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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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FORENSIC VETERINARY ASSESSMENT OF THE EXPERT INFORMATIVENESS OF BIOTRANSFORMATION PATTERNS OF DOG AND CAT CORPSES IN VARIOUS STATES OF DECOMPOSITION

Kazantsev R. H., Yatsenko I. V.

State Biotechnological University, Kharkiv, Ukraine, e-mail: trilobite@ukr.net

Summary. Currently, there is no universal algorithm for determining the time of death of an animal. The purpose of the study was to provide a comprehensive argumentation of the forensic veterinary diagnostic significance of the biotransformation phenomena of 28 dog and cat corpses with justification based on their thorough assessment of expert criteria for the duration of postmortem intervals. The study used special and logical-philosophical methods: physical, observation, cyto/histomorphological, forensic veterinary autopsy, analysis, synthesis, deduction, and induction. Early mortalities: rigor mortis, drying, spots, cooling, and late mortalities: decay, skeletalization, fragmentation, patterns of biotransformation, their time ranges, and morphological characteristics are identified. The criterion informativeness of the 'idiomuscular' and 'pupillary' supravital reactions has been proved. The dynamics of disorganization of venous blood of dog and cat corpses within 48 h after death was determined. The sequence of postmortem succession by the entomofauna is shown. According to the concept of 'evidence-based' veterinary medicine, the key stages of postmortem decomposition of dog and cat corpses at different levels of structural organization are illustrated. Based on the analysis of the results of the empirical study, it is substantiated that in the interval of more than 72 h from the moment of death, the answers to the questions in the expert's opinion, due to the large number of complex processes that occur in the tissues of dog and cat corpses, are often only probable

Keywords: forensic veterinary thanatology, corpse phenomena, postmortem intervals, postmortem decomposition, prescription of death, animals

Introduction. In recent years, forensic veterinary examination has rapidly developed into a distinct area of veterinary practice due to increasing public awareness of animal cruelty and health crimes. Despite the pronounced lag in the development of forensic veterinary medicine compared to forensic medicine, over the past decade forensic veterinary thanatology has been significantly enriched by numerous facts that expand our understanding of the biotransformation of animal carcasses, and in-depth research in this area is catalyzing the accumulation of experience and expansion of the evidence base for determining the cause of death and prescribing its onset (Parry and Stoll, 2020). In the context of animal cruelty issues, Yamada et al. (2023) suggest that a 'virtual' autopsy using radiation research methods should be performed on all sub-expert corpses in criminal cases.

The determination of the time of death of an animal remains a priority task in the forensic veterinary examination of its corpse as questions from pretrial investigation bodies regarding the duration of the postmortem period are found in the vast majority of decisions. In most forensic cases, the approximate time and circumstances of the animal's death are known from the case file, but in some cases, especially when it is not obvious, determining the duration of the post-mortem period has a significant impact on the further course of the investigation and helps to solve crimes against animals that led to their death. Taking into account the different nature and circumstances of the animal's death, as set out in the investigator's decision, the most correct approximation of the duration of the post-mortem interval is one of the most important problems of forensic veterinary medicine.

Many researchers have devoted their work to determining the criterion informativeness of early and late corpse phenomena, structural changes in organs and tissues, biochemistry of animal body fluids, thermometry, supravital reactions, saprotrophic fauna, and animal corpses in various states, including fragmented, skeletalized, and drowned corpses, to establish the duration of the postmortem period. Among them: Sanford (2015) notes the differences in entomological species colonizing human and animal bodies, which can be used to establish postmortem intervals; Brooks (2016) in a systematic review considered the process of biotransformation of animal corpses and methods of its control; Touroo and Fitch (2016) report that for the correct analysis of postmortem phenomena, it is necessary to focus on information and evidence during a thorough examination of the site of the animal corpse; Omond et al. (2017) reports on the prevalence of postmortem necrophagy of dog corpses by their own kind; Listos et al. (2018) evaluated postmortem thermometry of dog corpses; Piegari et al. (2019) studied the animal corpses that died as a result of drowning;

Panasiuk-Flak et al. (2021) determined the diagnostic informativeness of supra-rectal pupillary reactions of dog corpses; Stern and Muralidhar (2022) analyzed the dynamics of electrolyte, creatinine, and urea concentrations in the blood and vitreous of cats, dogs, and horses; Li et al. (2022) determined the entomofauna of dog corpses.

