutrition (Durham, 2017). At the same time, the role of the gastrointestinal microbiota and endotoxins in the pathogenesis of metabolic syndrome and laminitis is being increasingly studied (Milinovich et al., 2010).

Keeping horses in an unfavorable microclimate contributes to chronic stress, increased cortisol secretion, decreased levels of immunoglobulins (IgA, IgM), and, as a result, reduced natural resistance (Pritchard and Whay, 2010). Under conditions of high microbiological load, the risk of endotoxemia increases significantly, which, according to Noble et al. (2013), is a trigger for the development of laminitis.

In recent years, scientific studies have emphasized the importance of regularly sanitizing horse facilities to maintain hygiene, normalize the microclimate, and reduce the risk of developing metabolic syndrome and related complications. Disinfectants based on essential oils and bioactive complexes, such as Barez, are particularly promising because they are non-toxic, immunomodulatory, and reduce the bacterial load on mucous membranes and skin (Kovalenko et al., 2018).

In horse breeding, phenolic disinfectants are used because they are effective in the presence of organic matter and can control outbreaks of diseases such as rotavirus diarrhea and salmonellosis. Other disinfectants, such as chlorhexidine, iodophores, and alcohol solutions, are also used, but they may be less effective in the presence of organic matter. Effective preventive disinfection of horse housing requires an integrated approach that includes using various disinfectants, complying with hygiene standards, and developing action plans for infectious disease outbreaks. This approach helps reduce the risk of pathogen spread and ensures animal health (Halatiuk, 2009; Nedosiekov et al., 2021).

A hygiene plan should be developed that includes general biosecurity procedures and standard operating procedures for the event of an infectious disease outbreak, a zoonotic disease outbreak, or multidrug-resistant bacterial colonization. Enhanced hygiene measures, including the use of protective clothing, cleaning, disinfection, and isolation of potentially infected animals, should be taken as soon as a disease is suspected. Rapid confirmation of the pathogen by testing appropriate samples is crucial. All safety measures must be adjusted according to the infectivity of the pathogen in question and the main routes of transmission (Paliy et al., 2024b). In addition to locking down the stables, clinic, or showground, it is important to segregate horses. Extensive hygiene measures should be maintained until all animals test negative and do not show clinical signs of disease for a certain period (Kovalenko et al., 2018).

Currently, many disinfectants and antiseptics are used for disinfection in equine clinics, racetracks, and breeding farms, but these antimicrobials are generally not tested for commonly encountered pathogens, and their antimicrobial efficacy is unknown. The antimicrobial efficacy of ethanol, chlorhexidine, povidone iodine, sodium hypochlorite, peroxymonosulfate compound, and benzalkonium chloride was analyzed by the scientists using the quantitative suspension test method against field isolates of *Escherichia coli, Pseudomonas aeruginosa, Salmonella spp, Streprococcus zooepidemicus, Streptococcus equi, Rhodococcus equi*, and *Staphylococcus auerus*, which are the most common pathogens of horses, in the presence of organic load (Mete, 2019; Frees, 2018; Adler et al., 2016).

When choosing disinfectants, their potential impact on workers and animals should be considered, and personnel should be provided with personal respiratory protection (Paliy, 2018). Before using any disinfectant in the presence of horses, the manufacturer's instructions must be carefully read, and a veterinarian must be consulted to avoid possible negative consequences for animal health.

Horses are treated for insects with insecticides and repellents that repel or kill pests such as horseflies, mosquitoes, ticks, and midges. These are applied to the horse's coat and last for several days. These products kill and repel flying and crawling insects. Repellent shampoos and gels have a short-term effect and are often used after training or bathing. Collars containing repellents are easy to use, especially in field conditions, as they release their active ingredients slowly. Regular indoor spraying helps to reduce the insect population (Hopka, Khomenko and Pavlenko, 2004; Paliy et al., 2021; Nedosiekov et al., 2021).

It is always necessary to test the product on a small area of the horse's skin. Do not use the product near the eyes, mucous membranes, or genitals.

