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**Editorial Board Address:**
NSC 'Institute of Experimental and Clinical Veterinary Medicine'
83 Hryhoriia Skovorody Str., Kharkiv, Ukraine, 61023
tel. +38 (057) 707-20-53, 704-10-90
E-mail: nsc.iecvm.kharkov@gmail.com, inform@vet.kharkov.ua

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## ENVIRONMENTAL AND BIOETHICAL STANDARDS FOR THE TREATMENT OF METABOLIC ACIDOSIS IN WILD ANIMALS: EXPERIENCE WITH THE USE OF SODIUM BICARBONATE

**Sachuk R. M. [1], Velesyk T. A. [1], Stravskyy Ya. S. [2], Katsaraba O. A. [3], Barylo B. S. [3]**

[1] Rivne State Humanitarian University, Rivne, Ukraine, e-mail: sachuk.08@ukr.net
[2] Ivan Horbachevsky Ternopil National Medical University, Ternopil, Ukraine
[3] Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies of Lviv, Lviv, Ukraine

**Summary.** The article presents the results of a study on the effectiveness of the veterinary drug 'Acidostop' (60 mg/ml sodium bicarbonate) in treating metabolic acidosis in European fallow deer (*Dama dama*) in semi-free conditions. The study was conducted in aviaries on a private farm, utilizing both clinical and laboratory methods. Typical symptoms of acidosis, such as depression, diarrhea, and dehydration, were successfully alleviated within three to seven days of treatment. A comparative analysis with a 5% sodium bicarbonate solution revealed that 'Acidostop' has comparable therapeutic efficacy, is well tolerated, and does not cause adverse effects. The study was conducted in accordance with the requirements of GLP, GCP, and in compliance with the European Convention for the Protection of Vertebrate Animals. Special attention was paid to bioethical aspects: minimizing stress, environmental safety, and humane treatment of animals. The drug is officially registered in Ukraine for use in European fallow deer, has no analogues outside the country, which emphasizes its scientific novelty. The study has practical significance for ecology and veterinary medicine in conditions of semi-free keeping of wild animals. It is also a valuable teaching material for the educational programs 'Ecology', 'Veterinary Medicine', and 'Zoophysiotherapy'. The results obtained contribute to the formation of an environmentally responsible approach to the treatment of animals among students

**Keywords:** European fallow deer, 'Acidostop', hematological and biochemical blood parameters

**Introduction**. Hunting farms play an important role in preserving biodiversity, regenerating wild animal populations, and maintaining ecological balance in forest ecosystems (Di Minin et al., 2021; Kalábová et al., 2025). One popular species kept in semi-free or caged conditions is the European fallow deer, *Dama dama* (Linnaeus, 1758) (Artiodactyla: Cervidae), an animal with high adaptability and significant ecological value (Bijl and Csányi, 2022). However, even under appropriate conditions, these animals remain vulnerable to stress factors, feeding disorders, and infectious diseases (Tajchman et al., 2019; Ny et al., 2023). In hunting farm practice, metabolic disorders, including ketosis, acidosis, hypocalcemia, as well as bacterial and parasitic infections, are of particular concern. Insufficient control over the diet, excessive feeding of cereals or silage can provoke the development of diseases that, without timely diagnosis and correction, lead to animal losses (Campbell and VerCauteren, 2011; Vicente et al., 2014; Sultana et al., 2021; Huaman et al., 2023). In this context, the need to implement effective, environmentally safe, and bioethical approaches to treatment becomes paramount. Ensuring the environmental safety and bioethics of veterinary interventions in wild animal populations is of particular importance in the context of the current environmental crisis and anthropogenic pressure on natural ecosystems. An important aspect is the development and implementation of eco-oriented standards for the diagnosis and treatment of diseases accompanied by metabolic disorders in wild animals. One of these conditions is metabolic acidosis, which occurs in animals with digestive disorders, intoxications, stressful influences, or feeding errors (Fox, 1995; Åkerfeldt et al., 2020; Hunchak et al., 2024).

Currently, there is a need for effective, environmentally safe methods to correct the acid-base state of wild animals in semi-free conditions or aviaries. 'Acidostop', a veterinary drug produced by DEVIE LLC in the form of an infusion solution, has been shown to positively impact the acid-base balance of European fallow deer, as evidenced by clinical study results.

Successful correction of metabolic acidosis in wild animals requires the pharmacological effectiveness of the drugs and consideration of the ethical aspects of therapeutic intervention. Basic requirements for veterinary drugs used in wildlife include conditions of detention, minimization of stress during manipulations, and an absence of side effects on the environment (Faria, 2023; Dougherty et al., 2024). Sodium bicarbonate, in the form of the drug 'Acidostop', meets these requirements since it is not toxic to the environment, is quickly excreted from the body, and does not accumulate in tissues. This allows treatment to be integrated into environmental protection programs without harming ecosystems and wildlife, which is an important condition for the sustainable development of veterinary science.

The drug 'Acidostop' has a valid state registration certificate (No. AB-09832-01-25 dated 04.06.2025), which meets the requirements of the current legislation of Ukraine and is a mandatory condition for compliance with modern environmental and bioethical standards of veterinary practice.

It should also be noted that the veterinary drug 'Acidostop' is the only officially registered in Ukraine for use specifically in European fallow deer. Currently, there are no analogues of this drug with a similar registration for this species of animals in any other country in the world, which emphasizes its uniqueness in the international context. This opens up broad prospects for further research, in particular, to study the adaptive potential of the drug when used in other species of wild animals kept in semi-free conditions.

The **purpose of this work** is to highlight the experience of using 'Acidostop' in the treatment of metabolic acidosis in wild animals, using the example of European fallow deer, and to formulate proposals for the implementation of environmental and bioethical standards in veterinary practice for wild fauna.

**Materials and methods**. Experiments were conducted in an aviary on a private farm in the Shubkiv Forestry Department of Rivne District in Rivne Region. The aviary housed 30 adult European fallow deer (ages 7–10 years), 18 young deer (ages 10–14 months), and 12 five-day-old deer. The fallow deer's diet consisted of 1.6 kg of grain, 0.7 kg of oats, 1.2 kg of corn, and 1.5 kg of silage. They were also given up to 7 kg of branches per day: willow (60.0% edible), mountain-ash (30.0%), and bird cherry (10.0%).

Eight European fallow deer, aged 9–10 years, exhibited the following symptoms: depression, loss of appetite, rumen atony (the rumen was dense and distended), tachycardia, teeth grinding, severe thirst, normal body temperature, cessation of chewing, and frequent defecation of watery feces, followed by dehydration. Analysis of the animals' diets revealed that large quantities of wheat and silage were included in the grain mixture fed to each animal daily, resulting in metabolic acidosis.

The diagnosis of metabolic acidosis was established based on the clinical picture of the disease and laboratory tests, which confirmed high acidity of urine — pH 5.6, rumen content — 5.3, and the level of lactic acid in the blood — more than 40 mg/%.

To correct metabolic acidosis in European fallow deer, the veterinary medicinal product 'Acidostop' (solution for infusion) was used in the study, containing sodium bicarbonate — 60 mg/ml as the active substance and auxiliary components: sodium acetate, sodium citrate, and water for injection. The drug belongs to the group of electrolyte solutions for intravenous administration (ATCvet code: QB05XA02).

'Acidostop' ensures the restoration of the alkaline state of the blood by increasing the level of bicarbonate in the plasma, binding hydrogen ions, normalizing the pH and volume of circulating blood. It has a diuretic and expectorant effect, and helps to remove toxins from the body.

The therapeutic efficacy of the drug 'Acidostop' (solution for infusion) was studied in 9–10-years-old European fallow deer, suffering from metabolic acidosis, weighing from 68 to 72 kg, which were selected according to the principle of analogues and divided into two experimental groups (n = 4): animals of the first experimental group were intravenously administered the drug 'Acidostop' (solution for infusion) at a dose of 1,0 ml/kg of body weight; animals of the second experimental group were intravenously administered the comparison drug 'Sodium bicarbonate solution 5%' (solution for infusion) at a dose of 1,0 ml/kg of body weight. The drugs were administered to the animals once a day for 5 days.

The sick animals were treated with a complex treatment: washing the rumen through a probe with a soda solution at the rate of 450 g of the substance per 3 liters of water, oral calcium carbonate in the amount of 80 g per head, Ringer's solution intravenously in a dose of 500 ml. When the general condition improved, 100 g of yeast and 1 liter of milk were administered orally per animal. The doe was fed in the optimal ratio: 60% oats, 10% wheat, 30% corn, and no more than 0.5 kg of silage. The observation period for the animals lasted 14 days.

Laboratory studies of animal blood were conducted in the Laboratory for quality control, safety, and registration of veterinary drugs and feed additives of DEVIE LLC. In stabilized blood of animals, the number of erythrocytes, leukocytes, hematocrit level, and total hemoglobin content were determined using the automatic hematological analyzer BC-6000 (Mindray). In blood serum, the activity of indicator enzymes — alanine aminotransferase (ALT) and aspartate aminotransferase (AST), as well as the levels of total protein, glucose, total cholesterol, urea, and creatinine, were studied using the biochemical analyzer FUJI DRI-CHEM NX600, which works on the principle of 'dry chemistry' using slides. To determine the acid capacity, we measured the total $CO_2$ ($TCO_2$) parameter in serum, which is the total concentration of carbon dioxide in a biological sample and is important for assessing acid-base balance. The acid capacity was calculated using the appropriate formulas in laboratory conditions, taking into account the pH level, $TCO_2$ concentration, and, if necessary, the content of $Na^+$, $K^+$, $Cl^-$ ions, and total $CO_2$.

The determination of lactic acid was performed on the 'Accutrend Plus' device based on the enzymatic reaction with lactate oxidase, which oxidizes lactate to pyruvate with the formation of hydrogen peroxide. This product interacts with the chromogen in the test strip, causing a color change that is recorded photometrically. Capillary blood was used for the analysis, which was applied directly to the test zone of the strip. The device automatically calculates the lactate concentration and displays the result in mmol/l after 60 seconds (Kotsiumbas, 2009, 2013; Vlizlo, 2012).

Since the experiments involved concomitant treatment of animals, the use of veterinary medicinal products was carried out in accordance with the indications and protocols used in the farms for the relevant conditions.

Clinical studies of the veterinary medicinal product were conducted taking into account the guidelines for conducting clinical studies of veterinary medicinal products on target animal species and the requirements set out in the publication 'Guidelines for Conducting Clinical Trials of Veterinary Drugs on Target Animal Species' by Kotsiumbas et al. (2021).

It is important to emphasize that the treatment was carried out in compliance with the principles of humane treatment of animals in accordance with '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).

The results obtained were processed by methods of variational statistics using the StatPlus 7.6.5.0 software package. Data were presented as mean values with standard deviation at a confidence level of 95%, and the reliability of the results was assessed using Fisher's exact test (Tian et al., 2022).

**Results.** During clinical trials in European fallow deer patients with metabolic acidosis, it was found that the use of the experimental drug 'Acidostop' (solution for infusion) and the comparison drug 'Sodium bicarbonate solution 5%' (solution for infusion) in the complex therapy regimen provided normalization of basic metabolic processes and eliminated metabolic acidosis (Table 1).

**Table 1 —** Comparative efficacy of drugs for the treatment of metabolic acidosis in 9–10-years-old European fallow deer (n = 4)

| Indicator | Term of research, day | Group | |
|---|---|---|---|
| | | Experi-mental 1 | Experi-mental 2 |
| Presence of clinical signs, % | 1 | 100.0 | 100.0 |
| | 3 | 25.0 | 25.0 |
| | 5 | missing | missing |
| | 7 | missing | missing |

Notes: Experimental 1 — 'Acidostop' (solution for infusions); Experiment 2 — 'Sodium bicarbonate solution 5%' (solution for infusions).

The incidence of animals in the two experimental groups decreased by 75.0% on the 3rd day of treatment, and on the 5th day, the animals recorded complete recovery.

The results of clinical and biochemical blood tests of experimental European fallow deer before and after therapy are given in Table 2.

**Table 2** — Level of hematological and biochemical blood parameters of 9–10-years-old European fallow deer during treatment of metabolic acidosis (M ± m; n = 4)

| Research groups | Terms of research, day | | |
|---|---|---|---|
| | before treatment | 3 | 7 |
| **Total hemoglobin (HGB), g/l** | | | |
| Experimental 1 | 120.17±2.89 | 119.79±2.38 | 114.93±2.07 |
| Experimental 2 | 121.43±2.64 | 119.37±2.52 | 114.60±2.15 |
| Reference level | 110.0–130.0 | | |
| **Erythrocytes (RBC), $10^{12}$/l** | | | |
| Experimental 1 | 12.94±0.19 | 11.35±0.17* | 10.81±0.14* |
| Experimental 2 | 12.82±0.17 | 11.21±0.18* | 10.69±0.13* |
| Reference level | 9.0–13.0 | | |
| **Acid capacity, mg%** | | | |
| Experimental 1 | 369.49±7.29 | 436.72±8.27* | 457.62±8.65* |
| Experimental 2 | 370.12±8.67 | 435.96±8.34* | 456.79±8.34* |
| Reference level | 420.0–600.0 | | |
| **Total proteins, g/l** | | | |
| Experimental 1 | 67.73±0.84 | 69.58±0.75 | 69.89±0.83 |
| Experimental 2 | 67.60±0.70 | 69.36±0.89 | 69.71±0.87 |
| Reference level | 68.0–72.0 | | |
| **Urea, mmol/l** | | | |
| Experimental 1 | 9.19±0.19 | 5.41±0.16* | 4.68±0.15* |
| Experimental 2 | 9.21±0.17 | 5.45±0.13* | 4.74±0.14* |
| Reference level | 3.3–6.7 | | |
| **Glucose, mmol/l** | | | |
| Experimental 1 | 1.69±0.09 | 2.37±0.10* | 2.51±0.11* |
| Experimental 2 | 1.63±0.10 | 2.31±0.11* | 2.48±0.10* |
| Reference level | 1.94–3.33 | | |
| **Lactic acid, mg%** | | | |
| Experimental 1 | 43.12±0.36 | 17.68±0.21* | 11.27±0.11* |
| Experimental 2 | 42.98±0.41 | 18.13±0.23* | 11.38±0.13* |
| Reference level | 5.0–20.0 | | |

Notes: Experimental 1 — 'Acidostop' (solution for infusions); Experimental 2 — 'Sodium bicarbonate solution 5%' (solution for infusions); * — the difference in the values of the indicators is significant at $p < 0.05$ relative to the corresponding indicators before treatment.

Thus, before treatment, the concentration of total hemoglobin and the number of erythrocytes in the blood were within the reference levels, but were slightly increased, while the acid capacity of the blood, the concentration of total proteins and glucose were reduced relative to the reference level, and the concentrations of urea and lactic acid exceeded it, which indicated the presence of minor dehydration and metabolic disorders of an acidogenic nature in the body of European fallow deer.

Starting from the 3rd day of the experiment, the average clinical and biochemical blood parameters of European fallow deer in both experimental groups came to the limits of the physiological level, but completely stabilized only on the 7th day after the start of treatment:

— with the introduction of the drug 'Acidostop' (solution for infusions) the number of erythrocytes, the concentration of total hemoglobin, the acid capacity, the concentration of total proteins and glucose increased by 14.4% ($p < 0.05$), 2.4%, 3.0%, 11.1% ($p < 0.05$), and 44.4% ($p < 0,05$) respectively, while the concentration of urea and lactic acid decreased ($p < 0,05$) by 45.1% and 66.4%;

— with the administration of the drug 'Sodium bicarbonate solution 5%' (solution for infusions), the number of erythrocytes, the concentration of total hemoglobin, acid capacity, the concentration of total proteins and glucose increased by 14.6% ($p < 0.05$), 3.7%, 2.9%, 12.0% ($p < 0.05$), and 46.9% ($p < 0.05$) respectively, while the concentration of urea and lactic acid decreased ($p < 0.05$) by 44.7% and 65.7%.

The use of 'Acidostop' in the complex therapy of metabolic acidosis in European fallow deer ensured the restoration of clinical and biochemical blood parameters to physiological limits without any detected side effects, which indicates its eco-safe nature.

Therefore, the veterinary drug 'Acidostop' (solution for infusions) in terms of therapeutic efficacy in the complex treatment of metabolic acidosis in European fallow deer is not inferior to the comparison drug 'Sodium bicarbonate solution 5%' (solution for infusions) and has a positive effect on the clinical and biochemical blood parameters of treated animals.

The use of 'Acidostop' in the complex therapy of metabolic acidosis in European fallow deer ensured the restoration of clinical and biochemical blood parameters to physiological limits without any detected side effects, which indicates its eco-safe nature.

All studies were conducted in accordance with the methodological recommendations for good Laboratory Practice (GLP) and good Clinical Practice (GCP) in veterinary medicine. The studies were conducted in compliance with the principles of the humane treatment of animals and the ethical standards defined by the 'European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes'.

Special attention was paid to minimizing stress in animals, the ecological safety of the drugs used, and the absence of toxic effects on the environment. All manipulations were performed by qualified personnel in accordance with standard operating procedures and regulatory requirements. The studies were conducted with mandatory consideration of bioethical principles, including informed consent of animal owners. The results obtained have not only scientific value but also practical significance for the development of safe, ethical approaches in veterinary therapy.

This approach meets modern ecological and bioethical standards of veterinary practice and can become the basis for interdisciplinary research, taking into account the principles of bioethics, physiology, and animal rehabilitation.

In the context of the educational programs 'Ecology', 'Veterinary Medicine', and 'Zoophysiotherapy', such studies play an important role in developing practical skills in animal diagnostics and treatment in compliance with ecological and bioethical principles. Their results are valuable educational material and a scientific basis for training a new generation of specialists.

**Discussion** of the results of the clinical use of the drug 'Acidostop' in the treatment of metabolic acidosis in European fallow deer allows us to better assess its effectiveness in the context of modern veterinary practice. Analysis of the obtained data in combination with literary sources allows us to determine the key aspects of the pharmacological action of the drug, its bioethical acceptability, and environmental safety. It is also important to consider the influence of housing conditions, feeding, and concomitant treatment on the clinical and biochemical parameters of animals.