The issue of the expert value of postmortem changes in organs and tissues for forensic veterinary diagnosis of the time and circumstances of animal death has been discussed by many scientists. For example, the dynamics of postmortem decomposition of artificially injured skeletal muscles of dog corpses under the influence of sea water was substantiated (Stacy, Costidis and Keene, 2015); the dynamics of postmortem proteolysis in the pectoral muscle of a female duck was established (Liao et al., 2016); the possibility of estimating the time of death of animals by the development of microflora in the calf muscle of a dog was found (Listos et al., 2017); the metabolic profile of the rat thigh muscle was characterized at different periods after death (Du et al., 2018); the relationship between electrical conductivity and chemical content of the solution for skeletal muscle impregnation in rats to determine the duration of the postmortem period was substantiated (Zheng et al., 2019); the duration of the postmortem interval was estimated by determining the activity of catalase and aminolevulinate dehydratase in the tissues of the liver, kidneys, skeletal muscle and brain of Swiss mice (Paltian et al., 2019); the expression of an autophagy-associated protein in rat muscle tissue after ante- and postmortem injury was recorded (Shi et al., 2020); postmortem protein degradation of skeletal muscle in pig limbs was studied (Geissenberger et al., 2021); the informativeness of postmortem changes in the electrical conductivity of skeletal muscles of *Dicentrarchus labrax* for assessing the duration of the postmortem period was determined (Abbate et al., 2022); the potential use of muscle proteins (desmin and dystrophin) as biomarkers for assessing postmortem intervals in dogs was shown (Piegari et al., 2023). In recent years, the observations of Ukrainian researchers on corpse cooling have gained priority in determining postmortem phenomena, in particular, a correlation between destructive changes in internal organs and the time of death in cats (Serdioucov, Shkundia and Kruchynenko, 2023) and postmortem destructive changes in muscle tissue, in particular, the postmortem dynamics of skeletal muscles according to histomorphological and histochemical parameters of dogs and cats whose death occurred as a result of acute hypoxia in different environmental conditions (Yatsenko and Kazantsev, 2024).

Referring to the achievements of modern science, we can certainly state that the accumulated body of facts has contributed to the solution of some problems. However, it should be emphasized that the issue of finding expert criteria for the duration of the post-mortem interval is still relevant because due to the considerable number of factors that simultaneously affect the animal corpse and determine the development of post-mortem changes (environmental conditions, age and diseases of the animal, etc.), the researchers mentioned above had difficulties in establishing uniform criteria for the time of death.

Despite the numerous experimental studies on the biotransformation of animal corpses reported in scientific journals, modern veterinary medicine has a large amount of incomplete material, the generalization of which can be successfully implemented in the practice of forensic veterinary medicine. The authors of this publication believe that a systematic approach will greatly expand the possibilities for studying the postmortem processes of animal corpses and will allow them to be more thoroughly substantiated from the standpoint of evidence-based veterinary medicine.

The **aim of the study** is therefore to provide a comprehensive forensic veterinary evaluation of biotransformation phenomena in dog and cat corpses and to substantiate expert criteria for determining the duration of postmortem intervals.

Materials and methods. The prospective experiment was conducted for two years, from the middle of May 2021 to the end of May 2023, at the municipal veterinary medicine institution in Kharkiv, by observing and analyzing the phenomena of biotransformation of carcasses in the period from the onset of animal death to skeletonization and partial fragmentation of carcasses. The design of the experiment reproduced the meteorological environmental conditions in which animals die under non-obvious circumstances.

The objects of the empirical part of the study were 28 corpses of dogs (n = 16) and cats (n = 12). All corpses were divided into 4 experimental groups (n = 7 per group) according to the criterion of the immediate cause of death of the animal. Each group contained corpses with the following distribution: by taxonomic species (cats, n = 3; dogs, n = 4), sex (females, n = 3; males, n = 4), age (neonatal, n = 2; mature, n = 3; geriatric, n = 2). Group 1 included corpses of animals with sudden death due to cardiac pathology or poisoning; they were stored outdoors at a temperature of +18°C and 59% humidity. Group 2 included the corpses of animals that died from injuries and hyperthermia; they were placed in a tight plastic bag, buried in the ground, and stored at a temperature of +18°C and 64% humidity for 7 days. The death of animals in Group 3 was due to mechanical asphyxiation, in particular, aspiration with liquid during drowning, and the corpses were stored in tap water at a temperature of +2°C and humidity of 73%. Group 4 included the corpses of animals that died of general hypothermia; they were stored in thermal bags at a temperature of –18°C and humidity of 92%. Under the conditions of the experiment, after 7 days of postmortem interval, the corpses of the 2nd, 3rd, and 4th groups were placed in an open space at an average daily temperature of +18°C and relative humidity of 59% and no direct exposure to precipitation for 14 days. Further, the variability of temperature and relative humidity was not