Horses are treated for worms with anthelmintics, which can be administered as pastes, gels, tablets, or injections. Such treatments are required at least two to four times a year, depending on the conditions of the facility, the horse's age, and the region. Before deworming, it is advisable to perform a faecal test to determine the type of parasite. The dosage depends on the horse's weight — it is important not to exceed the recommended amount. Preventative treatments are

usually carried out in spring, summer, and autumn. After treatment, it is advisable to monitor the state of the gastrointestinal tract (Kovalenko et al., 2017).

To keep horses at the proper level, it is necessary to follow special procedures and a plan for sanitary work, especially adjusting the rotation of disinfectants during disinfection.

Our work **aims** to evaluate the effectiveness of a set of biosafety measures in preventing metabolic disorders. In particular, we focus on the use of biocides in housing environments and their effect on microclimates and horses' bodies.

Materials and methods. A study was conducted to evaluate the efficacy and safety of three products: 'DZPT-2' based on glutaraldehyde, 'Iodesol' based on iodine, and 'Geocide' based on benzalkonium chloride and deltamethrin. The study took place at the Rymchuk private horse farm in Bashtanka District of Mykolaiv Region, as well as at the Institute of Veterinary Medicine of NAAS in Kyiv and the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' in Kharkiv.

The objects of the study were seven horses of the thoroughbred English breed (first group), which were treated with the biocidal drug 'Iodesol', and seven horses (second group), which were treated according to the scheme with the biocidal drug 'Iodesol' and the drug 'Geocide', and kept indoors. A separate control group of seven horses was formed, which was not treated with any drugs. The animals were selected for the experiments according to the principle of analogous pairs, taking into account age, live weight, sex, and physiological condition. The average age of the animals was 5.4 ± 2.3 years, and their average body weight was 505 ± 45.3 kg. The feeding and housing conditions of the experimental and control groups corresponded to the technological processes adopted on each farm.

The clinical trials were conducted following the recommendations of 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'.

According to the 'insert leaflet', the disinfectant 'DZPT-2' was used to treat enterobacteria, Gram-positive cocci, Gram-negative bacilli and bacilli in an aqueous solution at a concentration of 0.5% for six hours in the animal-free premises.

For preventive purposes, the premises were disinfected in the presence of horses with the drug 'Iodezol' once every two weeks, after thorough mechanical and sanitary cleaning of the surfaces of the facilities. A 0.2% solution of the product was prepared at a rate of 0.3 l of solution per 1 m^2 and left to dry completely for 30 min.

After feeding the horses, the premises and equipment were mechanically cleaned and washed with tap water. In the experimental group, the premises were disinfected once a week using a cold fog generator with the 'Geocide' aerosol method and a 0.5% working solution for a one-hour exposure at a consumption rate of 0.05 l/m^3. Disinfection was carried out with ventilation turned on. Sanitary and hygienic studies were conducted according to current methods.

The study of the general parameters of the indoor microclimate, in particular temperature, humidity, and air velocity, was conducted using an aspiration psychrometer MV 41 L, a weekly thermograph, a hygrometer M 16, a hygrograph M 21, an anemometer wing ASO 13, and a layer catathermometer. The concentration of harmful gases was measured using a universal gas analyser UG 2; the level of carbon dioxide was measured using the Hess method; illumination was measured using a lux meter YU 16 (Kovalenko et al., 2017); dust contamination was measured using the weight method; and microbial contamination was measured using the sedimentation method (Harkavenko et al., 2020).

Flushes were collected twice: once after cleaning and again after disinfection (three hours after treatment). Six samples were taken from each of the test surfaces (floor, walls, windows, machines, and feeders). The cultures were then plated on diagnostic thioglycol medium and MPA. The cultures were then incubated at 37°C for 48–72 h. The colonies that grew on each plate were then counted separately and their average number calculated.

The following were determined in whole blood: haemoglobin concentration by the haemoglobin cyanide method; total leukocytes and erythrocytes by counting under a microscope in a chamber with a Goryaev grid. Biochemical studies were performed according to generally accepted methods, as described in the reference book (Vlizlo, 2012).

Statistical analysis of the data was performed using Minitab 19 and Minitab Inc. Based on the results of statistical processing, the following indicators are presented in the tables: mean ± SD. A significant difference between the study groups was established based on the Mann–Whitney test ($P < 0.05$).