The results of our study correlate well with the scientific observations presented in the work of Tajchman et al. (2019), which proved that disturbances in mineral metabolism, in particular calcium and phosphorus deficiency, can cause metabolic disorders in fallow deer. This point of view is also supported by the study of Ny et al. (2023), which noted the increased sensitivity of these animals to changes in feeding and immunological factors, which can negatively affect behavioral and physiological reactions.

In addition to physiological aspects, ethical requirements for the treatment of wild animals are no less important. The works of Fox (1995) and Åkerfeldt et al. (2020) emphasize that stress factors can change the results of therapy; therefore, a bioethical approach is critically important. A similar opinion is expressed by Huaman et al. (2023), emphasizing the need to use eco-safe drugs in the practice of keeping wild deer in captivity. Thus, the results obtained by us confirm the effectiveness and feasibility of using 'Acidostop', which not only does not cause side effects, but also complies with modern principles of veterinary bioethics. Separate attention should be paid to the potential of the drug within preventive programs. Based on the data obtained, it can be argued that 'Acidostop' is advisable to use not only for treatment, but also as a means of preventing metabolic disorders in animals that are subjected to increased feeding loads. This opens up new prospects for forming standards for keeping and caring for wild ungulates in aviary conditions while adhering to an eco-oriented approach.

**Conclusions**. The veterinary drug 'Acidostop' (a solution for infusion) is based on sodium bicarbonate. When used in complex therapy, it restores the acid-base balance of the blood in cases of metabolic acidosis and ketosis after calving in European fallow deer. Its effectiveness is comparable to that of the reference drug.

The uniqueness of 'Acidostop' lies in its official registration for use in wild ungulates, particularly fallow deer. This is an innovation in Ukrainian veterinary practice and has no direct analogues outside the country. This emphasizes the drug's scientific and practical value for further developing eco-oriented veterinary medicine.

The study was conducted in accordance with the international GLP and GCP standards and in compliance with the European Convention for the Protection of Vertebrate Animals. Special attention was paid to bioethical aspects, such as minimizing stress, ensuring environmental safety, treating animals humanely, and obtaining informed consent from owners. These measures ensure the integration of veterinary interventions into the broader context of nature conservation and sustainable development.

The results are of great importance for the training of specialists in ecology, veterinary medicine, and zoophysiotherapy, contributing to the formation of environmentally responsible thinking and an interdisciplinary approach to the treatment of wild animals.

Therefore, 'Acidostop' is a promising, environmentally safe, and bioethically justified drug that can be recommended for widespread implementation in veterinary practice for the treatment of metabolic acidosis in wild animals in semi-free conditions.

**Prospects for further research.** Further scientific research should focus on improving treatment regimens for metabolic acidosis in animals, particularly regarding the dosage and frequency of eco-friendly drugs.

It is also promising to study the impact of adjusting the feeding ration on the prevention of acidosis in semi-free conditions for wild animals. Particular attention should be paid to the integration of the obtained results into the educational process within the educational program 'Zoophysiotherapy', which will ensure the formation of practical skills in the application of physiotherapeutic methods among students.

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**2025 © Sachuk R. M. 0000-0003-4532-4220, Velesyk T. A. 0000-0003-3201-9323, Stravskyy Ya. S. 0000-0001-6541-9097, Katsaraba O. A. 0000-0001-8520-224X, Barylo B. S. 0000-0002-0075-8159**

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# FEATURES OF CLINICAL MANIFESTATIONS AND PATHOMORPHOLOGICAL CHARACTERISTICS OF BORDETELLOSIS IN DOGS

**Hadzevych D. V.**
National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Kharkiv, Ukraine, e-mail: d5195681@gmail.com

**Summary.** The results of the studies showed that the clinical signs of *Bordetella* infection in dogs are pronounced and have characteristic manifestations. These signs typically last for three to four months and then disappear. The most pronounced symptom was coughing, ranging from a rare, mild, productive cough to a severe, dry cough accompanied by suffocation attacks and vomiting. We recorded the most pronounced damage and typical pathomorphological changes in the respiratory organs of all dead dogs. The mucous membranes of the trachea and bronchi were hyperemic, and their lumens contained foamy sputum. Signs of catarrhal or catarrhal-purulent inflammation, emphysema, and hyperemia were detected in the lungs. A heterogeneous, spotted, marbled pattern was observed on the surface of the lungs, especially in the dorsal regions, which is characteristic of this infection. According to our studies, the infection was found in animals of all age groups. However, it was more frequently recorded and proceeded particularly severely in puppies aged two to five weeks and six months. In most cases, bordetellosis resulted in recovery following adequate etiological and symptomatic therapy; however, complications and animal deaths were frequently reported. According to our observations, mortality was low in complicated cases, ranging from 7% to over 22%

**Keywords:** *Bordetella bronchiseptica*, tracheobronchitis, bronchoalveolar lavage, nasopharyngeal secretion

**Introduction.** Currently, bordetellosis is recognized as an independent nosological entity caused by the bacterium *Bordetella bronchiseptica* (Versalovic et al., 2011). This bacterium is a medium-sized, Gram-negative, ovoid rod that was first isolated by N. S. Ferry in 1910 from the respiratory tract of a dog in the early stages of the disease (Avramenko, 1999). This pathogen causes respiratory diseases in a wide range of mammals, including dogs, horses, rabbits, pigs, rodents, and cats (De la Torre et al., 2012; Gueirard et al., 1995). In dogs, it causes tracheobronchitis, also known as 'kennel cough.' In rabbits, it is the main cause of rhinitis. In cats, it causes pneumonia. In piglets, it causes atrophic rhinitis (Huebner et al., 2006; Khanna et al., 2005; El Khatib et al., 2015). Infection with this pathogen can lead to chronic, asymptomatic colonization of the upper respiratory tract in animals (Versalovic et al., 2011). In humans, disease caused by *B. bronchiseptica* is uncommon. More often, it is characterized by asymptomatic carriage of the bacterium on the mucous membrane of the respiratory tract (Gueirard et al., 1995). *B. bronchiseptica* is an opportunistic pathogen that can be detected in humans with other infections. Severe manifestations and relapses are more characteristic of immunocompromised individuals, such as premature infants, patients with acute leukemia, cystic fibrosis, and HIV infection, as well as individuals after bone marrow and lung transplantation. The onset of infection is often associated with contact with sick animals (Gueirard et al., 1995; Huebner et al., 2006; El Khatib et al., 2015; Spilker, Liwienski and LiPuma, 2008).

Data from animal studies indicate that the pathogen binds to cells of the ciliated respiratory tract epithelium due to the presence of pertactin, fimbriae, the tracheal colonization factor, and filamentous hemagglutinin. The dermonecrotic factor, adenylate cyclase hemolysin, and tracheal cytotoxin cause local epithelial damage and increased mucus production. Consequently, mucociliary clearance is impaired, resulting in the accumulation of viscous secretions. This reduces the patency of small bronchi and bronchioles, causing atelectasis, bronchopneumonia, and emphysema (Avramenko, 1999). The pathogen evades the host's protective mechanisms by inhibiting phagocytosis through adenylate cyclase toxin. This contributes to its prolonged persistence in epithelial cells of the respiratory tract and alveolar macrophages. Such intracellular parasitism prevents the pathogen from being rapidly eliminated from the body and leads to bacteriocarrier status and chronic and recurrent infection (Gueirard et al., 1995; MHU, 2005, 2007; Hadzevych, 2024).

Although bordetellosis in domestic animals is a pressing global issue, the disease has not been adequately studied in our country. There is a severe lack of data on the spread, detection, and elimination of the disease. Many questions remain unanswered. These questions are about its epizootic characteristics and epidemiological role. The primary reservoir of *B. bronchiseptica* remains unknown, and the intraspecies and interspecies transmission of the pathogen in nature is not well understood. Additionally, there is conflicting data on the seasonality of the disease and the role of rodents, wild birds, and synanthropic birds in its spread. There is no clear information on the pathogenicity of *B. bronchiseptica* for laboratory animals. In fact, the available information is contradictory. There is no objective data on the ability of the bordetellosis causative agent to kill white mice in bioassays. Furthermore, the

pathomorphology of this disease has only been described in a few reports in foreign specialized publications. In our opinion, the clinical presentation of the infection, pathogenesis, and pathomorphological characteristics require further study and broader coverage. Information about the characteristics of the clinical picture and pathomorphological changes in sick animals is the primary basis for making a preliminary diagnosis and deciding on further actions. These actions include implementing the necessary anti-epizootic measures, such as applying appropriate diagnostic, preventive, and therapeutic procedures. These procedures help prevent or locate the source of infection promptly.

This stage of the study **aimed** to investigate the clinical manifestations of bordetellosis in dogs of different ages and to determine the patterns of pathomorphological changes in acute and chronic forms of the disease. To achieve this goal, the following tasks were set: (1) study and analyze available literature on related issues; (2) determine the epidemiological situation of bordetellosis in kennels in Kharkiv Region; (3) investigate clinical features in dogs with bordetellosis; (4) perform pathological autopsies on carcasses of animals that died from a *Bordetella* infection; and (5) determine macroscopic characteristics of the disease.

**Materials and methods.** The following methods were used in the research: epizootiological, medical history collection, clinical, morphological, and biochemical studies of animal blood, pathological, bacteriological, serological, and virological studies, and statistical analysis. Epizootiological research methods included studying the patterns of occurrence, spread, and dynamics of infection, as well as developing preventive measures and means of combating the disease. These methods included observation, comparison, measurement, experimentation, analysis, and synthesis. The isolation and identification of *B. bronchiseptica* clinical isolates from dogs with or suspected of having bordetellosis were carried out in accordance with the methodological guidelines for the microbiological diagnosis of whooping cough and paracough (MHU, 2005). During an epizootiological survey of kennels in Kharkiv Region of Ukraine, biological material was collected from sick and deceased animals for laboratory testing. This material included parenchymal organs, nasal and conjunctival swabs, stabilized blood, blood serum, scrapings, milk, and nasopharyngeal secretions. Nasopharyngeal secretions were sampled for *Bordetella* isolation using a sterile, disposable probe swab from the mucous membranes of the tonsils and pharynx. During the epizootiological examination, special attention was paid to retrospective epizootiological analysis (Korniienko et al., 2008; Gerlach et al., 2001; Milanko, Kholodylo and Dushkyn, 1995).

In cases of severe tracheobronchitis in animals, bronchoscopy was performed, and tracheal and bronchoalveolar lavage samples were collected. The resulting fluid was examined bacteriologically to determine the sensitivity of the microflora to antibiotics. We determined and recorded antibiotic sensitivity using the disc diffusion method in accordance with the methodological guidelines 'Determination of the Sensitivity of Microorganisms to Antibacterial Drugs' (MHU, 2007).

To exclude other microorganisms and viruses associated with respiratory tract damage accompanied by coughing, appropriate bacteriological, mycological, virological, and serological methods, as well as PCR diagnostics, were performed. First, *B. bronchiseptica* was differentiated from canine adenovirus, canine parainfluenza virus, canine respiratory coronavirus, influenza A virus, carnivore plague virus, reovirus types II-III, herpesvirus, *Mycoplasma cynos*, β-hemolytic streptococci, and *Pasteurella multocida* (Versalovic et al., 2011; Gadzevich, 2024; Mylanko and Dushkin, 1996; Milanko, Gerilovich and Dushkin, 1995).

During pathological autopsies of dead animals, macroscopic changes in organs and tissues were recorded and described. During autopsy, pathological material was collected for further histological examination by means of incomplete evisceration.

**Results and discussion.** The results of the studies showed that the clinical signs of canine bordetellosis are pronounced and have characteristic manifestations. The signs are prolonged and disappear within three to four months. The manifestation of symptoms depends on the extent of respiratory system damage, the type of inflammation, the course of the disease, the aggressiveness of the pathogen, complications from other pathogens, and the dog's age. Sometimes, the disease manifested only as a cough, with or without phlegm. In other cases, severe, life-threatening pneumonia developed. The initial signs of infection are small drops of serous exudate forming on the dog's nasal wings (Fig. 1) and difficulty breathing through the nose, accompanied by snorting sounds. After three to seven days, the animals developed depression, sneezing, anorexia, and pyrexia. Profuse serous discharge was observed from the eyes and nose, which then became purulent due to secondary infection. Initially, respiratory sounds were usually unchanged; however, in some cases, bronchial breathing was heard. Later, crepitus and wheezing were recorded. At the onset of uncomplicated bordetellosis, we most often observed only coughing, which appeared three to four days after contact with the pathogen. For example, this occurred after a dog visited a veterinary clinic or an exhibition where it came into contact with other sick animals. The cough could be dry (nonproductive) or wet (productive), rough or soft, and frequent or infrequent. Sometimes, a coughing fit was accompanied by phlegm, which was usually white or yellowish in color. Frequent regurgitation of large amounts of phlegm sometimes provoked vomiting. It should be noted that animal owners often mistook profuse phlegm for vomiting when it was actually a productive cough. After a few days, the animals' health typically worsens due to secondary infections and complications.

The results of the studies showed that bordetellosis in kenneled dogs in Kharkiv Region occurred in acute, subacute, chronic, latent, and atypical forms. The acute form was characterized by pronounced depression, frequent labored breathing, intermittent fever, an increase in body temperature to 40–41°C, refusal to eat, conjunctivitis, and rhinitis (Fig. 2 and Fig. 3). The most pronounced symptom was coughing, ranging from mild, productive coughs to severe, dry coughs accompanied by suffocation and vomiting. It should be noted that coughing in dogs with *Bordetella* infection had distinguishing characteristics. Initially, coughing was mainly observed at night in the form of short coughing fits. During the day, no coughing was observed. After a short time (two to three days), the frequency and nature of the cough usually worsen significantly. The cough became dry, raucous, and prolonged. Attacks of suffocation occurred, which were complicated by vomiting. Puppies often develop abdominal hernias due to excessive straining while coughing. The cough could easily be triggered by slight irritation or touching the trachea. Profuse, bilateral mucopurulent discharge was observed from the nasal cavity. The animals lost significant weight within one to three weeks. They lost their appetite, eventually refusing to eat altogether. Their 'voice' became hoarse. During examination of the oral cavity, redness and hyperemia of the soft palate and tonsils were noted. The cervical and submandibular lymph nodes were enlarged. The animals became lethargic and reluctant to get up; they also moaned. According to the results of clinical and morphological blood tests, neutrophilic leukocytosis with a left shift was noted, and pneumonia was diagnosed based on focal or diffuse opacity detected by chest X-ray.

The acute form lasted one to two weeks — this was the first wave of the endemic disease. During autopsies of dead animals, macroscopic changes were recorded and described in various organs and tissues. These changes were found in dogs infected with bordetellosis. During the external examination of carcasses, it was noted that the skin was dry and inelastic and that the visible mucous membranes were pale and dry. All animals showed signs of exhaustion (Fig. 4). We established that the most pronounced damage and typical pathomorphological changes were recorded in the respiratory organs of all dead dogs. The mucous membranes of the trachea and bronchi were hyperemic, and their lumens contained foamy sputum. The lungs showed signs of catarrhal or catarrhal-purulent inflammation (Fig. 5), emphysema, and hyperemia (Fig. 6).

The inflammatory process primarily spreads along the blunt edges of the diaphragmatic lobes of the lungs. A heterogeneous, spotted, marbled pattern was observed on the surface of the lungs, particularly on their dorsal surfaces, which is characteristic of this infection (Versalovic et al., 2011). Dense foci of bluish-rusty inflammation were found in various configurations in the apical and cardiac lobes of the lungs. Lesions in the extremities of the lungs were most often recorded and had clear demarcation. During autopsy, small necrotic foci of brown color were found. Additionally, an increase in the size of the bronchial lymph nodes, edema of the mucous membranes of the trachea and bronchi, and accumulations of mucus and pus in their lumens were noted. The enlarged bronchial lymph nodes were pinkish-red in color, both externally and on the parenchyma section. They were juicy, which are morphological signs of hyperplasia and serous-hemorrhagic inflammation. Other non-specific but consistent morphological signs resulting from circulatory disorders and heart failure included an enlarged, flaccid heart with right ventricular dilatation (Fig. 7). The liver was enlarged with signs of passive venous hyperemia. The gallbladder was filled with dark green bile (Fig. 8). In the later stages of the disease, dystrophic processes were observed in the liver (Fig. 9). The kidneys were enlarged and bean-shaped with signs of hyperemia. The spleen was enlarged and lanceolate, with various types of hemorrhages (spotted and striped).

Foci of serous-catarrhal inflammation were detected in the gastrointestinal tract (Fig. 10).

Upon examining the abdominal organs, enlarged mesenteric lymph nodes often attracted attention first. Sometimes, vascular hyperemia and hemorrhages in the serous membrane of the intestine were also observed. The enlarged lymph nodes were pinkish-red on the outside and in the parenchyma section. They were juicy, which are morphological signs of hyperplasia and serous-hemorrhagic inflammation.

In subacute cases, the infection was complicated by secondary microflora; prolonged, convulsive coughing, suffocation, wheezing, mucous discharge from the nasal cavity, thirst, and cracked paw pads were observed. The course of the disease in dogs was typical for bordetellosis. The animals showed a decrease or loss of appetite, lethargy, mucopurulent discharge from the nasal passages and conjunctivitis, enlarged submandibular lymph nodes, increased body temperature to 40–41°C, catarrhal and, subsequently, catarrhal-purulent inflammation of the upper respiratory tract; shortness of breath, frequent sneezing, and a suffocating, painful cough. The cough intensified when standing or moving, changing from wet to dry and severe, accompanied by suffocation and vomiting. As pathogenic microflora accumulated, pulmonary emphysema and pneumonia developed (Fig. 11). The animals exhibited severe abdominal breathing accompanied by wheezing. When a large number of animals were involved, the infection was much more severe, resulting in generalized lung damage. The sick animals were stunted in growth and development, lost weight (Fig. 12), and died en masse. Auscultation of the chest during inhalation revealed distinct noises and wheezing. Starting at four weeks of age, the development of the bone base of the upper jaw in puppies was delayed, and their incisor bite was affected.

In kennels with a history of *Bordetella* infection, a second wave of infection was often observed among animals after weaning. This wave of endemic disease

developed into a chronic form. In chronic cases or when the disease was complicated by secondary microflora, the inflammatory process involved other organs and tissues. Pleurisy, pericarditis, and lung abscesses were detected in such cases, and the affected areas became grayish-yellow in color. The foci of inflammation were both localized and diffuse, and the pathological changes depended on the duration of the process. In the localized form, separate foci were found in the lungs. In the diffuse form, which lasted more than two months, lobar foci were detected that covered the anterior, cardiac, accessory, and sometimes diaphragmatic lobes.