taken into account. After reaching the cadaveric 'plateau', the dynamics of biotransformation and features of succession by necrophilic entomofauna in all groups were documented once a month until the end of the observation period.

The time ranges of supravital reactions and early and late cadaveric changes in the corpses of animals of Group 1 were recorded separately. Supravital reactions were determined in a certain sequence: first, the 'pupillary' reactions to atropine and pilocarpine of the iris muscles were studied, then the 'idiomuscular' reaction of *m. quadriceps femori* to mechanical irritation after impact with an oblong object with a narrow surface. With a syringe with a thin, fixed needle, 0.1 cm^3 of 1% solution of *atropini sulfati* (Darnitsa, Ukraine) was injected into the anterior chamber of the eye of cats every 6 h during the day, followed by 0.1 cm^3 of 1% solution of *pilocarpini hydrochloridi* (Darnitsa, Ukraine), and similar solutions were injected into the eye of dog corpses in the reverse sequence. The height of the muscle roller of the lateral surface of *m. quadriceps femori*, which was formed after a transverse blow with the back of a large sectional knife. Mercury (Medicare, Ukraine) and electronic (Medicare, Ukraine) thermometers with a measuring range of 35.0–42.0°C were used to observe the dynamics of cooling of dog and cat corpses; the sensors were immersed through an artificial hole in the lateral abdominal wall in the projection of the left or right lobe of the liver at an angle of 75° to the segmental plane; the process of cooling the skin surface of animal corpses was visualized using a *ULIRvision TI120* non-contact thermal imaging camera (China).

The onset and development of rigor mortis was determined by touch by the presence of complete contracture of the muscles of the animal carcasses' cervical, thoracic, and pelvic limbs. For the objective examination of the liver, the method of observing the disappearance, pallor, recovery, or absence of a color change of the cadaveric spot when pressing on its visual center and recording the time of color change with a stopwatch was used. For metrological examination of the carcasses, the longitudinal (linea median anterior from the first cervical vertebra to the first caudal vertebra) and circumferential dimensions of the trunk (in the mid-chest and neck region), neck (in the mid-region) and limbs (in the thigh and forearm region) were measured daily for 10 days after the onset of death of the animals using a tape measure (Ukraine).

For the cytomorphological study of the dynamics of destruction of the formative elements and the growth of the number of bacterial colonies in the blood, samples of unclotted blood from the heart chambers of cats and dogs of Group 1 were taken within 48 h after death every 6 h by the vacuum method in *Vacusera* tubes (Ukraine) with a *K_3EDTA* capillary. Smears were made from it, stained by Romanowsky–Giemza, and, using an optical microscope *Granum R50* (China), blood cytograms were examined at a field of view of ×1000 (Zaporozhan et al., 2002). A forensic veterinary necropsy was performed by the method of partial evisceration. For histomorphological assessment of the severity of liver biotransformation in Group 2 animals, 1 cm^3 samples were used, slides were made, stained with hematoxylin and eosin, and examined at ×400 microscope magnification using the standard method (Horalskyi, Khomych and Kononskyi, 2015). The informative areas were photographed using a digital nozzle *ToupCam UCMOS03100KPA* (China) integrated with the microscope. The obtained information was processed on a personal computer using the software package *'Photo Frame Studio 3.0'*. The definition of 'patterns' in this study refers to any signs of macroscopic decomposition of the animal corpse or elements of microscopic tissue destruction, which were described in the obtained cytograms and histotopograms according to the recommendations of Ressel (2017) and Raskin, Meyer and Boes (2022).

Photogrammetry was carried out using a forensic ruler designed for large-scale photographic recording of objects. The forensic veterinary examination of animal corpses was carried out in four stages: preparatory (preliminary examination), analytical (separate examination), comparative, and synthesizing, which help to trace and evaluate the results obtained when analyzing the expert's opinion (Yatsenko, 2022).