Results and discussion. The results of the studies on the microclimate in the premises of the two experimental groups of horses undergoing treatment with 'Iodezol' at a concentration of 0.2% (group 1), treatment according to a scheme involving the biocidal preparation 'Iodezol' and the preparation 'Geocide' (group 2), and the standard control group are shown in Table 1. The results of the experimental studies revealed that the microclimate indicators differed depending on the room in which the samples were taken. Microbial contamination in stables should not exceed 150 thousand microbial bodies/m^3. Total microbial contamination is determined by a complex association of bacterial, viral, and fungal

microorganisms. The estimated level of contamination before disinfection with 'DZPT-2' was about 352.3–420.3 thousand CFU/cm² of area, as determined by quantitative analysis of serial dilutions of samples. After the premises and equipment in the production areas were disinfected using an aerosol, the number of microorganisms in the analyzed samples decreased significantly.

Table 1 — Microclimate parameters of horse stables after treatment with biocidal drugs (mean ± SD, n = 7)

Characteristics	Animal group		
	control	1st experimental	2nd experimental
Air temperature, °C	14.20 ± 0.11	12.30 ± 0.52	11.30 ± 0.14
Relative air humidity, %.	75.20 ± 3.14	68.30 ± 2.10*	65.20 ± 1.70*
Air velocity, m/s	0.8 ± 0.002	0.9 ± 0.001	0.9 ± 0.001
Ammonia, mg/m³	10.1 ± 0.01	6.50 ± 0.02**	5.20 ± 0.05**
Hydrogen sulfide, mg/m³	5.30 ± 0.02	3.09 ± 0.01**	2.12 ± 0.01**
Carbon dioxide (CO_2), %	0.3 ± 0.0022	0.19 ± 0.001**	0.15 ± 0.004**
Dust, mg/m	8.72 ± 0.08	4.12 ± 0.03**	3.14 ± 0.04**
Artificial lighting, lux	50	50	50
Microbial contamination thousand microbial bodies/m³	180.5 ± 12.1	70.3 ± 1.43**	52.12 ± 0.11**

Notes: * — $P < 0.01$, ** — $P < 0.001$ relative to control indicators.

The problem of microbiological contamination in horse housing includes establishing permissible limits for the number of microorganisms that could negatively impact their health. The lack of standardized norms across different countries makes it difficult to determine specific numerical values. However, research highlights important aspects of microclimate management that mitigate risks associated with microorganisms (Colavita et al., 2016).

Studies analyzing indoor microbial levels, for example, emphasize controlling the accumulation of dust and gases, such as CO_2 and ammonia, which can lower air quality and create favorable conditions for microbial growth (Carrillo Heredero et al., 2024). The type of ventilation, the bedding materials used, and the general cleanliness of the space affect the quality of the microflora (Yarnell et al., 2017).

Another aspect to consider is the diversity and seasonality of microbial contamination, which affect the composition of the microflora. Studies have shown that the number of microorganisms is significantly higher in the summer. This may require a different management approach depending on the season (Witkowska et al., 2012).

Although there are no specific standards for the concentration of microorganisms in equine facilities, general recommendations for managing microclimate conditions, such as ventilation, humidity, and dust control, are essential for reducing the risk of pathogen development and ensuring the health of horses.

Disinfecting with the drug 'Iodesol' effectively reduced microbial contamination in the air in all areas where animals were kept. The number of microorganisms in the air decreased by 61% in the first experimental section and by 71% in the second experimental section, which used a combination of two biocides, 'Iodesol' and, later, 'Geocide'. There was a significant difference ($P < 0.01$).

Table 1 shows that disinfection with the iodine-based product 'Iodesol' and the benzalkonium chloride-based product 'Geocide' has a positive effect on optimizing the main parameters of the microclimate of livestock premises. The relative humidity decreased by 10% and 13%, respectively, in rooms where disinfection was carried out in the presence of horses in the first and second experimental groups ($P < 0.01$).

The air temperature in all experimental horse rooms was between 11°C and 14°C, which is within the normal range. Air velocity ranged from 0.8 to 0.9 m/s, with no significant difference between experimental and control groups.