Bordetellosis was most commonly observed in young, old, or chronically ill animals. According to our research, the infection was found in animals of all ages, but it was more frequently recorded, and was particularly severe, in puppies aged from two–five weeks to six months. We diagnosed the disease in dogs ranging in age from birth to two years and older; however, the percentage of dogs diagnosed with bordetellosis decreased with age. Typically, bordetellosis in adult dogs manifests only as sneezing and rare coughing, which could periodically intensify or subside. Our research showed that *Bordetella* infection sometimes caused abortions (Fig. 13) in pregnant dogs with bordetellosis or resulted in the birth of non-viable offspring. In kennels unaffected by the disease, animals were most often able to carry and give birth to puppies. However, we often recorded exacerbations of *Bordetella* infection and increased coughing in bitches during the first days after giving birth. During this time, the puppies also developed symptoms characteristic of bordetellosis (within 2–5 days). It should be emphasized that, when signs of the disease appeared in a bitch or at least one puppy in the litter, *Bordetella* infection was usually observed among all animals in the litter that had been in contact with each other within a short period of time (2–5 days).

The main sources of infection were sick animals and convalescents. One trigger of the disease was endothelium damage caused by toxins or infectious agents, such as a viral infection. This was followed by the accumulation of bacteria, fungi, mycoplasma, and other pathogens. Congenital respiratory system abnormalities, chronic bronchitis, bronchiectasis, and tracheal collapse also contributed to the onset of the disease. Bordetellosis in dogs most often occurred after they visited mass events, such as pet shops, kennels, breeding farms, and animal exhibitions.

Our research has shown that the following breeds of dogs are most susceptible to bordetellosis: Pinscher, Spaniel, Eastern European Shepherd, Boxer, and Doberman. The following breeds are less susceptible: Royal Poodle, Airedale Terrier, Laika, and Irish Setter. However, it is too early to conclude whether a dog's susceptibility to *Bordetella* infection depends on its breed. Additional and more detailed observations are needed.

It should also be noted that a significant proportion of infected animals were asymptomatic. Some animals that recovered from the disease remained carriers of the bacteria for a long time. Both categories posed an increased risk of infection to susceptible animals in contact with them because they were undetectable sources of infection. According to our research, the carrier state can last from four to five weeks to several months. However, according to the literature, the carrier state can last for several years.

In most cases, bordetellosis resulted in recovery following adequate etiological and symptomatic therapy. However, complications and animal deaths were frequently reported. Risk factors for developing complications included having an impaired immune system or chronic respiratory diseases, such as bronchitis, ciliary dyskinesia, and tracheal collapse. Complications of the infection included chronic rhinitis, sinusitis, pneumonia, conjunctivitis, keratitis, cough, exhaustion, dehydration, skin lesions, abortions, and gastrointestinal diseases presenting as vomiting and diarrhea. According to our observations, mortality was low, ranging from 7% to 22%, even in complicated cases.

According to the research, the bacterial agent is most actively released into the environment during the acute phase of the disease. Therefore, samples should be taken from sick animals at an early stage of the disease (but no later than three weeks). At a later stage, the pathogen's viability decreases sharply. The literature indicates that the pathogen can survive for up to 20 days or longer in both external and indoor environments. It has been established that *Bordetella* can survive for a prolonged period in stagnant water, suggesting the potential for interspecies transmission without direct contact.

The infection is transmitted from sick animals by air or contact. Indirect transmission of the pathogen through infected feed, water, rodents, and stray cats is also possible (Fig. 14).

Research has shown that a high concentration of dogs in a confined area (kennel, animal hotel, neighborhood, house, apartment) was one of the main factors contributing to the spread of the disease. The infection should be considered highly contagious, as infection often occurs after brief contact with its source, for example, when visiting a veterinary hospital.

Peaks in enzootic disease were observed in kennels in Kharkiv Region during periods of two to five weeks after birth and after weaning puppies from their mothers. Due to adverse effects from various stress factors (e.g., temperature, biology), repeated outbreaks of infection were often recorded, accompanied by mortality rates of 22% or higher. The severity of the disease was associated with pathogenic factors in *B. bronchiseptica*, such as adhesins (filamentous hemagglutinin, pertactin, and fimbriae), and toxins (dermanecrotic, adenylate cyclase-hemolysin, and lipopolysaccharide).

Due to a sharp decrease in overall immunobiological resistance and the presence of hidden carriers in the kennel, the disease can spontaneously onset at any time of the year. However, the infection was most prevalent during the winter-spring and autumn-winter periods.

**Figure 1.** Formation of small drops of serous exudate on the wings of a dog's nose.

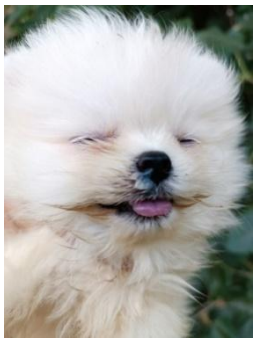

**Figure 2.** Mucopurulent exudate from the nasal canal and conjunctivitis in a puppy with bordetellosis.

**Figure 3.** Mucopurulent exudate from the nasal canal and conjunctivitis in a puppy with bordetellosis.

**Figure 4.** Signs of exhaustion in a dead animal with pale, dry, and dull visible mucous membranes and dry, inelastic skin.

**Figure 5.** Catarrhal-purulent pneumonia.

**Figure 6.** Emphysema and lung hyperemia.

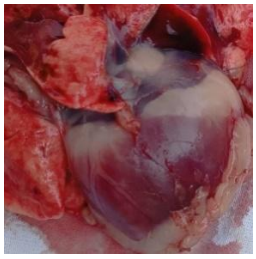

**Figure 7**. Flaccid, enlarged heart with right ventricular dilatation.

**Figure 8.** Venous hyperemia of the liver and an overfilled gallbladder.

**Figure 9.** Dystrophic processes in the liver.

**Figure 10.** Bloating and serous-catarrhal inflammation in the small intestine.

**Figure 11.** Catarrhal-purulent pneumonia.

**Figure 12.** Signs of exhaustion, depression, vomiting, and diarrhea in an animal sick with bordetellosis.

**Figure 13.** Aborted fetus from a pregnant dog with bordetellosis.

**Figure 14.** Signs of rhinitis and conjunctivitis in a homeless cat with bordetellosis.

In crowded animal husbandry conditions, bordetellosis most often co-occurs with mycoplasmosis, adenovirus, parainfluenza, and other infections. At the same time, the clinical presentation of the disease and the extent of pathological changes were more pronounced, and the percentage of young animals dying was significantly higher. In cases of associated bordetellosis in dogs, an avalanche-like spread of the infection among kennel animals was often observed. Animals with mixed infections were more severely ill, and etiological and symptomatic therapies were often ineffective.

**Conclusions**. 1. The results of the studies showed that the clinical manifestations of bordetellosis depended on the extent of respiratory system damage, type of inflammation, course of the disease, aggressiveness of the pathogen, complications from other pathogens, and age of the dog. The most pronounced sign of infection was coughing, ranging from mild, productive coughs to severe, dry coughs accompanied by suffocation and vomiting.

2. The most pronounced damage and typical pathomorphological changes in all dead dogs were recorded in the respiratory organs. The mucous membranes of the trachea and bronchi were hyperemic, and their lumens contained foamy sputum. The lungs showed signs of catarrhal or catarrhal-purulent inflammation, emphysema, and hyperemia. We observed a heterogeneous spotted marbled pattern on the surface of the lungs, especially in their dorsal parts, which is characteristic of this infection.

3. In most cases, bordetellosis resulted in recovery following adequate etiological and symptomatic therapy. However, complications and animal deaths were frequently reported. According to our observations, mortality was low, ranging from 7% to 22%, even in complicated cases.

**Prospects for further research.** The data we obtained indicate that, under crowded animal husbandry conditions, bordetellosis most often co-occurred with mycoplasmosis, adenovirus infection, parainfluenza, and other infections. These co-occurrences proceeded in accordance with the nature of the associated disease. At the same time, the clinical presentation of the disease and the extent of pathological changes were more pronounced, and the percentage of young animals that died was significantly higher. Animals with mixed infections were severely ill, and etiological and symptomatic therapies were often ineffective. According to the literature, a mixed course of many diseases, including respiratory diseases caused by two or more viral-bacterial agents, has been observed more often in recent years. The polyetiology of respiratory diseases in dogs, the insufficient study of their progression, imperfect diagnostic methods, and the lack of specific preventive measures mean that generally accepted health measures do not always yield positive results. These circumstances highlight the need for a more thorough examination of the epidemiological situation of respiratory diseases in dogs, their characteristics, and the development of treatment and prevention strategies. These issues require careful study and research. The results will serve as the foundation for developing a set of anti-epizootic measures, including appropriate diagnostic, preventive, and therapeutic procedures. These measures will help prevent or promptly identify the source of *Bordetella* infection.

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# ASSESSMENT OF BIOCHEMICAL MARKERS OF CONNECTIVE TISSUE METABOLISM IN HORSES WITH LAMINITIS

**Borovkov S. B. [1], Tymoshenko O. P. [2], Matsenko O. V. [2], Shchepetilnikov Yu. O. [2], Suietskyi O. V. [2]**
[1] Institute of Veterinary Medicine of the National Academy of Agrarian Sciences of Ukraine, Kyiv, Ukraine, e-mail: serg_b78@ukr.net
[2] State Biotechnological University, Kharkiv, Ukraine

**Summary.** This study aimed to evaluate biochemical changes in urine associated with mineral and connective tissue metabolism in horses diagnosed with laminitis, and to identify potential markers for early diagnosis and monitoring of this condition. Twenty horses participated in the study: 10 clinically healthy (control group) and 10 animals with laminitis. A clinical examination was conducted, assessing behavior, posture, response to palpation, and physiological indicators. Concentrations of calcium (Ca), inorganic phosphorus (P), uronic acids, hydroxyproline, and creatinine were determined in urine samples. To eliminate the influence of variations in urine output, the ratio of each indicator to the creatinine level was calculated. Animals with laminitis exhibited the following characteristic clinical signs: weight transfer to the hind limbs; shortened gait; lameness; increased pulsation of the palmar arteries; pain; and localized temperature increase in the hoof area. Biochemical analysis of the urine revealed significant increases in calcium (+31.1%), phosphorus (+78.8%), uronic acids (+49.4%), and hydroxyproline (+50.6%) levels ($p < 0.001$). The Ca/P ratio decreased by 24% ($p < 0.001$). Relative indicators also increased: Ca/creatinine (30.2%), P/creatinine (75.0%), uronic acids/creatinine (47.4%), and hydroxyproline/creatinine (50.0%). The obtained data showed that laminitis in horses is accompanied by local and systemic metabolic disorders. Urine markers and their creatinine ratios can be used as additional, sensitive indicators of laminitis severity and treatment effectiveness

**Keywords:** horses, urine, calcium, phosphorus, hydroxyproline, uronic acids, creatinine

**Introduction.** Metabolic disorders of lipid metabolism in horses are often observed alongside internal diseases and can act as a marker of disease as well as an independent risk factor for complications (Bertin, 2023; Durham et al., 2019). These disorders accompany endocrine dysfunction, hepatogenic pathologies, and chronic inflammatory processes, and also occur in a state of negative energy balance (Perez-Ecija et al., 2021).

Laminitis is one of the most common and clinically significant manifestations of metabolic disorders in horses (Morgan, Keen, and McGowan, 2015). It is one of the most complex orthopedic pathologies in horses, involving damage to the laminar apparatus of the hoof and leading to significant functional disorders, prolonged lameness, and loss of working capacity. It is characterized by inflammation and degenerative changes in the connective tissue structures that attach the hoof horn to the distal phalanx, creating a risk of irreversible anatomical deformities (De Laat et al., 2019; Luomala, 2022). Key risk factors for laminitis include carbohydrate metabolism disorders, insulin resistance, obesity, systemic inflammatory conditions, and technological disorders in the feeding and housing of animals (De Laat et al., 2016). The mechanisms of laminitis development are multifactorial, including vascular, endocrine, and immunoinflammatory components, as well as an imbalance in the matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs), which leads to the destruction of the basal membrane of the hoof apparatus (Clutterbuck et al., 2010).

Early diagnosis is particularly challenging because clinical manifestations typically emerge when significant tissue damage has already occurred. Therefore, it is important to study molecular and biochemical markers that would enable metabolic disorders of connective tissue to be detected in time, even before clinical symptoms develop. Promising biomarkers include extracellular matrix degradation, particularly focal adhesion proteins (talin 1, vinculin, and cadherin 13), glycoproteins (α2-macroglobulin), and heat shock proteins (HSP90), as well as components of the complement and blood coagulation systems (Campolo et al., 2020; Espinosa-López et al., 2025). Despite significant progress in studying the pathophysiology of laminitis, many questions remain regarding the mechanisms of extracellular matrix damage and the role of systemic metabolic disorders in developing this pathology. Further research in this area is necessary to improve early diagnosis and develop effective approaches to the prevention and treatment of laminitis (Serteyn et al., 2024).

In clinical practice, assessing the lipid profile (including triglycerides, cholesterol, free fatty acids, HDL, LDL, and VLDL lipoproteins) is becoming increasingly important. However, as Zemek et al. (2024) have noted, even in animals with obvious clinical abnormalities, deviations from reference values are not always recorded. This highlights the need for greater attention to be paid to interpreting such indicators in combination with clinical symptoms.

Hyperlipidemia can develop in ponies and miniature horse breeds even with moderate stress or energy deficiency, causing particular clinical concern (De Laat et al., 2016).

Disorders of lipid metabolism in horses and ponies commonly accompany internal diseases, particularly metabolic syndrome, endocrinopathies, and liver pathologies. At the same time, hypertriglyceridemia can complicate the underlying disease and have independent clinical significance. Some animals, especially ponies, quickly develop dyslipidemic changes in response to stress or energy deficiency that remain unnoticed without timely diagnosis (Bertin, 2023).

Although methods for assessing lipid metabolism in horses are available, they are not widely used in veterinary practice. The absence of systematic reference values and diagnostic algorithms, as well as the limited number of studies, makes the clinical interpretation of results difficult (Zemek, Kemp and Bertin, 2024). This is why studying lipid metabolism in normal and pathological conditions is relevant and has practical significance for improving diagnostic approaches.

This study **aimed** to evaluate biochemical changes in urine associated with mineral and connective tissue metabolism in horses diagnosed with laminitis, and to identify potential markers for early diagnosis and monitoring of this condition.

**Materials and methods.** The study was conducted on two groups of horses: Group I comprised clinically healthy animals (n = 10), while Group II comprised animals with a confirmed diagnosis of laminitis (n = 10). All animals underwent a clinical examination, including an assessment of their general condition and measurement of basic physiological parameters, as well as an examination involving palpation, percussion, and auscultation of organs and systems. A comprehensive diagnosis of laminitis and associated metabolic disorders was made based on anamnestic data, characteristic clinical changes, and laboratory test results, by the recommended criteria (Geor, 2009).

The selection of biochemical indicators for study was based on the pathogenetic mechanisms of laminitis. Determining calcium and phosphorus concentrations in urine, as well as their ratio (Ca/P), enabled us to evaluate disorders of mineral metabolism, which are crucial for bone and connective tissue remodeling. Uronic acid and hydroxyproline are sensitive markers of extracellular matrix and collagen degradation, while creatinine levels reflect kidney function, enabling other indicators to be adjusted for concentration capacity.

Urine samples were collected during physiological urination in dry sterile containers. After collection, the biomaterial was immediately delivered to the laboratory for biochemical analysis. The following indicators were determined in urine: hydroxyproline, which was assessed using a spectrophotometric method after acid hydrolysis. This method is based on the formation of a colored complex involving chloramine T and para-dimethylaminobenzaldehyde. This allowed for a quantitative assessment of the hydroxyproline content at a wavelength of 540 nm. This method has been adapted for use in veterinary practices and is recommended in studies of collagen metabolism (McIlwraith et al., 2018). The level of glycosaminoglycans in biological fluids was quantitatively assessed using a modern modification of the classic carbazole method, which allowed for the determination of uronic acids via photometry (Zhang et al., 2016).

The calcium concentration was determined using a photometric method with an arsenazo III reagent. At a pH of 6.5, the reagent forms a colored complex with calcium, enabling an accurate measurement of calcium content in urine (Morozenko and Leontieva, 2016).

Inorganic phosphorus was studied using a photometric method based on the formation of a phosphomolybdenum complex, which in the presence of a reducing agent (ascorbic acid) acquires an intense blue color with a maximum absorption at a wavelength of 700–720 nm (Morozenko and Leontieva, 2016; Baylor, Chandler and Marshall, 1982).

Creatinine concentration was determined by the photometric method using the classic Jaffe reaction with a standard set of reagents produced by Reagent PJSC (Ukraine), which is widely used in veterinary laboratory diagnostics (Morozenko and Leontieva, 2016).

To improve the accuracy of the interpretation of the results, the ratio of the studied substances to the creatinine concentration was calculated: calcium/creatinine, phosphorus/creatinine, uronic acids/creatinine, and hydroxyproline/creatinine, which reduced the influence of daily diuresis variability and is widely recognized in connective tissue metabolism studies.

The research was 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 State Biotechnological University (Kharkiv, Ukraine).

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

**Results and discussion.** A clinical examination revealed a significant difference in behavior between horses diagnosed with laminitis and those that were clinically healthy. Healthy horses exhibited active behavior and a symmetrical posture, as well as a natural gait with an even distribution of body weight across all limbs. Their pulse and respiratory rates, body temperature, hoof condition, and response to palpation were all within physiological limits (Wylie et al., 2016). In contrast, horses with laminitis exhibited characteristic clinical symptoms caused by pain and inflammation in the hoof area. During the examination, an altered posture was observed: the front limbs were extended forwards and the hind limbs were tucked under the torso. This is a typical reaction designed to reduce the

load on the affected areas. Limited motor activity, lameness, and a cautious, tense gait were observed, which worsened when changing direction. In some cases, forced immobility was observed (Pollitt, 2010; Van Eps, Collins and Pollitt, 2010). Palpation revealed an increase in local hoof wall temperature, pronounced pain in the coronet area, and, when pressure was applied to the sole. The pulsation of the palmar artery was intensified, further indicating the development of an inflammatory process (Belknap and Geor, 2012). Some animals showed general depression, an increased body temperature (up to 38.9–39.4 °C), tachycardia (up to 52–58 beats/min), and rapid breathing (up to 24–28 breaths/min), indicating a systemic response to inflammation. These indicators remained stable in healthy horses: body temperature was within the range of 37.5–38.2 °C; heart rate was 28–40 beats/min; and respiratory rate was 10–14 breaths/min. Due to severe pain, percussion examination was limited, but in acceptable cases, a change in sound conductivity in the sole area was recorded. This indirectly indicated soft tissue edema or displacement of the coffin bone (Orsini, 2014).