Results and discussion. Our clinical practice has identified a sequential stage that precedes the terminal state of animals: the pre-agonal state, terminal pause, and agony. Our findings indicate that hypoxia is the primary thanatogenetic factor in the onset of terminal states, while circulatory hypoxemia is the initial trigger in the process of thanatogenesis. It is recognized that the causes of terminal circulatory hypoxia can vary. However, refractory disorders of the vital triangle organs (cardiac paralysis, respiratory arrest, and cessation of brain function), which are documented during terminal conditions in animals, should be considered probable signs of death. These conditions directly or indirectly result in clinical death.

We argue that immediately after the onset of biological death, in the early postmortem period, it is advisable to differentiate between the processes inherent in a living animal that cause supravital reactions and those that occur in the postmortem period and cause cadaveric phenomena. In our opinion, the degree of severity of certain supravital reactions and cadaveric phenomena is due to the prevalence of a certain stage of the terminal state.

It is necessary to focus on defining the 'early postmortem period' itself because the literature analysis revealed controversial and sometimes contradictory statements. Therefore, if we consider cadaver autolysis to be intermediate between early and late cadaveric phenomena, then the duration of the early postmortem period should be understood as a period not exceeding 24 h after the onset of death because according to the results of microscopy of parenchymal organs of cat corpses (Kazantsev and Yatsenko, 2021) and dog corpses (Yatsenko and Kazantsev, 2022) proved that the degree

of decomposition after 24 h of the postmortem period does not allow the use of the cytomorphological method to monitor the further biotransformation of the animal corpse at the cellular level. In this regard, the authors of the study evaluated certain cadaveric phenomena and supravital reactions to establish the time range of diagnostic informativeness of the postmortem interval from the moment of biological death of the animal. There is no doubt that damage to corpses, in particular as a result of cannibalism (Fig. 1), and cadaveric changes that are noted by a veterinary specialist by examining the animal corpse at the place of its discovery (Fig. 2) or removal (Fig. 3) the cadaveric bed, during the analysis of the case file, based on the results of a comprehensive examination of the corpse using additional research methods, are the basis for modeling an expert hypothesis about the cause of death and, further, determining the syndromic forensic veterinary diagnosis currently used in expert practice (for example, profuse bleeding, chemical poisoning, pulmonary edema, etc.)

Given the above, we focus on the early phenomena of biotransformation, in particular, cadaveric rigor mortis, cadaveric desiccation, cadaveric staining, and cadaveric cooling. The phenomenon of postmortem rigor mortis is detailed by the authors of this publication in a prospective study of skeletal muscles of the neck of dog and cat corpses (Yatsenko and Kazantsev, 2024). It is associated with the denaturation of actin and myosin in myofilaments and the subsequent redistribution of calcium ions in them. It was noted that immediately after the death of the animal, a state of atony was recorded in all skeletal muscles, however, in 2–6 h after death, they contracted, became denser, and had passive movements due to postmortem contracture in the joints were not reproduced. The pace and intensity of such phenomena were more pronounced in the corpses of animals of Group 2, because, probably, as a result of damage to the brain stem due to general hyperthermia, rapid processes of postmortem contraction of myofilaments occur. Postmortem contracture of the joints of animal corpses of all groups started in the form of a sequential contraction in the direction from the masticatory muscles, neck, thoracic, and pelvic limbs to the skeletal muscles of the trunk with a peak 12 h after death, then gradually disappeared in a similar sequence and was not observed after 72 h of postmortem interval. It should be emphasized that the least pronounced variant of rigor mortis was recorded in the corpses of animals of Group 1 with a short agonal period and neonatal animals, and the most pronounced — with well-developed skeletal muscle. When moving the cadaver by dragging the thoracic limbs, we found a discrepancy in the degree of rigor mortis compared to the joints of the pelvic limbs, which remained completely immobile.

The early absolute signs of biotransformation of the corpse were confirmed: the phenomena of drying and cornea opacity (Fig. 4) and cadaveric spots (Fig. 5), which occurred immediately after the onset of death.

Undoubtedly, the phenomenon of drying out of the animal corpse is caused by the evaporation of moisture from the surface of the general cover. Signs of cadaveric desiccation were recorded on the cornea, which was not covered by eyelids, in the corpses of animals of all groups except Group 4, indicating that they could not be formed in a humid environment. 6 h after death, the formation of irregular triangular spots of grayish-brown color (Larsche's sign) was observed on the cornea, while the sclera remained shiny.