The concentration of harmful gases (ammonia, hydrogen sulfide, and carbon dioxide) decreased in the indoor air of all experimental groups compared to the control group. The ammonia content in the room of the first experimental group decreased by 36% after using the preparation. The hydrogen sulfide concentration in the room of the second experimental group decreased by 60% after using the two preparations.

The results of the research show that disinfection with a complex of three biocidal preparations contributed to the optimization of the microclimate in the premises for keeping horses. At the same time, a decrease in humidity, as well as the presence of harmful gases (ammonia, carbon dioxide, and hydrogen sulfide), dust contamination, and microbial contamination of the air, was observed.

After 30 days of observation to determine the prolonged effect of the disinfectant complex, 'DZPT-2', 'Iodesol', and 'Geocide', an effective bactericidal effect was revealed, which maintained the microbial background of the facility within the normal range during the study period.

The effectiveness of the drugs is due to their compositions: iodine in 'Iodesol', benzalkonium chloride as a detergent in 'Geocide', and glutaraldehyde in

'DZPT-2'. These components provide the drugs with a long-lasting bactericidal effect against infectious agents.

Experiments show that 'Iodesol' disinfection solutions are effective and economically feasible for preventing respiratory diseases in horses in livestock facilities. The economic benefits of using the disinfectant preparation 'Geocide' for disinfection include reducing the duration of disinfection, the number of staff required, and the cost of drugs due to its multifaceted action (disinfection, disinsection, and disinfestation).

High-quality mechanical preparation of premises for disinfection reduces the amount of solution required per unit area, is cost-effective, and protects the environment from excessive chemical exposure.

Studies of the physiological state of horses in the experimental groups show that, during the observation period, the body temperature of horses increased slightly under the influence of the drugs 'Iodesol' and 'Geocide' by an average of 0.4°C.

A statistically significant increase in respiratory movements was found in the first days of the experiment. In the first experimental group, this increase compared to the control group amounted to five respiratory movements; in the second experimental group, it amounted to three. It should be noted that, at the same time, breathing became deeper. Compared to the control group, pulse beats tended to increase. In particular, the studies noted that, within one hour of applying the drug, the pulse beat frequency increased by an average of 10–15%, and, 1.5–2 h after disinfection, these indicators differed by only 1–2%.

Thus, disinfection with 'Iodesol' and 'Geocide' resulted in a slight increase in body temperature, heart rate, and respiration, which is associated with an increase in the intensity of redox processes in tissues and organs, as well as stress.

Subsequent studies on horses were conducted to investigate the safety of using 'Iodesol' and 'Geocide' for aerosol disinfection on the hematological and immunological parameters of animals. The goal was to assess the safety of the drugs and justify their use in production conditions. Clinical observations and hematological studies (hemoglobin content and number of red and white blood cells) showed that exposure to 0.2% and 0.5% solutions of the biocidal preparations 'Iodesol' and 'Geocide' did not cause changes in hemoglobin content, a decrease in the number of red blood cells, or a decrease in the number of white blood cells compared to the norm for the species and age group and the control group. The results of these studies are shown in Table 2.

The results revealed that, 30 days after the premises were treated with the drugs, positive changes in the animals' blood morphology and biochemistry were observed in the experimental groups.

Thus, the hemoglobin level in the second experimental group increased by 3.5% ($p < 0.05$) compared to the control group. This increase may indicate the activation of hematopoiesis and an increase in oxidation-reduction reactions. The number of red blood cells increased by 9.1% ($p < 0.05$), which indicates an increase in the blood's oxygen capacity.

The decrease in leukocytes by 5.7% indicates an absence of inflammation or decreased immune system load after treatment of the premises. Concurrently, a 7% increase in total protein in the blood suggests increased liver synthetic function or metabolic activation.

ALT activity in experimental group 2 increased by 16.2% ($p < 0.05$) and AST by 17.7% ($p < 0.05$) compared to control, which is within the physiological norm and may reflect the metabolic activity of hepatocytes. Glucose and albumin remained at a stable level, which confirms the absence of hyperglycemia or protein metabolism disorders.

Thus, the results indicate that the drugs 'Iodesol' and 'Geocide' are safe and do not negatively affect the main hematological and biochemical parameters.