Urine analysis revealed changes in the metabolic status of horses with laminitis, showing significant biochemical parameter deviations compared to clinically healthy animals (Table 1).

**Table 1** — Results of biochemical studies of horse urine

| Indicator | Animal group | | Δ |
|---|---|---|---|
| | I clinically healthy (n = 10) | II with laminitis (n = 10) | |
| Ca, mg/l | 136.8 ± 3.0 | 179.4 ± 2.5 | +42.6*** |
| P, mg/l | 27.4 ± 1.3 | 49.0 ± 2.9 | +21.6*** |
| Ca/P | 5.0 ± 0.2 | 3.8 ± 0.2 | −1.2*** |
| Uronic acids, mg/l | 8.3 ± 0.4 | 12.4 ± 0.6 | +4.1*** |
| Hydroxyproline, mg/l | 31.4 ± 1.4 | 47.3 ± 2.1 | +15.9*** |
| Creatinine, mmol/l | 8.7 ± 0.3 | 8.7 ± 0.3 | 0.0 |

Note: *** — $p < 0.001$.

Biochemical analysis of the urine of horses with clinically confirmed laminitis revealed systemic changes in their metabolic status, which differed significantly from the parameters observed in healthy animals. The obtained data indicated active disruption of mineral and connective tissue metabolism, likely to be both localized to the hoof and systemic. The concentration of calcium in the urine of animals with laminitis was significantly higher (by 31.1%) than in clinically healthy horses ($p < 0.001$). This hypercalciuria suggests a reduction in tubular calcium reabsorption efficiency or an increase in filtration due to endocrine changes associated with the inflammatory process. It is known that, in response to chronic inflammation (including laminitis), osteolysis mechanisms are activated and parathyroid hormone levels increase. This stimulates the mobilization of calcium from bone tissue and enhances its excretion by the kidneys (Alexander, Fuster and Dimke, 2022; Etemadi et al., 2023; Messa et al., 2023). Additionally, at the local level, the destruction of calcium-containing structures in affected tissues may further contribute to increased calcium levels in biological fluids.

At the same time, a significant increase in inorganic phosphorus levels was observed, rising by 78.8% ($p < 0.001$). This phosphaturia may be due to the inhibition of phosphate reabsorption in the proximal tubules and hormonal imbalances, particularly increased FGF23 activity and decreased calcitonin sensitivity (Diniz, 2013; Tsuboi et al., 2020). Excessive phosphorus excretion in urine during laminitis reflects profound disturbances in mineral homeostasis, which are often associated with signs of secondary hyperparathyroidism.

These changes, taken together, contributed to a shift in the Ca/P ratio in urine. In the control group, the ratio was 5.0 ± 0.2; in the sick animals, it was 3.8 ± 0.2 (a difference of −24.0%; $p < 0.001$). A decrease in this ratio is considered a more sensitive indicator of mineral metabolism disorders than absolute values of individual macronutrients. It may result from changes in hydroxyapatite crystal formation, decreased osteogenesis, and bone tissue resorption. Similar dynamics are also associated with the dysfunction of vitamin D-dependent regulation of phosphorus-calcium metabolism (Hans and Levine, 2024).

Additionally, a substantial 49.4% ($p < 0.001$) increase in uronic acid content was observed in the urine of horses with laminitis, indicating the activation of glycosaminoglycan degradation processes in the intercellular substance. Uronic acids are components of sulphated mucopolysaccharides, and excessive excretion of these acids indicates the destruction of the structural matrix of connective tissue. This matrix plays a key role in the formation and maintenance of the functional state of the hoof horn (Williams, Anastasopoulou and Sapra, 2024).

Hydroxyproline, a stable fragment of degraded collagen, was also significantly elevated in the urine of animals with laminitis, increasing by 50.6% ($p < 0.001$). This confirmed the presence of active collagen breakdown in hoof tissues, consistent with the morphological changes described in chronic laminitis, particularly lamellar layer disintegration and ground substance edema (Kaneko, Harvey and Bruss, 2008; Torres Sánchez et al., 2024). Therefore, uronic acids and hydroxyproline can be considered early biochemical markers of connective tissue destruction in this condition.

Creatinine levels remained stable in both groups (8.7 ± 0.3 mmol/l; $p > 0.05$), indicating no significant impairment of renal filtration function. This enabled us to interpret changes in other parameters as reflecting systemic inflammation and metabolic disorders rather than nephropathy (Kamińska et al., 2020).

To reduce the impact of variations in daily urine volume, animal hydration status, and glomerular filtration rate, relative indices were used, i.e., the ratios of key biochemical markers to creatinine levels (Table 2). This approach is widely used in clinical biochemistry because, as a stable end product of muscle metabolism that is filtered exclusively by the glomeruli and not reabsorbed in the renal tubules, creatinine serves as a reliable internal standard (Onwuka et al., 2021).

**Table 2** — Ratio of biochemical indicators in urine to creatinine level

| Indicator | Animal group | | Δ |
|---|---|---|---|
| | I clinically healthy (n = 10) | II with laminitis (n = 10) | |
| Ca/creatinine | 15.9 ± 0.5 | 20.7 ± 0.7 | +4.8*** |
| P/creatinine | 3.2 ± 0.2 | 5.6 ± 0.3 | +2.4*** |
| Uronic acids/ creatinine | 0.97 ± 0.07 | 1.43 ± 0.06 | +0.46* |
| Hydroxyproline/ creatinine | 3.6 ± 0.1 | 5.4 ± 0.2 | +1.8*** |

Notes. *** — $p < 0.05$; *** — $p < 0.001$.

Our study found that in horses with laminitis, the calcium-to-creatinine ratio (Ca/creatinine) increased significantly, rising from an average of 15.9 ± 0.5 in the healthy group to 20.7 ± 0.7 (an increase of 30.2%; $p < 0.001$). This indicates increased calcium excretion relative to baseline renal filtration function, and could result from impaired endocrine control of calcium homeostasis (in particular, parathyroid hormone activation), as well as osteolysis due to chronic inflammation.

A similar pattern was observed for the P/creatinine ratio. Its level in sick animals was 5.6 ± 0.3, which is 75% higher than in healthy horses (3.2 ± 0.2; $p < 0.001$). This phosphaturia, accompanied by a preserved filtration function, also indicates the activation of tissue demineralization processes that accompany laminitis (Kanepa et al., 2024).

The uronic acid/creatinine and hydroxyproline/ creatinine ratios were particularly valuable for diagnosis. In animals with laminitis, the uronic acid/creatinine ratio increased to 1.43 ± 0.06, compared to 0.97 ± 0.07 in the control group (+47.4%; $p < 0.05$). The hydroxyproline/ creatinine ratio increased to 5.4 ± 0.2, compared to 3.6 ± 0.1 in healthy animals (+50.0%; $p < 0.001$). These changes indicated intense connective tissue degradation and intercellular matrix catabolism activation, particularly of glycosaminoglycans and collagen. The obtained indicators reflected deep degenerative-inflammatory processes in hoof tissue accompanied by the destruction of the basal lamina, the disorganization of the lamellar structure, and the formation of fibrosis (Galantino-Homer and Brooks, 2020; Paes, Carino and Aguiar, 2024; Belknap and Geor, 2016; Pollitt, 2019).

Calculating the ratio of the concentrations of the studied markers to the amount of creatinine in the urine reduced the influence of physiological variations, ensuring a more accurate interpretation of the results. This approach provides a more accurate and reproducible assessment of calcium, phosphorus, uronic acid, and hydroxyproline excretion levels since creatinine is filtered exclusively in the glomeruli, not reabsorbed in the tubules, and is a stable marker of kidney function and urine concentration capacity (Kamińska et al., 2020; Onwuka et al., 2021).

Using relative indicators avoids errors associated with accidental dilution or concentration of urine. This method is widely recognized in human medicine for monitoring metabolic disorders, bone tissue disorders, fibrosis, and osteolysis (Kanepa et al., 2024; Williams, Anastasopoulou and Sapra, 2024). While these methods are not yet routine in veterinary medicine, they have high potential for implementation in diagnostic practices, especially for cases involving systemic connective tissue and kidney damage, as well as mineral metabolism disorders.

Thus, the results of the study confirmed that horses with laminitis experience profound and complex systemic disorders that affect mineral and connective tissue metabolism. These disorders manifested as a significant increase in the absolute concentrations of calcium, phosphorus, uronic acids, and hydroxyproline in urine, accompanied by a corresponding increase in their ratios to creatinine. The increase in these markers' relative values allowed for a more objective assessment of pathological processes, regardless of physiological fluctuations in urine volume or the kidneys' functional state.

The detected changes indicated not only local damage to the hoof tissue, including degradation of the extracellular matrix and destruction of the basement membrane and lamellar architecture, but also a systemic metabolic imbalance resulting from chronic inflammation. Disruption of mineral homeostasis and breakdown of connective tissue components in laminitis are not isolated, local reactions; rather, they are a complex, multi-level pathology affecting the skeletal, endocrine, and extracellular systems (McGrath et al., 2024, Belknap and Geor, 2016; Pollitt, 2019; Torres Sánchez et al., 2024).

The obtained indicators can serve as sensitive additional diagnostic markers for evaluating the activity and severity of laminitis, detecting systemic complications early, and monitoring the effectiveness of therapeutic and preventive interventions. Using them in conjunction with clinical, morphological, and instrumental methods can significantly enhance the informative value of a comprehensive diagnosis and monitoring of laminitis in horses.

The detected changes can be used as additional laboratory markers to comprehensively diagnose and monitor laminitis (Paes, Carino and Aguiar, 2024; Patterson Rosa et al., 2020; Belknap and Geor, 2016;

Pollitt, 2019), which indicates a systemic metabolic imbalance (Hans and Levine, 2024).

Markers such as uronic acids and hydroxyproline provide information about the extent of degradation of the connective tissue component, as confirmed by foreign and domestic studies (Torres Sánchez et al., 2024; Xenoulis, Steiner and Monnet, 2023).

**Conclusions.** 1. A clinical examination of horses with laminitis revealed the following symptoms: a forced posture with unloading of the front limbs; a cautious and shortened gait; lameness, which was especially noticeable when turning; local hyperthermia of the hooves; pain in the coronet area; and increased pulsation of the palmar arteries. Some animals showed systemic manifestations, such as hyperthermia, tachycardia, and polypnea. These signs confirmed the development of an acute pain syndrome and an inflammatory response, which are characteristic of the clinical course of laminitis.

2. Biochemical analysis of urine in horses with laminitis revealed a significant increase in calcium (+31.1%), inorganic phosphorus (+78.8%), uronic acids (+49.4%), and hydroxyproline (+50.6%) compared to clinically healthy animals ($p < 0.001$), indicating impaired mineral and connective tissue metabolism.

3. The Ca/P ratio in the urine decreased by 24% ($p < 0.001$), which indicates an imbalance between calcium and phosphorus, impaired mineralization processes, and increased bone resorption.

4. Calculating the ratios of biomarkers to creatinine concentration in urine (Ca/creatinine, P/creatinine, uronic acids/creatinine, and hydroxyproline/creatinine) provided a more accurate interpretation of the results. This method reduced the influence of individual variations in urine output and allowed for an objective assessment of the degree of metabolic disorders in laminitis.

5. An increase in the relative values of uronic acids and hydroxyproline to creatinine indicates the activation of glycosaminoglycan and collagen degradation in the extracellular matrix structures of hoof tissue. This confirms the development of a destructive inflammatory process in connective tissue.

6. The obtained biochemical urine parameters can be used as additional laboratory markers for the comprehensive diagnosis of laminitis. They can also be used to stratify the severity of the disease, detect systemic complications early, and monitor the effectiveness of therapeutic measures.

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**2025 © Borovkov S. B. 0000-0003-3021-2410, Tymoshenko O. P. 0000-0001-9696-1698, Matsenko O. V. 0000-0002-1782-4650, Shchepetilnikov Yu. O. 0000-0001-7275-0079, Suietskyi O. V. 0009-0005-2780-9664**

# Part 2. Biotechnology

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## TRISYMMETRONS AND TUBULAR FORMS OF IRIDOVIRUS FROM THE MOSQUITO *AEDES* (*OCHLEROTATUS*) *CANTANS* (MEIGEN, 1818) (DIPTERA: CULICIDAE)

**Buchatskyi L. P.** [1, 2]

[1] D. K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine, Kyiv, Ukraine, e-mail: iridolpb@gmail.com
[2] Taras Shevchenko National University of Kyiv, Kyiv, Ukraine

**Summary.** Mosquito iridescent viruses (MIV) were isolated in Ukraine from the larvae of the bloodsucking mosquito *Aedes* (*Ochlerotatus*) *cantans* (Meigen, 1818) (Diptera: Culicidae) that were infected. Examination of ultrathin sections of infected mosquito fat body cells revealed that MIV virion maturation occurs in the cytoplasm. The virions were spherical with pentagonal and hexagonal outlines, indicating icosahedral symmetry. In addition to spherical virions with a diameter of 190 ± 5 nm, smaller tubular structures were present in the cytoplasm of infected cells. Upon destroying the purified virus, a large number of trisymmetrons were identified. Each trisymmetron consists of 55 hexagonally arranged subunits that form an isosceles triangle with an edge length of 60 nm

**Keywords:** MIV, IIV-3, electron microscopy, morphology, mosquito larvae

**Introduction.** Mosquito iridescent virus (MIV, or IIV-3) is a member of the *Chloriridovirus* genus, which is part of the Iridoviridae family. This family includes large (120–350 nm) DNA-containing spherical viruses from many invertebrates (beetles, butterflies, mosquitoes, midges, ticks, bees, isopods, crabs, oysters, spiders, scorpions, nematodes) and vertebrates (fish, amphibians, reptiles). The name of the iridovirus family comes from the word 'iris', which means 'goddess of the rainbow' in Greek. The name of the genus *Chloriridovirus* comes from the word chloros, which means 'green' in Greek.

For the first time, the mosquito iridescent virus of the *Ochlerotatus* subgenus was detected almost simultaneously in 1965 in the Czech Republic in the larvae of *Aedes* (*Ochlerotatus*) *annulipes* (Meigen, 1830) and *Aedes* (*Ochlerotatus*) *cantans* (Meigen, 1818) (Weiser, 1965) and in the USA in the larvae of the black salt marsh mosquito, *Aedes* (*Ochlerotatus*) *taeniorhychus* (Wiedemann, 1821) (Clark, Kellen and Lum, 1965). It was then isolated from many species of this subgenus in various countries of Europe, Asia, North America, and South America, including Ukraine (Buchatskiy and Sheremet, 1974; Buchatskyi, Kaniuka and Lebedinets, 1976).

Because of the large size and complex structure of MIV spherical virions, many details about their structure and the mechanism of virion maturation in infected cells remain unresolved. In this work, the tubular forms and trisymmetrons of the iridovirus from the mosquito *Ae. cantans* are described for the first time using the method of electron microscopy.

**Aim** of this research was to study the ultrastructure of purified mosquito iridovirus virions during their degradation during long-term storage in distilled water and to perform electron microscopic analysis of ultrathin sections of fat body cells of *Ae. cantans* mosquito larvae infected with iridovirus.

**Materials and methods.** Mosquito iridescent virus was isolated from fourth-instar larvae of mosquitoes *Ae. cantans*, which were found in one of the reservoirs in Kyiv Region. The virus was cultivated in larvae of the honeycomb moth, *Galleria mellonella* (Linnaeus, 1758) (Lepidoptera: Pyralidae) and cell cultures of *Ae. aegypti* (Mos 20A) as described earlier (Buchatskyi, Victorov-Nabokov and Sheremet, 1976; Sutugina et al., 1995).

MIV was purified by differential centrifugation and subsequent centrifugation in a sucrose density gradient (10–50%) for 40 min in a Beckman L5-50B ultracentrifuge in a SW-40 rotor (Buchatskiy and Sherban, 1976). To obtain trisymmetrons, the purified mosquito iridovirus was stored for two months in distilled water at a temperature of 4°C.

For electron microscopy, purified MIVs were negatively stained with 2% phosphotungstic acid (PTA) adjusted to pH 6.8 with 1 M KOH. For thin layer electron microscopy, infected larvae were fixed in phosphate-buffered 2.5% glutaraldehyde, post-fixed in 1% osmium tetroxide, dehydrated in graded alcohols, and embedded in 812 Epon resin. Ultra-thin sections were double-stained in uranyl acetate and lead citrate. Examinations were performed with JEMB-100B electron microscope.

**Results.** After long-term storage in distilled water, purified MIV virions disintegrated into separate components. As for other iridoviruses of insects and fish, the most numerous components of their degradation were trisymmetrons (Fig. 1). Each of them consisted of 55 hexagonally arranged subunits, forming an isosceles triangle with a rib length of 60 nm. Ten protein subunits

were placed on each rib. MIV trisymmetrons were often located close to each other and had the same number of such subunits (Fig. 1). In contrast, pentasymmetrons, which are another component of icosahedrons, did not have a clear image of capsomers, because they are three-dimensional structures and their capsomers overlap each other in electron micrographs. It is known from crystallographic conditions that an icosahedron with triangulation number T = 147, including MIV, can be built from 20 trisymmetrons and 12 pentasymmetrons (Caspar and Klug, 1962; Stoltz, 1971, 1973; Parvez, 2020).

As in other insect iridoviruses (Wrigley, 1969, 1970), each pentasymmetron contains thirty-one protein subunits. Therefore, the total number of capsomeres in the MIV can be calculated by the formula: N=(55×20)+(31×12)=1,472.