In fact, the formation of cadaveric spots and hypostases in the internal organs of animals is associated with myocardial paralysis, which results in blood flowing through the vessels to the lowest parts of the body. A certain sequential stage in the development of cadaveric spots has been established. The hypostasis stage began 1–3 h after death and lasts up to 12 h. When pressing on the red center of the spot, the color disappears but is fully restored after 1 second. In animals that were moved or changed position, the spots moved to other parts of the body where they could not form naturally. The stasis stage lasted in the range of 12–24 h after the death of the animal. It is obvious that the plasma of liquid blood from the blood vessels impregnated the adjacent tissues, concentrated over time, and after pressing on the center of the spot, partially disappeared and recovered more slowly within 3 seconds. The stage of imbibition was most pronounced in the period of 24–48 h after death. It was found that when pressing on the center of the spot in the third stage, its color did not change, and the rate of onset of the stage depended on the surrounding temperature in the following way: in the low-temperature regime, the rate of onset of imbibition increased. It is important to emphasize that in the corpses of animals of Group 1, spots of intense blue color with diffuse localization were recorded, but in the corpses of animals of Groups 3 and 4, on the contrary, they were pinkish-red. During the forensic veterinary autopsy of the animals, the formation of cadaveric spots in the viscera was observed as a result of postmortem redistribution of blood to the lowest parts of the viscera, which, in our opinion, is the cadaveric norm, but, taking into account the information in the case file, requires differentiation from pathological conditions.

As for the corpses of animals of Group 3, during prolonged exposure to water, in cases of intentional drowning, fatal injuries, throwing the corpse into the water immediately after death, etc., we noted permanent non-random signs in the form of pink-red cadaveric spots and epidermal maceration at 24 h postmortem. In all corpses of animals that died as a result of drowning, we recorded congestive venous hyperemia and severe pulmonary emphysema, pulmonary vasodilation, rupture of the alveolar walls, alveolar edema, the presence of alveolar transudate, multifocal intra-alveolar hemorrhages, which was confirmed by histomorphological examination.

Figure 1. Fragment of a dog corpse with injuries caused by cannibalism.

Figure 2. Dog corpse at the place of its discovery with postmortem pigmentation.

Figure 3. The corpses of a dog and a cat immersed in tap water containers.

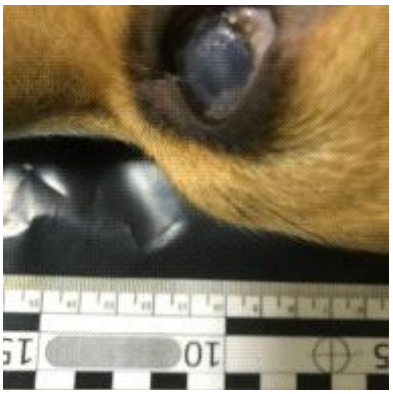


Figure 4. Corneal opacity with Larsche's sign in the medial angle of a dog corpse.

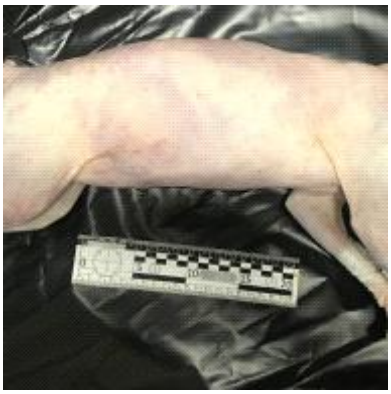


Figure 5. Postmortem spots on a cat corpse 12 h after death.

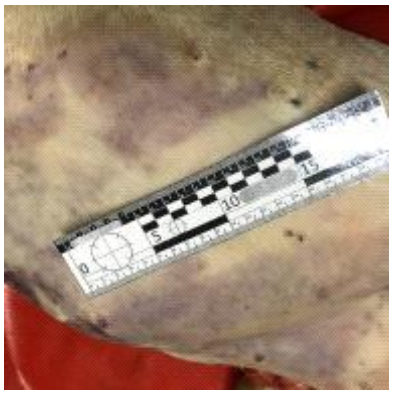


Figure 6. Postmortem stains on a dog corpse 24 h after death.