Based on the data in Table 3, it was determined that the drugs did not cause inhibition of nonspecific resistance in animals. On the contrary, there was a tendency to increase it. Specifically, the phagocytic activity of leukocytes in the second experimental group increased by 4.3%, reaching 44.2% ($p < 0.01$), indicating the activation of cellular immunity. The bactericidal activity of the blood serum increased by 4.9%, reaching 55.7%, which indicates stimulation of the humoral link of nonspecific immunity. The lysozyme activity in the second experimental group exceeded the control values by 3.3% and the lymphocyte content by 7.4% ($p < 0.01$). This may indicate the stabilization or activation of the adaptive immune response. Compared to control animals, the level of T-lymphocytes increased by 2.6% ($p < 0.05$), and the level of B lymphocytes increased by 10% ($p < 0.01$), confirming the activation of both major lymphocyte populations.

According to the results shown in Table 3, there were no statistically significant differences in total phagocytic activity and phagocytosis intensity among the experimental animals.

Thus, according to the immunopharmacological characteristics of the preparations 'Iodesol' and 'Geocide', they do not exhibit a negative immunotropic effect and have no contraindications for use in sanitizing the premises of a horse breeding complex in the presence of animals.

Therefore, when choosing disinfectants for use in the presence of horses, it is important to consider their effectiveness and safety for animals (Ponomarenko et al., 2021). Phenolic compounds are effective against many bacteria, viruses, and fungi, and they remain active in the presence of organic contaminants.

However, they can be toxic to other animal species, such as cats and pigs, so care should be taken when using them. Iodine-based products (iodophores), such as povidone iodine, are often used to disinfect hands and equipment. They are effective against many microorganisms but may be less suitable for large areas (Kovalenko et al., 2017).

Table 2 — The effect of aerosol treatment of premises with 'Iodesol' and 'Geocide' on the morphological and biochemical parameters of horse blood (mean ± SD, n = 7)

Indicators	Observation period, days	Animal groups		
		control	1st experimental	2nd experimental
Hemoglobin, g/l	1	134.87 ± 10.42	137.51 ± 12.06	140.28 ± 11.39
	15	136.62 ± 9.76	139.08 ± 11.44	141.73 ± 10.27
	30	135.94 ± 10.23	138.36 ± 10.58	140.65 ± 9.88*
Erythrocytes, T/l	1	9.14 ± 1.23	9.33 ± 1.15	9.61 ± 1.08
	15	9.21 ± 1.18	9.45 ± 1.07	9.72 ± 1.02
	30	9.03 ± 1.26	9.51 ± 1.04*	9.85 ± 0.93*
Leukocytes, G/l	1	8.48 ± 0.97	8.29 ± 1.12	8.15 ± 1.19
	15	8.61 ± 1.07	8.12 ± 1.06*	8.04 ± 1.03*
	30	8.39 ± 1.14	7.97 ± 1.09	7.91 ± 1.08
Total protein, g/l	1	69.84 ± 4.12	72.09 ± 3.47	74.18 ± 3.25*
	15	70.97 ± 3.83	73.42 ± 3.19	75.46 ± 2.91
	30	70.23 ± 4.05	73.88 ± 3.36	75.15 ± 2.87
Glucose, mol/l	1	4.91 ± 0.38	5.07 ± 0.36	5.29 ± 0.31
	15	4.86 ± 0.44	5.03 ± 0.41	5.17 ± 0.36
	30	4.94 ± 0.35	5.08 ± 0.39	5.24 ± 0.33
Albumin, %,	1	38.97 ± 2.41	38.52 ± 2.83	37.95 ± 3.08
	15	38.75 ± 2.66	38.18 ± 2.94	38.04 ± 2.65
	30	38.42 ± 2.59	38.03 ± 2.87	37.88 ± 2.91
ALT, mmol×h/l	1	0.76 ± 0.144	0.79 ± 0.180	0.83 ± 0.180
	15	0.72 ± 0.144	0.79 ± 0.144	0.82 ± 0.180
	30	0.68 ± 0.108	0.76 ± 0.144	0.79 ± 0.144*
AST, mmol×h/l	1	1.40 ± 0.216	1.51 ± 0.180	1.58 ± 0.216
	15	1.44 ± 0.252	1.58 ± 0.216*	1.66 ± 0.180
	30	1.47 ± 0.180	1.65 ± 0.180	1.73 ± 0.216*

Note: * — $p < 0.05$ relative to the control indicators.