Examination of ultrathin sections of infected mosquito fat body cells showed that maturation of MIV virions occurs in the cytoplasm. Virions had a spherical shape, pentagonal and hexagonal outlines, indicating an icosahedral type of symmetry. Next to the mature forms of virions with a diameter of 195 ± 5 nm in the cytoplasm there were a large number of immature virions that were at various stages of the formation of the viral capsid and tubular forms of virions (Fig. 2). The diameter of these tubular forms was much smaller than that of virions, ranging from 110 to 165 nm. Their length could reach up to 1.2 μm (Fig. 3). One of the ends of the tubular form of the virus was often closely connected with the incomplete form of the virion (Figs 4–6), which may testify in favor of the hypothesis of the formation of the spherical capsid of the virus from tubular forms. On ultrathin sections of infected cells, such tubular forms of the virus were often located near mitochondria (Fig. 7).

**Discussion.** The presence of trisymmetrons among the destroyed virions of large spherical DNA-containing viruses and the phylogenetic relationships between them has been described in many works (Wrigley, 1969; Xiao and Rossmann, 2011; Yutin and Koonin, 2012; Zhao et al., 2023). In terms of morphology, trisymmetrons of other viruses did not differ from those described by us, except for the length of the ribs. For the iridovirus of the European crane fly, *Tipula paludosa* Meigen, 1830 (Diptera: Tipulidae), the length of the ribs of the trisymmetrons was 70 nm (Wrigley, 1970; Manyakov, 1977), and for MIV it is 60 nm, that is, less.

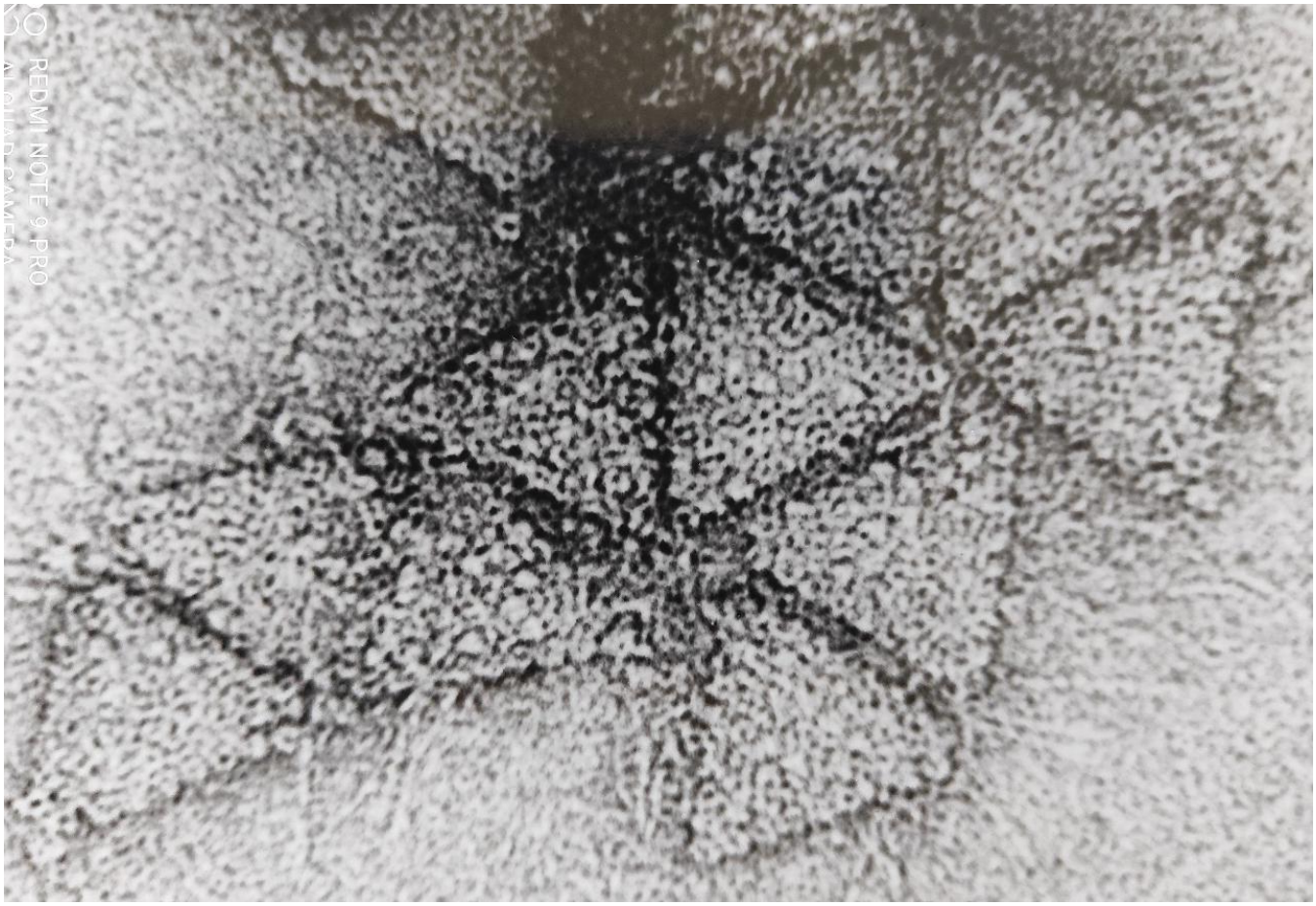

**Figure 1.** Trissymetrons of the iridovirus from the mosquito *Ae. cantans*. The length of the ribs is 60 ± 2 nm.

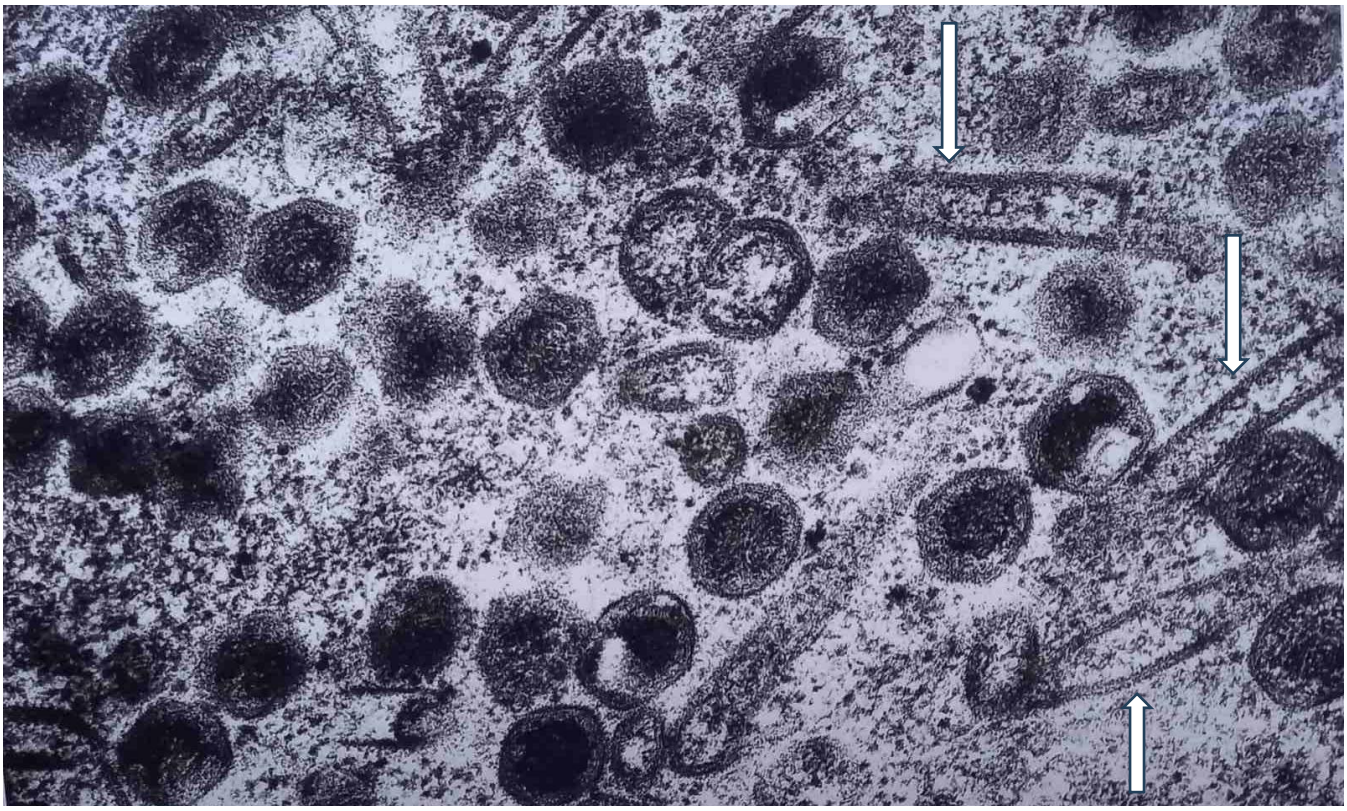

**Figure 2.** Tubular forms (indicated by arrows) of *Ae. cantans* iridovirus among mature and immature forms of the virus. Diameter of virions is 195 ± 5 nm.

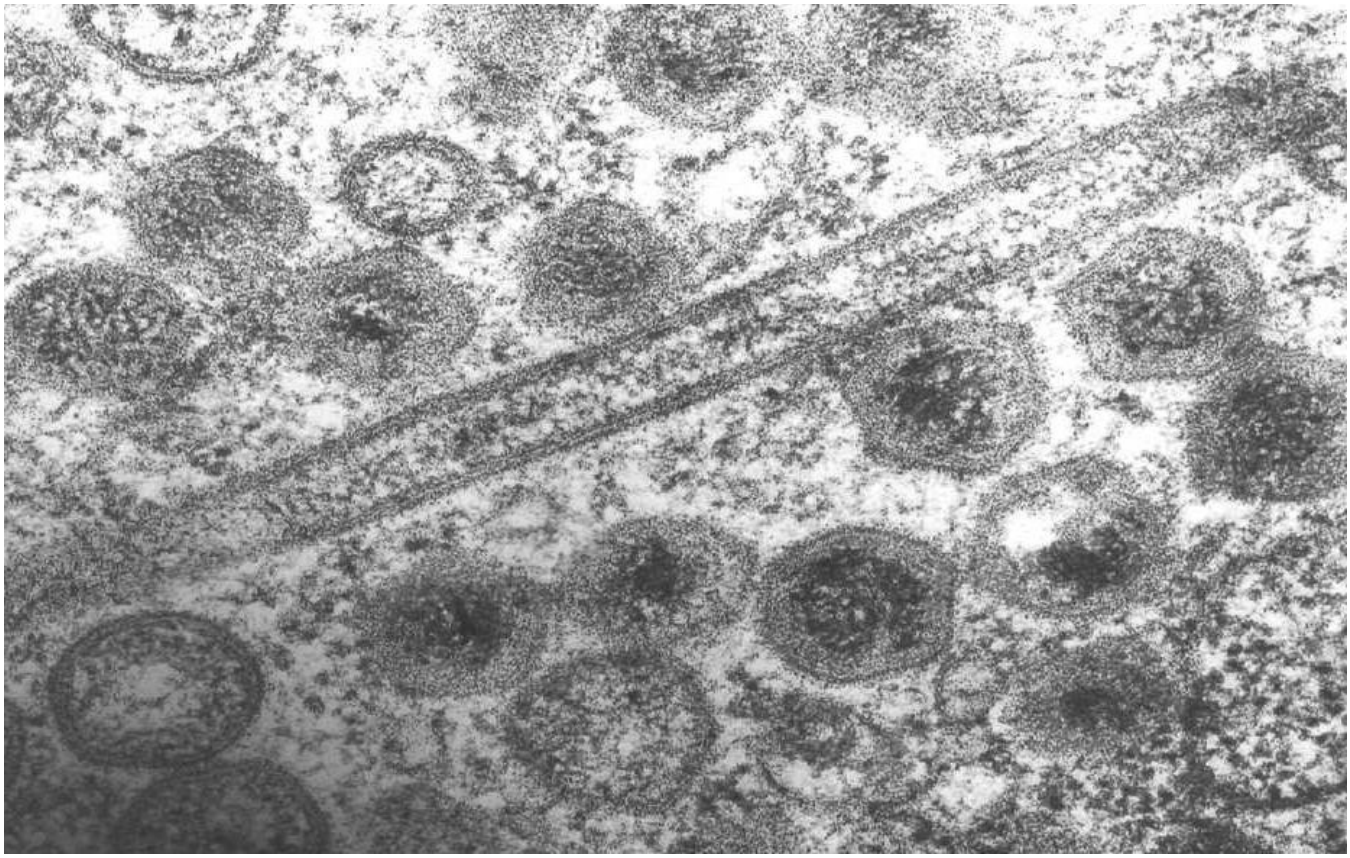

**Figure 3.** The tubular form of the iridovirus from the mosquito *Ae. cantans* is more than 1 μm in length. Diameter of virions is 195 ± 5 nm.

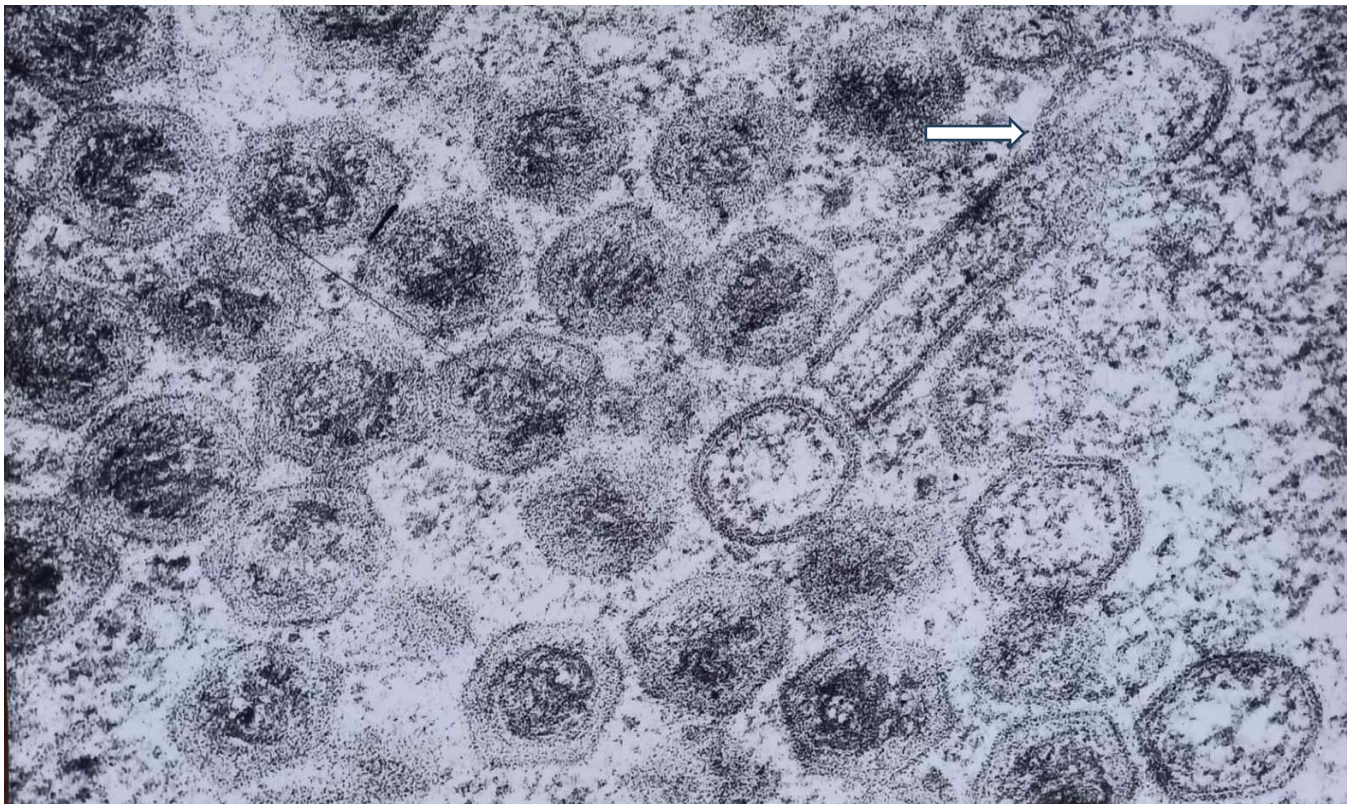

**Figure 4.** The tubular form of the iridovirus of the mosquito *Ae. cantans*, at one end of which a viral capsid is formed. Diameter of virions is 195 ± 5 nm.

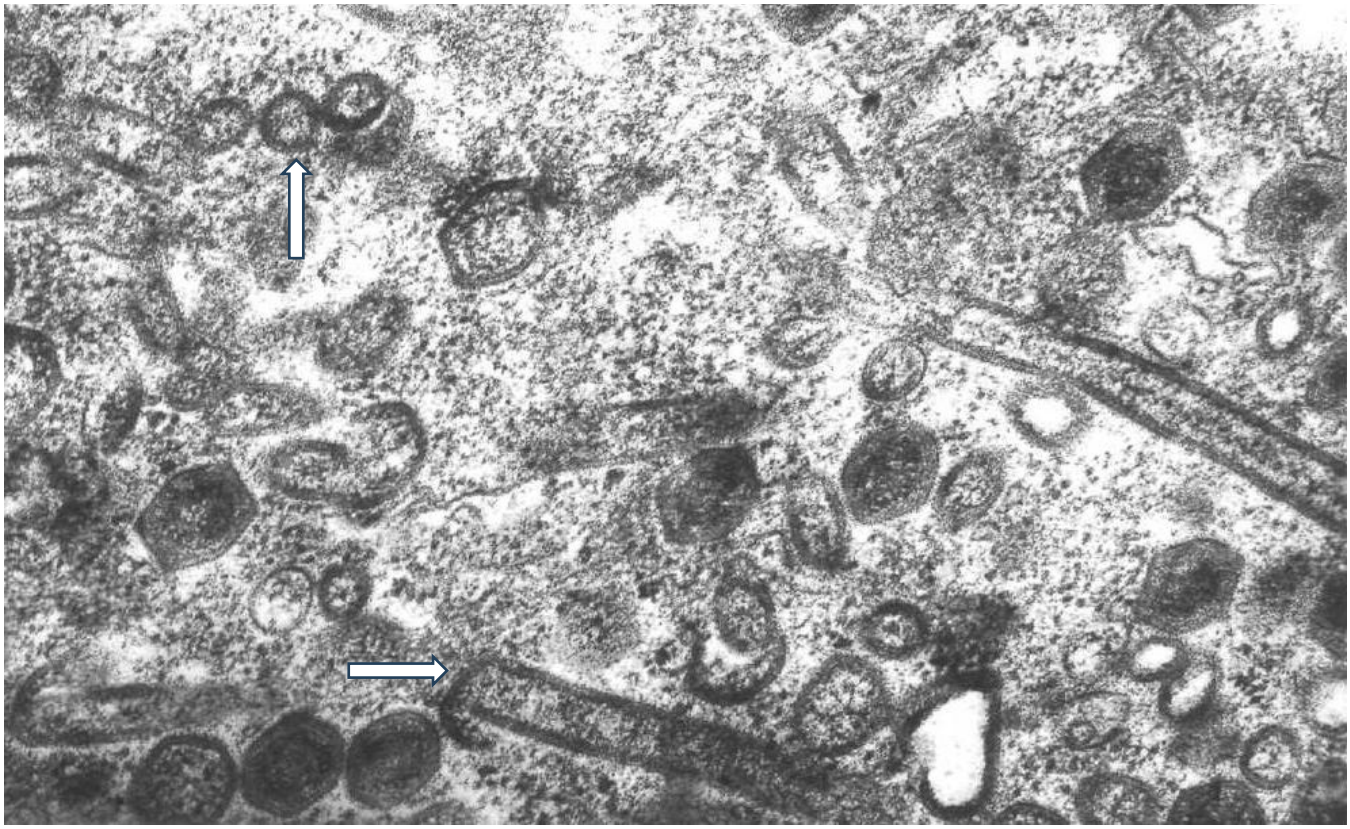

**Figure 5.** Tubular forms of iridovirus of the mosquito *Ae. cantans.* A viral capsid is formed at one end of the tubular form (horizontal arrow). A cross-section of the tubular form of the MIV is marked with a horizontal arrow. Diameter of virions is 195 ± 5 nm.

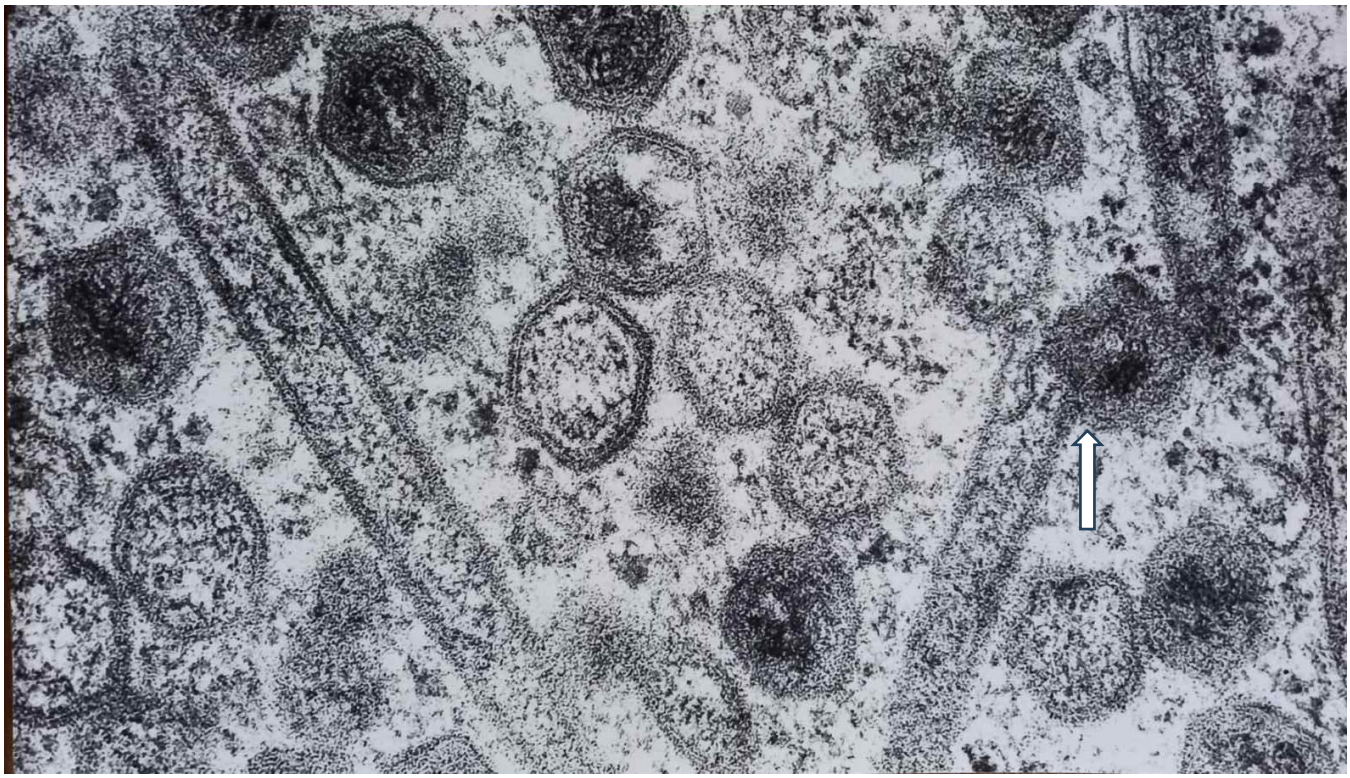

**Figure 6.** Tubular forms of iridovirus of the mosquito *Ae. cantans*. At one of the ends of the tubular form, the viral capsid is formed (arrow). Diameter of virions is 195 ± 5 nm.

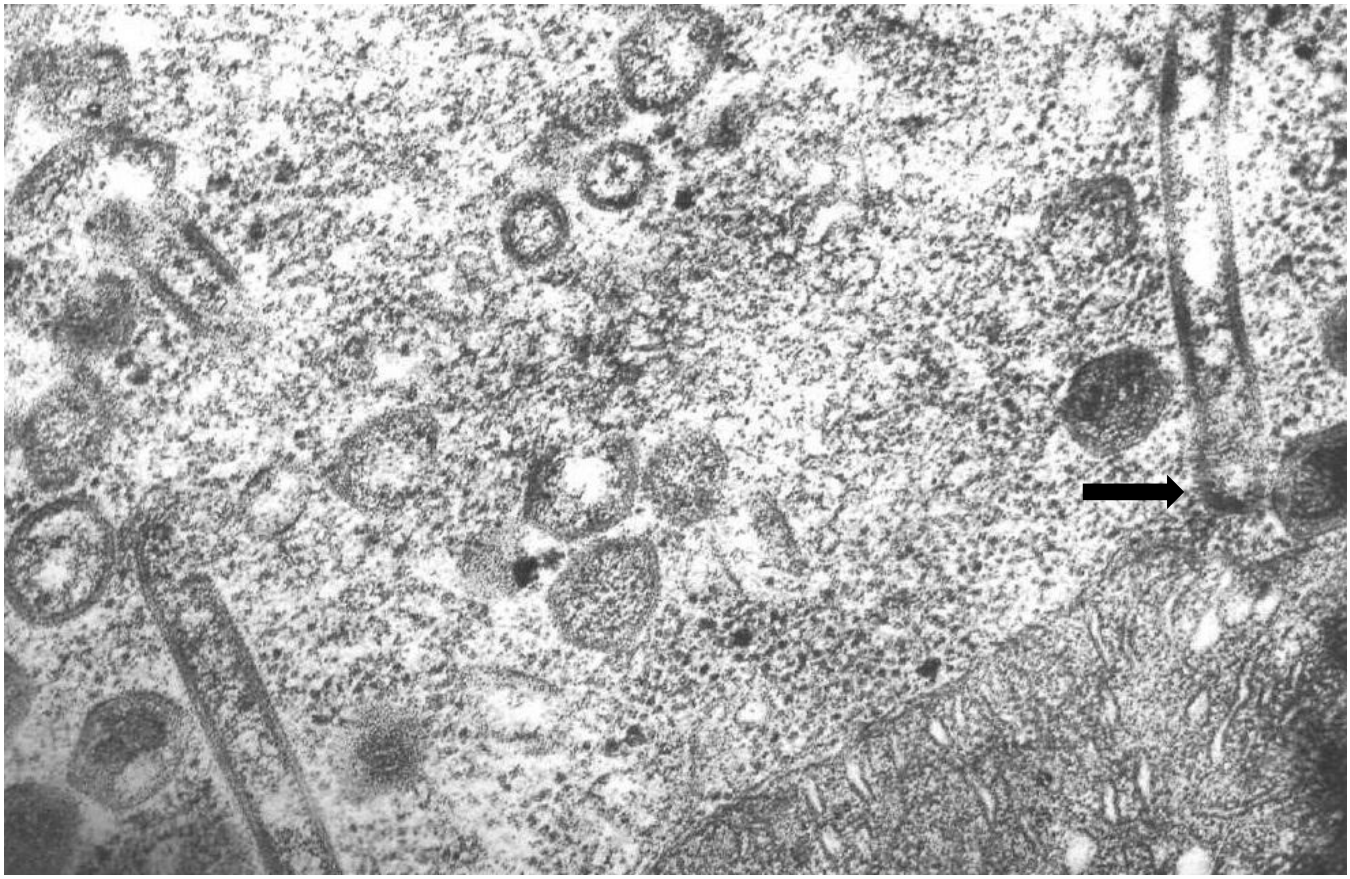

**Figure 7.** The tubular form of the iridovirus of the mosquito *Ae. cantans* is found near the mitochondria with broken cristae. Diameter of virions is 195 ± 5 nm.

The presence of tubular forms of viruses in infected cells has been established for many viruses. They have been described both for plant viruses (Bancroft, Hills and Markham, 1967; Hitchborn and Hills, 1967; Chen et al., 2012) and for animal and human viruses such as African swine fever virus (Epifano et al., 2006), foot-and-mouth disease virus (Ghosh, Borca and Roy, 2002), bovine reovirus (Kimura and Hase, 1987), herpes virus (Iwasaka, Mori and Oda, 1980), alphavirus (Kril et al., 2024), papovaviruses (Arnold, 1979; Keef, Taormina and Twarock, 2005), hepatitis B virus (Neurath et al., 1976), human immunodeficiency virus HIV-1 (Bharat et al., 2014) and other viruses. Until now, the mechanisms of maturation of iridoviruses have not been fully studied. There are two alternative views on this: (1) an envelope forms around a pool of DNA; (2) first, the envelope is formed, then DNA penetrates through the unfinished capsid. However, it is difficult to decide in favor of one of these two mechanisms of virion maturation of iridoviruses. Considering the presence of a large number of enzymes in the composition of iridoviruses (Eaton, Ring and Brunetti, 2010), it can be assumed that both points of view are valid, that is, these two processes can go simultaneously. We also assume that one of the alternative ways of forming the capsid of MIV virions is their 'slicing' from the pool of viral proteins existing in the form of tubes.

We found such tubular forms of MIV not only in mosquitoes *Ae. cantans*, but in other mosquito larvae infected with this virus — *Aedes* (*Ochlerotatus*) *dorsalis* (Meigen, 1830), *Aedes* (*Ochlerotatus*) *caspius* (Pallas, 1771), which indicates their universal importance.

**Conclusions**. During the degradation of MIV, its capsid breaks up into trisymmetrons with 60 nm edge lengths. Each edge contains ten protein capsomeres. There are 1,472 capsomeres in a virion. Tubular forms of the iridovirus were detected in the fat body cells of infected *Aedes* (*Ochlerotatus*) *cantans* mosquito larvae.

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# Part 3. Biosafety

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## ASSESSMENT OF THE SAFETY AND QUALITY OF COMBINED FEED FOR POULTRY FARMS IN UKRAINE IN 2023–2025

**Yaroshenko M. O., Koreneva Yu. M., Boiko V. S., Rudenko O. P., Kovalenko L. V.**

National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Kharkiv, Ukraine, e-mail: margarita.yaroshenko.69@ukr.net

**Summary.** In the context of developing market relations in the livestock sector of agricultural production, ensuring high productivity of animals and poultry and obtaining high-quality products at the lowest cost is the main task. Therefore, violations of feed safety and quality indicators and the search for ways to correct them are relevant. The aim of this study was therefore to identify violations of the most important safety and quality indicators for compound feed in Ukraine's poultry industry between 2023 and 2025. To determine the degree of microfungal contamination, 47 samples of compound feed for agricultural poultry were tested. The results showed that 78.7% of the feed was substandard, while 21.3% was of good quality. The main contaminants in the feed were representatives of the genera *Aspergillus* (38.3%), *Penicillium* (16.4%), *Fusarium* (4.7%), and Mucoraceae (13.7%). Potentially toxin-producing taxa of the genus *Aspergillus* were represented by *A. flavus*, *A. fumigatus*, *A. niger*, *A. glaucus*, and *A. oryzae*; *Penicillium* — *P. lanosum* and *P. commune*; *Fusarium* — *F. moniliforme*; the family Mucoraceae — *Mucor* and *Rhizopus*, and the genus *Trichothecium* — *T. roseum*. Monitoring of the quality indicators of 58 feed samples revealed the following: in compound feeds, there was a decrease in crude protein content by an average of 16.3%, crude fiber content by 33.8%, crude fat content by 15.6%, total calcium concentration by 13.3%, and inorganic phosphorus by 13.4%; in concentrated feed, there was a decrease in crude protein content by an average of 7.0% and an increase in crude fiber content by 11.3%. These results suggest that the sanitary conditions of the feed for agricultural poultry are unsatisfactory, which is probably due to non-compliance with processing regimes for compound feed components and the consequences of military operations in Ukraine

**Keywords:** mold fungi, contamination, compound feed components

**Introduction.** Feed and feed additive safety involves minimizing or eliminating risks at all stages of production, distribution, disposal, and destruction. Risk management to prevent possible harmful effects is carried out during the cultivation and harvesting of feed, and at all stages of the technological processes involved in its production, transportation, and storage.

The use of raw materials from regions affected by particularly dangerous animal diseases for the production of feed and feed additives is prohibited. In such regions, the mandatory removal and destruction of animals, products, and raw materials of animal origin that pose a particular danger to the health and life of animals and humans is carried out.

The harvesting, storage, and transportation of raw materials used in the production of feed and feed supplements must comply with the requirements set out in regulatory acts and normative documents relating to veterinary medicine and food safety (VRU, 2018).

Feeding poultry is an important part of modern poultry production technology. Animal productivity is 55–60% determined by the level and adequacy of feeding, while breed accounts for 25–30%, and keeping methods and technology account for 15–20%. The main condition for achieving high productivity is to organize a complete, high-quality and balanced feeding regimen using diets that meet the birds' needs in terms of essential nutrients and biologically active substances (VRU, 2018; Arroyo-Manzanares et al., 2014; Sentin, 2005).

If feeding conditions do not meet the bird's physiological needs, all types of metabolism will be profoundly disturbed, resulting in decreased resistance and productivity, as well as clinically apparent diseases. Statistics show that a significant proportion of animal diseases are non-contagious and associated with feed poisoning. Providing animals with safe, high-quality feed ensures genetically determined productivity and high feed conversion efficiency (Sentin, 2005; Cegielska-Radziejewska, Stuper-Szablewska and Szablewski, 2013).

Of the many biological contaminants that pollute feed and food products, microscopic fungi are of particular concern. They are potential producers of mycotoxins, which most often affect grain feed and pose a real threat to human and livestock health. Feed quality indicators also remain an important area of research as changes in climatic conditions and agricultural production technologies lead to changes in quality and safety (Cegielska-Radziejewska, Stuper-Szablewska and Szablewski, 2013; Harčárová et al., 2018; Vasjanovych, Ruda and Jangol, 2017; Kutsan et al., 2020; Kyryliuk, 2019).

Poultry feed is most susceptible to the growth of microscopic fungi. The main source of fungal microflora in feed is its plant-based components, primarily grains

(Creppy, 2002; Kwiatek and Kukier, 2008). During the growing season, harvesting, transportation, storage, and preparation for feeding, feed is affected by moldy saprophytes, which give it a darker color and an unpleasant odor. In particular, during storage, the mycobiota of mill waste, grain, and mixed feed consists of facultative parasites and epiphytes capable of toxin production, namely representatives of the genera *Fusarium*, *Penicillium*, *Aspergillus*, *Alternaria*, *Botrytis*, *Helmintosporium*, *Nigrospora*, *Diplodia*, *Sclerotinia*, *Trichoderma*, *Trichothecium*, *Cephalosporium*, *Acremoniella (Monopodium)*, *Mucor*, *Rhizopus*, etc. (Kriuchkova, 1999; Pidoplichko M. and Bilai, 1946; Shareef, 2010; Oliveira et al., 2006).

During the development of mold fungi, the physical properties of feed change, and organic substances decompose to form toxic compounds that can cause poisoning if consumed by poultry. Broilers in particular show clinical signs such as decreased weight gain and appetite, digestive disorders, salivation, constipation or diarrhoea, liver and kidney damage, anorexia, decreased feed conversion efficiency, reduced egg production and poor eggshell quality (Rosa et al., 2006; Cegielska-Radziejewska, Stuper-Szablewska and Szablewski, 2013; Alkhursan, Khudor and Abbas, 2021).

The quality of agricultural raw materials has a significant impact on human health. The production of livestock products depends on various factors, such as the level of breeding work, the adoption of modern animal husbandry technologies, and, most importantly, the development of a high-quality feed base. Developing feed quality control is one of the most important tasks in modern animal nutrition science. Constant monitoring of feed quality violations and finding ways to correct them is important because, in the context of market relations developing in the livestock sector of agricultural production, the main task is to ensure animals and poultry are highly productive and to produce high-quality products at the lowest cost (Ibatullin and Zhukorskyi, 2016).

Poultry farming is particularly susceptible to excessive contamination by microscopic fungi and a decline in feed quality, since compound feed consists mainly of grains and food supplements such as concentrated protein, soybean meal, vitamins and minerals.

Therefore, under martial law, it is particularly relevant to conduct systematic mycological studies of feed and feed raw materials to detect mold saprophytes, as this will not only determine their taxonomic affiliation and identify species that produce toxins, but also contribute to the creation of a system of measures to prevent feed toxicosis in poultry.

Taking the above into account, the research **aimed** to determine the safety and quality of compound feed for poultry farms in various regions of Ukraine between 2023 and 2025, based on priority indicators.

**Materials and methods.** Feed testing was conducted at the Laboratory of Toxicology, Safety and Quality of Agricultural Products and the Laboratory of Clinical Biochemistry in the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Kharkiv, Ukraine).