Table 3 — The effect of aerosol treatment of premises with 'Iodesol' and 'Geocide' on the indicators of nonspecific resistance of horses (mean ± SD, n = 7)

Indicator	Observation period, days	Animal groups		
		Control	1 experimental group	2 experimental group
Phagocytic activity, %	1	41.3 ± 0.65	43.2 ± 0.87	42.8 ± 0.72
	15	41.8 ± 0.58	43.9 ± 1.01	43.1 ± 0.95
	30	42.0 ± 0.77	44.2 ± 0.90	43.8 ± 0.66**
Bactericidal activity, %	1	52.5 ± 0.88	54.3 ± 1.01	53.1 ± 0.94
	15	52.90 ± 0.74	54.9 ± 0.85	53.9 ± 0.71
	30	53.1 ± 0.67	55.7 ± 0.76	54.2 ± 0.80
Lysozyme activity, %	1	50.3 ± 2.10	52.1 ± 1.95	51.8 ± 1.87
	15	50.2 ± 1.85	52.9 ± 1.72	52.3 ± 1.68
	30	50.8 ± 1.94	53.1 ± 1.84	52.5 ± 1.75
Lymphocytes, %	1	9.2 ± 0.22	9.2 ± 0.20	9.3 ± 0.21
	15	9.4 ± 0.30	9.8 ± 0.27	9.9 ± 0.24**
	30	9.4 ± 0.29	10.1 ± 0.33	10.1 ± 0.31
T-lymphocytes, %	1	72.1 ± 1.94	72.9 ± 2.04	73.3 ± 2.08
	15	72.7 ± 1.86	73.8 ± 2.11*	74.0 ± 2.27**
	30	72.4 ± 2.00	74.3 ± 2.12	73.8 ± 2.34
B-lymphocytes, %	1	21.4 ± 2.05	22.5 ± 1.95	23.1 ± 1.84
	15	21.8 ± 2.10	22.9 ± 2.00	23.6 ± 1.98
	30	21.9 ± 2.20	23.4 ± 2.08*	24.1 ± 2.12**

Notes: * — $P < 0.05$, ** — $P < 0.01$ relative to the control indicators.

Disinfectants such as quaternary ammonium compounds, hypochlorites (e. g., bleach), chlorhexidine, and pine oil are less effective in the presence of organic materials. Formaldehyde disinfectants are highly toxic and not recommended for use around horses. Some products are designed for the dry disinfection of horse housing. These products can be used in the absence or presence of animals by spreading them evenly over the floor.

Before applying disinfectants, the following general recommendations should be followed: before applying disinfectants, surfaces should be thoroughly cleaned of dirt and organic materials to increase the effectiveness of the treatment. Always follow the recommendations for concentration, method of application, and exposure time for each product. The premises should be well ventilated during and after disinfection. When working with chemical disinfectants, appropriate protective equipment should be used to prevent contact with skin and mucous membranes (Gehlen et. al., 2022).

Always consult a veterinary professional before selecting and using disinfectants to ensure the health and safety of your horses. Some disinfectants can be dangerous or toxic to horses, especially if used improperly. Only apply disinfectant after removing the horses from the room (unless the product is approved for use in their presence). Be sure to ventilate the room before returning the animals.

Conclusions. According to the results of the quantitative analysis of the serial dilutions of the samples, the estimated level of contamination decreased to 85% of the colony-forming units per cm^2 of area after the premises were disinfected with 'DZPT-2'.

After disinfecting the facility with 'Iodesol' and 'Geocide' at concentrations of 0.2% and 0.5%, respectively, a decrease in the concentration of harmful gases was observed: ammonia decreased by 36% and hydrogen sulfide by 60%. Relative humidity decreased by 13%, and the number of airborne microorganisms decreased by 71%.

Using solutions of 'Iodesol' and 'Geocide' at 0.2% and 0.5% concentrations to disinfect premises occupied by horses is harmless to the animals. This is evidenced by the indicators of nonspecific resistance factors, such as bactericidal and lysozyme activity in the blood serum. These values remained within normal limits during the study period.

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