Forty-seven samples of compound feed for agricultural poultry from farms in Vinnytsia, Poltava, Sumy, and Kharkiv regions were tested for mycological contamination.

The samples were examined in accordance with the generally accepted methods of mycological analysis. In particular, the following were determined:

— the degree of contamination of feed with microscopic fungi, by primary isolation under culturing conditions in agar and Chapek nutrient media; the total number of fungal spores in 1 g of feed, isolated into a pure culture (Obrazhei et al., 1998).

— the species affiliation of microscopic fungal isolates by comparing their cultural and morphological characteristics (e. g. features of growth on different nutrient media, size, shape, width, edge and center structure, growth intensity, surface characteristics, color of colonies and reverse, mycelium, etc.) with descriptions in microfungal identification guides and museum strains of test cultures (Bilai, 1977; Pidoplichko N. and Milko, 1971; Pidoplichko N., 1972; Bilai and Koval, 1988; Danshina, Danshin and Timchuk, 1985).

The research results were interpreted by the 'List of Maximum Permissible Levels of Undesirable Substances in Feed and Feed Materials for Animals' (MAPFU, 2012).

Feed quality indicators (n = 58) were monitored and analyzed on farms in Vinnytsia, Poltava, Sumy, and Kharkiv regions.

The quality of feed was studied based on the crude protein content, which was determined using the Kjeldahl method (ISO, 2005), moisture content (ISO, 1999b), crude fiber content (ISO, 2000), crude fat (ISO, 1999a), calcium (ISO, 1985), phosphorus (ISO, 1998).

**Results and discussion.** Forty-seven samples of compound feed for agricultural poultry were subjected to mycological monitoring and determination of the degree of contamination with microscopic fungi (Fig. 1).

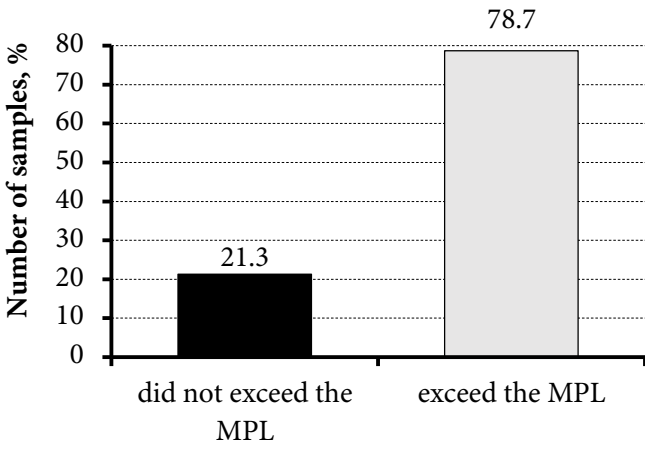


**Figure 1.** Degree of contamination of compound feed for agricultural poultry with micromycetes in 2023–2025.

It was established (Fig. 1) that 37 samples (78.3%) exceeded the permissible level of contamination with mold fungi (more than $5.0\times10^4$ CFU/g of feed), while 10 samples (21.3%) had permissible mycological contamination. When the maximum permissible level (MPL) was exceeded, the total contamination of feed with microscopic fungi ranged from $7.50\times10^4$ CFU/g of feed to $260.0\times10^4$ CFU/g of feed.

When determining the composition of the mycobiota of poultry feed, 342 isolates of mold and yeast-like fungi were isolated and identified (Fig. 2). The main isolates were mold saprophytes of the genera *Aspergillus* — 131 isolates, *Penicillium* — 56 isolates, Mucoraceae — 47 isolates, *Fusarium* — 16 isolates. Representatives of other genera accounted for 92 isolates.

Species identification of isolated mold fungi, taking into account the presence of toxin-producing taxa (Table 1), a large number of which in feed can contribute to increased feed toxigenicity due to the accumulation of secondary metabolites — mycotoxins (Vasjanovych, Ruda and Jangol, 2017; Kutsan et al., 2020; Kyryliuk, 2019; Creppy, 2002), was the next stage of our research.

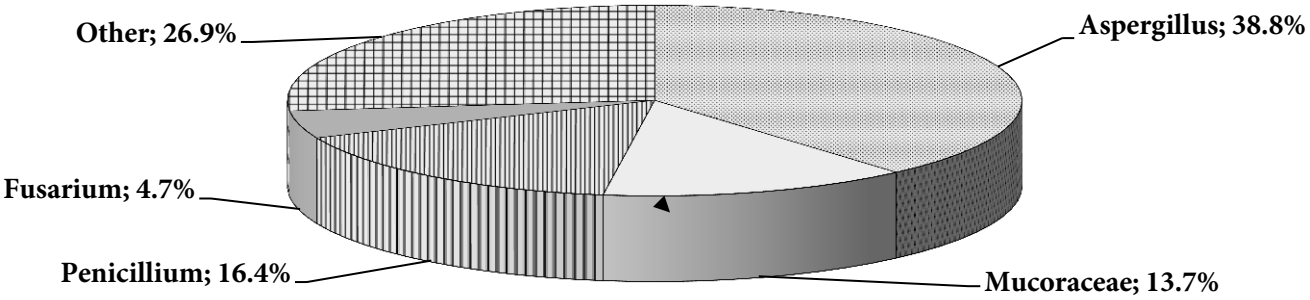


**Figure 2.** Generic composition of mycobiota in compound feed used in the poultry industry in 2023–2025.

**Table 1** — Species composition of toxin-producing micromycetes isolated from feed and feed raw material samples

| Species of toxin-producing micromycetes, name of toxic metabolite | Number of isolates | Total number of isolates within the genus, % |
|---|---|---|
| **Genus *Aspergillus*** | | |
| *A. flavus* — aflatoxins $B_1$, $B_2$, $G_1$, $G_2$, $H_1$, $H_2$, sterigmatocystin, penitrins, tremogens, ochalates, etc. | 48 | 36.6 |
| *A. fumigatus* — aflatoxin, fumigatin, fumitoxins A–D, fumitremorgins, etc. | 27 | 20.6 |
| *A. niger* — aflatoxin, ochalates | 16 | 12.2 |
| *A. glaucus* — aflatoxin, patulin, etc. | 11 | 8.4 |
| *A. oryzae* — aflatoxin, oryzoxolin, maltoricin | 10 | 7.6 |
| *A. amstelodami* — aflatoxin, sterigmatocystin | 6 | 4.6 |
| Other species | 13 | 10.0 |
| Total | 131 | 100.0 |
| **Genus *Penicillium*** | | |
| *P. lanosum* — citrinin | 24 | 42.8 |
| *P. commune* — ochratoxin, penitrem, aflatoxin, etc. | 19 | 33.9 |
| Other species | 13 | 23.3 |
| Total | 56 | 100.0 |
| **Family Mucoraceae** | | |
| Genus *Rhizopus* — aflatoxin, toxic properties | 26 | 55.3 |
| Genus *Mucor* — toxic properties | 21 | 44.7 |
| Total | 47 | 100.0 |
| **Genus *Fusarium*** | | |
| *F. moniliforme* — moniliformin, vomitoxin, T-2 toxin, etc. | 16 | 100.0 |
| **Genus *Trichothecium*** | | |
| *T. roseum* — roseotoxins and trichothecenes | 11 | 100.0 |

Based on the identification of the species of the isolated microscopic fungi (Table 1), it was determined that the predominant species of the genus *Aspergillus*, were the toxic species *A. flavus*, *A. fumigatus*, *A. niger*, *A. glaucus*, *A. oryzae*. The most frequently identified species among the representatives of the genus *Penicillium* were *Pn. lanosum* and *P. commune*. The Mucoraceae family was represented by the *Mucor* and

*Rhizopus* genera. The species *F. moniliforme* was isolated from the genus *Fusarium*.

It should be noted that, over the past three years, representatives of the genus *Trichothecium*, specifically *T. roseum*, have increasingly been identified in experimental compound feeds.

*T. roseum* is known to be saprotrophic and is found in soils in many countries. The main habitats of *T. roseum* are uncultivated soils, forest nurseries, forest soils under beech trees, teak, cultivated soils with legumes, citrus plantations, heaths, dunes, salt marshes, and garden compost. There are about 222 different host plants of *T. roseum* in the world.

*T. roseum* has also been isolated from barley, wheat, oats, corn, apples, grapes, beans, forest nuts, pistachios, peanuts, meat products, cheese, and coffee. *T. roseum* levels in foods other than fruits are usually low (Domsch, Gams and Anderson, 1980).

*T. roseum* produces a wide range of secondary metabolites, including mycotoxins such as roseotoxins and trichothecenes, which are the main mycotoxins found in agricultural products and animal feed. It can act as both a secondary and opportunistic pathogen, causing pink rot on various fruits and vegetables, and thus has an economic impact on the agricultural industry. Secondary metabolites of *T. roseum*, in particular trichothecinol A, are being investigated as potential antimetastatic drugs. *T. roseum* is a source for the production of enzymes (Freeman and Morrison, 1949; Zhang et al., 2010).

Thus, mycological monitoring of 47 samples of compound feed for agricultural poultry revealed an increase in the percentage of poor-quality feed in 2023–2025 compared to our previous studies (Yaroshenko and Kolchyk, 2022), reaching 78.7%. Feed that did not exceed the maximum permissible level of contamination with micromycetes accounted for 21.36%.

The main contaminants in the feed were mold fungi from the genera *Aspergillus* (38.3%), *Penicillium* (16.4%), *Fusarium* (4.7%), Mucoraceae (13.7%), and representatives of other genera accounted for 26.9%. Potentially toxin-producing taxa of the genus *Aspergillus* were represented by *A. flavus*, *A. fumigatus*, *A. niger*, *A. glaucus*, and *A. oryzae*; *Penicillium* — *P. lanosum* and *P. commune*; *Fusarium* — *F. moniliforme*; the family Mucoraceae — *Mucor* and *Rhizopus*, and the genus *Trichothecium* — *T. roseum*.

Thus, the data obtained indicate the unsatisfactory sanitary condition of feed for agricultural poultry. This is probably due to non-compliance with processing regimes for compound feed components (e. g., granulation and extrusion), as well as the consequences of military operations in Ukraine.

Therefore, poultry farms must conduct systematic mycological studies of stored feed at least once every two months to promptly identify spoilage and prevent negative effects on animal health.

According to the literature, mycological contamination of feed significantly impacts its zoochemical composition, including the destruction of proteins and vitamins, the acceleration of fat oxidation processes, and the reduction of feed digestibility (Chhaya, O'Brien and Cummins, 2021).

We therefore monitored the quality of feed (both concentrated and mixed feed for different age groups of poultry) from farms in various regions of Ukraine. It was found that 68% of the feed complied with current regulatory documents, while 32% was of poor quality for feeding (Klitsenko, 2001).

Analysis of the obtained data showed that the highest percentage of quality violations was found in compound feed intended for young laying hens aged between one and eight weeks (Fig. 3).

Chemical analysis of compound feed for young laying hens aged 1–8 weeks revealed an increase in moisture content of 5.2%, an increase in crude fibre content of 48.7%, and a decrease in crude protein content of 18.0%, as well as decreases in crude fat content of 13.4%, total calcium concentration of 12.6%, and inorganic phosphorus content of 17.5%. When analysing compound feed for young laying hens aged 9–17 weeks, a decrease was observed in the content of crude protein by 17.0%, crude fat by 9.4%, total calcium by 20.8% and inorganic phosphorus by 15.6%, as well as an increase in the content of crude fibre by 18.9%. The moisture content was within the limits established by DSTU 4120-2002 (DSSU, 2003).

In samples of compound feed for young laying hens aged 18–22 weeks, a decrease in the content of all the studied nutrients was determined: crude protein by 14.0%, crude fat by 24.0%, crude fibre by 6.6%, total calcium concentration by 6.5%, inorganic phosphorus by 7.2% and moisture by 11.0%. According to the literature, a decrease in calcium and phosphorus concentrations affects bone tissue formation and calcification, resulting in changes to other systems in the animal's body.

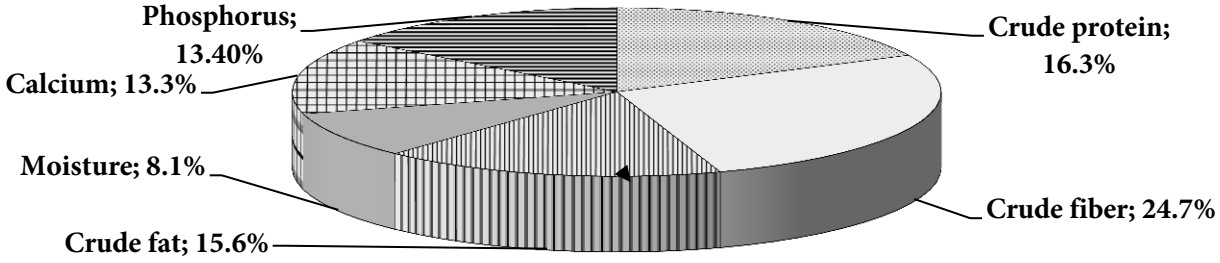


**Figure 3.** Analysis of the quality of compound feed for young laying hens.

When analyzing compound feed for 1–3-week-old broilers (Fig. 4), a decrease in crude protein content by an average of 30.2% was found in all samples, an increase in fiber content by 43.7% and crude fat by 16.2% relative to the reference level. According to the literature, an increase in fat in feed with a simultaneous decrease in protein can cause disorders in the digestive system, leading to the development of fatty liver dystrophy (Loza, 2012).

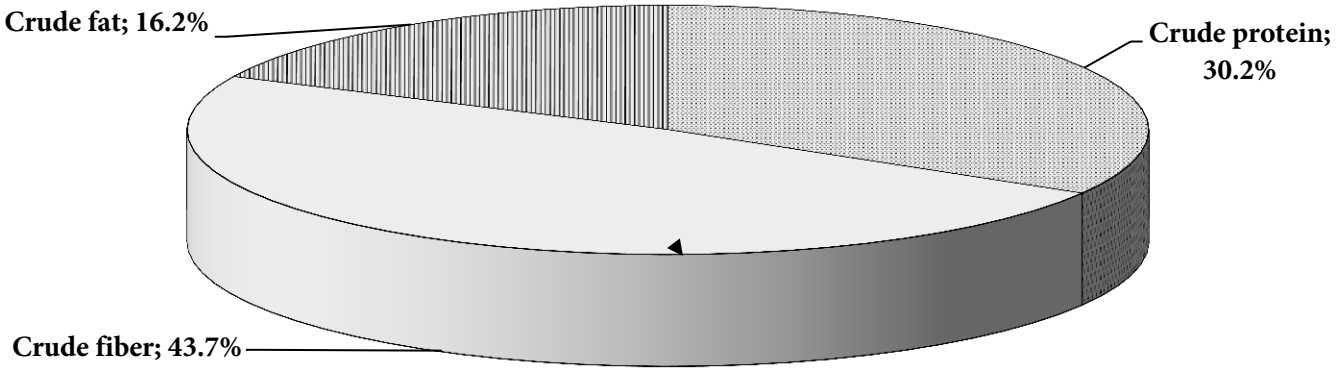


**Figure 4.** Analysis of the quality of compound feed for 1–3-week-old broilers.

As a result of establishing changes in the quality of compound feed for all age groups of poultry, the quality of the constituent components was monitored. The results show that 10.0% of the tested samples (including soybean and sunflower meal, wheat and corn grain) did not comply with current regulatory documents. In soybean meal samples, for example, an average decrease in crude protein content of 10.0% was noted. Sunflower meal samples showed an average decrease in crude protein content of 8.0% and an average increase in crude fibre content of 11.3%. Chemical analysis of grain samples revealed a slight decrease in crude protein content in wheat (3.0%) and an increase in crude fibre content (5.8%), and a decrease in crude protein content in corn grain (30.0%) and an increase in crude fibre content (16.8%). Based on these findings, it is evident that the components of the compound feed contain low-quality protein, which can negatively impact the digestibility and absorption of the feed. Additionally, poor-quality components are difficult to dose accurately, leading to an imbalance of essential nutrients (Kulik et al., 2020).

Therefore, monitoring feed quality through chemical analysis enables the creation of diets with high nutritional value, ensuring the provision of all necessary nutrients and facilitating rapid live weight gain and increased poultry productivity.

**Conclusions**. 1. Mycological monitoring of 47 samples of compound feed for agricultural poultry showed that the percentage of substandard feed increased in 2023–2025 and amounted to 78.7%. Feed that did not exceed the maximum permissible level of contamination with micromycetes accounted for 21.36%.

2. The main feed contaminants were representatives of mold fungi of the genera *Aspergillus* (38.3%), *Penicillium* (16.4%), *Fusarium* (4.7%), Mucoraceae (13.7%),, and representatives of other genera accounted for 26.9%. Potentially toxin-producing taxa of the genus *Aspergillus* were represented by *A. flavus*, *A. fumigatus*, *A. niger*, *A. glaucus*, and *A. oryzae*; *Penicillium* — *P. lanosum* and *P. commune*; *Fusarium* — *F. moniliforme*; the family Mucoraceae — *Mucor* and *Rhizopus*, and the genus *Trichothecium* — *T. roseum*.

3. As a result of monitoring the quality of feed, non-compliance with regulatory documents was established for the following indicators:

— compound feed: a decrease in crude protein content by an average of 16.3%, crude fibre content by 33.8%, crude fat content by 15.6%, total calcium concentration by 13.3%, and inorganic phosphorus by 13.4%;

— concentrated feed, there was a decrease in crude protein content by an average of 7.0%, and an increase in crude fibre content by 11.3%.

4. The results obtained indicate the unsatisfactory sanitary condition of feed intended for agricultural poultry. This is not only associated with non-compliance with processing regimes for compound feed components, but also with the consequences of military operations in Ukraine.

**Prospects for further research** lie in the systematic monitoring of safety and quality indicators for feed raw materials and feed used in poultry farming to prevent their negative impact on animal health and productivity and reduce economic losses in the poultry industry.

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**2025 © Yaroshenko M. O. 0000-0001-9040-6474, Koreneva Yu. M. 0000-0001-9401-7732, Boiko V. S. 0000-0002-8137-3399, Rudenko O. P. 0000-0001-5736-0654, Kovalenko L. V. 0000-0003-1856-1298**

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# INNOVATIVE METHODS OF DISINFECTING LIVESTOCK FACILITIES

**Paliy A. P. [1], Sumakova N. V. [1], Keleberda M. I. [1], Yemelianov A. V. [1], Pavlichenko O. V. [2]**

[1] National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Kharkiv, Ukraine, e-mail: paliy.dok@gmail.com
[2] State Biotechnological University, Kharkiv, Ukraine

**Summary.** Parasitic diseases in animals are widespread throughout the world and cause significant economic losses to the livestock industry. The most effective and economically justified measure for preventing these diseases among susceptible livestock is to implement high-quality veterinary and sanitary measures. To achieve this, it is essential to use effective disinfectants that have been proven to work in both laboratory and production environments. The work was carried out at the Laboratory of Veterinary Sanitation, Parasitology and Bee Diseases Study in the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Kharkiv, Ukraine). The effectiveness of the disinfectants was determined in accordance with existing regulatory documents. Based on these results, a method for disinfecting livestock facilities was developed. This method involves using a preparation containing peroxyacetic acid, hydrogen peroxide, acetic acid, stabilizing additives, and water. The exposure time ranges from 6 to 48 h, and the consumption rate is 500 ml/m². We propose a disinfection method involving a disinfectant containing potassium monopersulfate, sodium dichloroisocyanurate, sodium hexametaphosphate, sulfamic acid, malic acid, sodium alkylbenzyl sulfonate, sodium sulfate, and water. The exposure time is 3 h, and the consumption rate is 300 ml/m². Another method involves a preparation containing a mixture of quaternary ammonium compounds, glutaraldehyde, isopropyl alcohol, nonionic surfactants, and deionized water. This method requires an exposure time of 3–24 h and a consumption rate of 500 ml/m². The disinfectant, containing didecyldimethylammonium chloride, glutaraldehyde, benzalkonium chloride, surfactants, orthophosphoric acid, and water, has been proven effective at a 72-hour exposure rate of 500 ml/m² for soil disinfection. The proposed disinfection methods have been proven to meet biosafety and bioprotection requirements, and are easy to use, environmentally friendly, highly effective, and cost-effective. The results presented in this article significantly supplement existing sanitary and hygienic protocols in animal husbandry. Further research should focus on developing a comprehensive, scientifically based, integrated system for protecting farm animals

**Keywords:** disinfectant, concentration, exposure, soil, disinfection, effectiveness, helminth eggs

**Introduction.** In light of climate change and changes in livestock farming practices, addressing the issue of parasitic diseases in animals is becoming particularly relevant (Amoah et al., 2018; Bogach et al., 2020). Despite the success in controlling human and animal diseases, parasitic diseases remain a leading health concern (Kwong et al., 2021; Tadege et al., 2022; Elghryani et al., 2023). A variety of antiparasitic agents are used to treat parasitized animals. The effectiveness of these agents depends on the type of parasite, the animal's overall health, and the range of antiparasitic activity (Rolbiecki and Izdebska, 2024; Zhang et al., 2024). An infected animal is a constant source of pathogens and poses a threat to healthy livestock. Thus, a significant level of sanitary contamination with exogenous helminth forms (from 21.7% to 45.6%) and soil contamination (from 20% to 36.6%) was found during the examination of livestock facilities, including a pig farm, sheep farm, dairy farm, and dog training center (Paliy et al., 2018a). According to other reports, 41.3% of soil samples are contaminated with helminth eggs. The parasites detected were *Ascaris* spp. (39.0%), *Trichuris* spp. (26.0%), Ancylostoma/Strongyloides (22.0%), *Toxocara* spp. (4.0%), *Taenia* spp. (3.0%), and unidentified eggs (6.0%) (Paller and Babia-Abion, 2019). Gurmassa et al. (2023) report an even higher level of helminth contamination in the environment. According to their data, helminth eggs were present in 57.1% of soil samples and 18.6% of fecal samples. The most common types of helminths were *Ascaris lumbricoides* and *Trichuris trichiura* hookworms. In Kenya, 26.8% of households have soil contaminated with one or more types of helminth eggs, with *A. lumbricoides* being the most common (19.4%) (Steinbaum et al., 2016). In Chile, at least one parasite egg was found in 24.21% of the studied samples (Castro-Seriche, Fernández and Landaeta-Aqueveque, 2020). Additionally, 52% of 29 different source samples were found to have a prevalence of potentially infectious helminth eggs (Grego et al., 2018). The widespread distribution of helminth parasites and their potential danger to livestock necessitate antiparasitic measures, such as disinfecting the environment (Paliy, 2018). Various chemical compounds are used for this purpose, differing in composition, physicochemical and toxicological characteristics, and spectrum of antimicrobial activity (Ponomarenko et al., 2021; Paliy et al., 2024b). At the same time, it has been reported that the treatment of *A. suum* eggs with many commercially available disinfectants does not affect embryogenesis. While some disinfectants can delay or stop the development of invasive *A. suum* eggs, they are unlikely to completely kill them (Oh et al., 2016).

Therefore, it is crucial to identify effective disinfectants that align with modern livestock farming requirements, offering high efficacy and cost-effectiveness.

The study **aimed** to develop methods for disinfecting livestock facilities contaminated with exogenous helminth forms using innovative disinfectants.

**Materials and methods.** Experimental studies were conducted at the Laboratory of Veterinary Sanitation, Parasitology and Bee Diseases Study in the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Kharkiv, Ukraine).

Disinfectants with different contents of active and auxiliary substances were used to evaluate the effectiveness of disinfection and develop disinfection methods.

Disinfectant No. 1 contains peracetic acid, hydrogen peroxide, acetic acid, stabilizing additives, and water.

Disinfectant No. 2 contains potassium monopersulfate, sodium dichloroisocyanurate, sodium hexametaphosphate, sulfamic acid, malic acid, sodium alkylbenzyl sulfonate, and sodium sulfate in water.

Disinfectant No. 3 contains quaternary ammonium compounds (QACs), glutaraldehyde, isopropyl alcohol, nonionic surfactants, and deionized water.

Disinfectant No. 4 contains didecyldimethylammonium chloride, glutaraldehyde, benzalkonium chloride, surfactants, orthophosphoric acid, and water.

The effectiveness of the disinfectants was determined under current regulatory documents (Paliy et al., 2020b).

**Results and discussions.** The planned research evaluated the effectiveness of innovative disinfectants from various chemical groups for use in animal husbandry. Based on the results, scientifically proven methods of applying disinfectants were developed, along with appropriate usage methods.

First, a disinfectant composition containing optimal proportions of peracetic acid, hydrogen peroxide, acetic acid, stabilizing additives, and water was selected for the disinfection of *Ascaris suum* eggs.

Before disinfection, livestock facilities are cleaned of manure, feed residue, and debris. Technological equipment is cleaned of technical and organic contamination. The floor, ceiling, and walls are washed with pressurized water. After the water is removed from the premises, disinfection is carried out using a product with different compositions, exposure times, and consumption rates of 500 ml/m$^2$ (Table 1).

Composition 1: peroxyacetic acid — 0.2%, hydrogen peroxide — 0.3%, acetic acid — 0.48%, stabilizing additives — 0.1%, water — 98.92%.

Composition 2: peroxyacetic acid — 0.3%, hydrogen peroxide — 0.45%, acetic acid — 0.72%, stabilizing additives — 0.15%, water — 98.38%.

Composition 3: peroxyacetic acid — 0.4%, hydrogen peroxide — 0.6%, acetic acid — 0.96%, stabilizing additives — 0.2%, water — 97.84%.

Composition 4: peroxyacetic acid — 0.5%, hydrogen peroxide — 0.75%, acetic acid — 1.2%, stabilizing additives — 0.25%, water — 97.3%.

As shown in Table 1, disinfectants 1 and 2 are ineffective against exogenous helminth contamination in livestock facilities. Meanwhile, disinfectants 3 and 4 completely disinfect treated surfaces contaminated with animal helminth eggs when exposed for 48 h and applied at a rate of 500 ml/m$^2$. No corrosion was observed when the treated surfaces were examined after contact with the disinfectant.

**Table 1 —** Disinfection activity of disinfectant No. 1

| Composition | Terms for determining the viability of *Ascaris suum* eggs, days | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3 | | | 6 | | | 14 | | | 21 | | | 28 | | |
| | Exposure, h | | | | | | | | | | | | | | |
| | 6 | 24 | 48 | 6 | 24 | 48 | 6 | 24 | 48 | 6 | 24 | 48 | 6 | 24 | 48 |
| 1 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 2 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 3 | + | + | + | + | + | + | + | + | – | + | + | – | + | + | – |
| 4 | + | + | + | + | + | + | + | + | – | + | + | – | + | + | – |

Notes: '–' — egg death; '+' — egg development.

Based on these results, a Ukrainian patent for a utility model No. 144297 was obtained (Paliy et al., 2020a).

Such disinfectants are widely used in various fields (De Rezende, De Lima and Santos, 2023). The effectiveness of hydrogen peroxide-based disinfectants can reportedly be increased by adding silver and copper nanoparticles (Orta De Velásquez et al., 2008). The disinfecting effect of hydrogen peroxide can be enhanced through a synergistic effect with ozone (Ibáñez-Cervantes et al., 2024).

The next task involves selecting a disinfectant composition that contains potassium monopersulfate, sodium dichloroisocyanurate, sodium hexametaphosphate, sulfamic acid, malic acid, sodium alkylbenzyl sulfonate, sodium sulfate, and water. The task also involves developing a method for disinfecting veterinary control facilities. This method includes treating the facilities with a disinfectant by spraying it for 3 h at a consumption rate of 300 ml/m$^2$.

After pre-disinfection, mechanical cleaning, and water removal, disinfection is carried out with the preparation (Table 2).

Composition 1: potassium monopersulfate — 0.25%, sodium dichloroisocyanurate — 0.0125%, sodium hexametaphosphate — 0.125%, sulfamic acid — 0.0375%, malic acid — 0.0375%, sodium alkylbenzyl sulfonate — 0.025%, sodium sulfate — 0.0125%, water — 99.5%.

Composition 2: potassium monopersulfate — 0.5%, sodium dichloroisocyanurate — 0.025%, sodium hexametaphosphate — 0.25%, sulfamic acid — 0.075%, malic acid — 0.075%, sodium alkylbenzyl sulfonate — 0.05%, sodium sulfate — 0.025%, water — 99.0%.

Composition 3: potassium monopersulfate — 0.75%, sodium dichloroisocyanurate — 0.0375%, sodium hexametaphosphate — 0.375%, sulfamic acid — 0.1125%, malic acid — 0.1125%, sodium alkylbenzyl sulfonate — 0.075%, sodium sulfate — 0.0375%, water — 98.5%.

**Table 2 —** Disinfection activity of disinfectant No. 2

| Composition | Terms for determining the viability of *Ascaris suum* eggs, days | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3 | | | 6 | | | 14 | | | 21 | | | 28 | | |
| 1 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |

Notes: '–' — egg death; '+' — egg development.

As shown in Table 2, the disinfectant preparation of composition 1 is ineffective against veterinary control objects contaminated with exogenous helminth forms. Disinfectants with compositions 2 and 3 were proven to completely disinfect treated surfaces contaminated with animal helminth eggs when exposed for 3 h at a consumption rate of 300 ml/m². Upon inspection of the treated surfaces after contact with the disinfectants, no corrosion or discoloration was observed.

Based on these results, a Ukrainian patent for a utility model No. 156093 was obtained (Paliy et al., 2024a).

Our results expand the range of applications for disinfectants containing the active ingredient monopersulfate for disinfestation purposes. The effectiveness of this group of disinfectants against bacteria and viruses has previously been established (Sonthipet, Ruenphet and Takehara, 2018). These preparations are widely used in human medicine (Umemura et al., 2022).

Ren et al. (2024) reported that peracetic acid, hydrogen peroxide, and potassium monopersulfate are suitable for low-temperature environmental disinfection.

To develop a method for disinfecting surfaces contaminated with *Ascaris suum* eggs, the effectiveness of various compositions of active ingredients was investigated. These compositions contained a mixture of quaternary ammonium compounds (QACs), glutaraldehyde, isopropyl alcohol, nonionic surfactants (SASs), and deionized water. The exposure time ranged from 3 to 24 h, and the consumption rate was 500 ml/m².

After pre-disinfection, mechanical cleaning, and removal of water, the preparation is applied (Table 3).

Composition 1: mixture of QACs — 0.5%, glutaraldehyde — 0.22%, isopropyl alcohol — 0.16%, nonionic surfactants (SAS) — 0.1%, deionized water — 99.02%.

Composition 2: mixture of QACs — 0.75%, glutaraldehyde — 0.33%, isopropyl alcohol — 0.24%, nonionic surfactants (SAS) — 0.15%, deionized water — 98.53%.

Composition 3: mixture of QACs — 1.0%, glutaraldehyde — 0.44%, isopropyl alcohol — 0.32%, nonionic surfactants (SAS) — 0.2%, deionized water — 98.04%.

As shown in Table 3, treatment of the *Ascaris suum* test culture with preparations 1 and 2 did not affect the development of *Ascaris suum* eggs. Conversely, it was determined that preparation No. 3 delayed the development of the test culture eggs and ultimately led to their death; thus, the agent exhibited disinfestation properties.

**Table 3 —** Disinfection activity of disinfectant No. 3

| Composition | Terms for determining the viability of *Ascaris suum* eggs, days | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3 | | | 6 | | | 14 | | | 21 | | | 28 | | |
| | Exposure, h | | | | | | | | | | | | | | |
| | 3 | 6 | 24 | 3 | 6 | 24 | 3 | 6 | 24 | 3 | 6 | 24 | 3 | 6 | 24 |
| 1 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 2 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |

Notes: '–' — egg death; '+' — egg development.

Based on these results, a Ukrainian patent for a utility model No. 130430 was obtained (Paliy et al., 2018b).

The following utility model is based on developing a method to disinfect soil contaminated with *Toxocara canis* eggs. This method uses a disinfectant containing didecyldimethylammonium chloride, glutaraldehyde, benzalkonium chloride, surfactants (SAS), orthophosphoric acid, and water. The exposure time is 72 h, and the consumption rate is 500 ml/m².

After pre-disinfection mechanical cleaning and removal of water from the premises, disinfection is carried out using the following preparations (Table 4):

Composition 1: didecyldimethylammonium chloride — 0.0675%, glutaraldehyde — 0.45%, benzalkonium chloride — 0.24%, surfactants (SAS), orthophosphoric acid — 2.2425%, water — 97%.

Composition 2: didecyldimethylammonium chloride — 0.090%, glutaraldehyde — 0.60%, benzalkonium chloride — 0.32%, surfactants (SAS), orthophosphoric acid — 2.990%, water — 96.0%.

Composition 3: didecyldimethylammonium chloride — 0.1125%, glutaraldehyde — 0.75%, benzalkonium chloride — 0.40%, surfactants (SAS), orthophosphoric acid — 3.7375%, water — 95%.

**Table 4 —** Disinfection activity of disinfectant No. 4

| Composition | Terms for determining the viability of *Toxocara canis* eggs, days | | | | |
|---|---|---|---|---|---|
| | 3 | 6 | 14 | 21 | 28 |
| | Exposure, h / consumption rate, ml/m² | | | | |
| | 72 / 500 | 72 / 500 | 72 / 500 | 72 / 500 | 72 / 500 |
| 1 | + | + | + | + | + |
| 2 | – | – | – | – | – |
| 3 | – | – | – | – | – |

Notes: '–' — egg death; '+' — egg development.

As shown in Table 4, treating soil contaminated with *T. canis* eggs with compound 1 did not affect the development of the eggs. Conversely, it was found that applying compositions 2 and 3 for 72 h at a rate of 500 ml/m² delayed the development of *T. canis* eggs in the test culture and resulted in their death. In other

words, the compositions exhibited disinfection properties.

Based on these results, a Ukrainian patent for a utility model No. 137488 was obtained (Paliy et al., 2019).

Khorolskyi (2022) also recommends using glutaraldehyde-containing disinfectants to control and prevent rabbit pasalurosis, as they effectively disinfect environmental objects and animal housing facilities.

The results of the studies show that the proposed disinfestation methods meet modern agro-industrial production requirements, are effective and environmentally safe, and are economical and easy to use.

It is well-known that helminthiasis spreads among susceptible animals on livestock farms and complexes when veterinary and sanitary standards are not followed. This requires the scientifically-based use of highly effective deworming and disinfection agents (Paliy et al., 2018a; Labana et al., 2024).

Many antimicrobial agents are available for disinfection in animal husbandry, presenting practitioners with the task of selecting the most effective and cost-efficient option (Paliy et al., 2020b; Tyski, Bocian and Laudy, 2024). However, not all disinfectants meet modern requirements for effective disinfection in animal husbandry. Of the disinfectants tested, solutions containing 3.0% cresol, 0.2% sodium hypochlorite, and 0.02% sodium hypochlorite delayed, but did not prevent, the embryonation of purified *A. suum* eggs after three weeks of incubation. After six weeks, undeveloped eggs completed embryonation regardless of exposure time, except when 10.0% povidone-iodine was used. Nevertheless, a 10.0% povidone-iodine solution inactivates most eggs after 5 minutes, although it never completely kills them, even after 60 minutes of exposure (Oh et al., 2016). Therefore, prior evaluation of a disinfectant is required, along with the development of regulations for its use.

Based on the results of our research, we have established effective application methods for four modern disinfectants to destroy exogenous helminth forms.

Using the proposed disinfection methods will improve the effectiveness of health and preventive measures against parasitic diseases in susceptible animals in agricultural enterprises.

**Conclusions.** Regimens for using aldehyde- and acid-containing disinfectants to decontaminate livestock facilities contaminated with exogenous helminths have been developed. These regimens can be used on livestock farms and complexes.

These methods of disinfection in animal husbandry using these agents meet environmental protection requirements and ensure the production of safe, high-quality animal products. They are also easy to use, highly effective, and cost-effective.

Results obtained from the practical implementation of these methods will allow us to propose new, effective disinfection protocols.

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**2025 © Paliy A. P. 0000-0002-9193-3548, Sumakova N. V. 0000-0002-6092-6054, Keleberda M. I. 0009-0008-9144-5908, Yemelianov A. V. 0009-0009-6728-6692, Pavlichenko O. V. 0000-0002-6577-6577**

# Contents

## Part 1. Veterinary medicine



## Part 2. Biotechnology



## Part 3. Biosafety