Regarding the corpses of animals of Group 4, it should be emphasized that freezing of the corpse can occur only if it has been exposed to temperatures below 0°C for a certain period. In this regard, we believe that the freezing of a corpse and the death of an animal from hypothermia are not identical phenomena, and therefore have specific signs. Freezing causes postmortem artifacts in the form of gross soft tissue tears and cracking of the bone sutures of the skull, which must be differentiated from intravital injuries.

Obviously, cadaveric cooling is associated with the inhibition and cessation of heat production and a gradual decrease in body temperature due to the prevailing heat transfer. According to the images of the thermal imaging camera, the cooling of the surface occurs unevenly, both in the corpses of cats (Fig. 7) and dogs (Fig. 8). For example, the highest temperature peaks are recorded in areas with dense soft tissue, in particular, hypodermis and skeletal muscle.

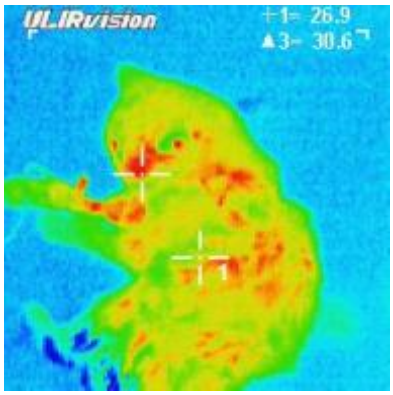


Figure 7. Thermography of the surface of a cat corpse with a thermal imaging camera.

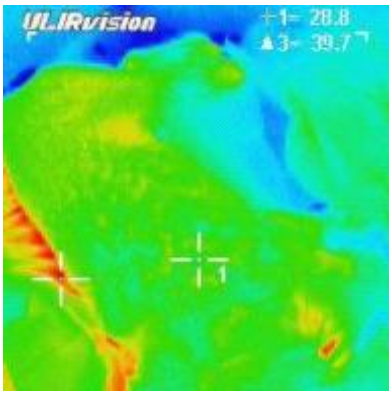


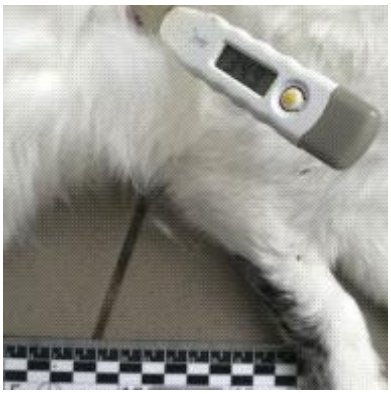
Figure 8. Thermography of the surface of a dog corpse with a thermal imaging camera.

During the terminal pause, a significant increase in temperature up to 40.0°C was recorded in the animals.

According to our data, the liver temperature of the corpses of animals in Group 1 and the environment finally equalized after 27 h postmortem. The results of hepatic thermography show that the temperature of the corpse decreased steadily by 3.0°C in the first hour after the death of the animal. At 2–3 h after death, the decrease in temperature level was about 1.0°C, in the range of 3–18 h — from 0.5°C to 1.0°C, then in the following hours — decreased by 0.1°C every hour until the end of the observation period. The liver temperature of the cat carcasses was measured with electronic (Fig. 9a) and mercury (Fig. 9b) thermometers. The liver temperature of the dog carcass was measured with a mercury thermometer (Fig. 10). The temperatures were not significantly different, but were higher than on the skin surface.

It has been shown that after the transverse impact of the *quadriceps femori* of animal corpses with the back of a large sectional knife in the early postmortem period, an 'idiomuscular' roll was formed (Fig. 11). In the first 2 h it was pronouncedly high, appeared and disappeared quickly, in the period of 2–6 h it was low, appeared and disappeared more slowly than in the previous period, and 6–8 h after the onset of the animal's death it appeared only in the form of a focal thickening at the site of mechanical irritation.

As for the reaction of the pupil to the injection of pilocarpine (Fig. 12) into the anterior chamber of the eye in the form of constriction and its reverse dilation after atropine (Fig. 13), it should be noted that such reflexes persisted for 24 h after death, but the duration of the reactions slowed down every 6 h.



a

b

Figure 9. Hepatic thermometry of a cat corpse: a — electronic thermometer; b — mercury thermometer.

Figure 10. Hepatic thermometry of a dog corpse with a mercury thermometer.

Figure 11. 'Idiomuscular' reaction of *m. quadriceps femori* of a dog corpse after mechanical stimulation.

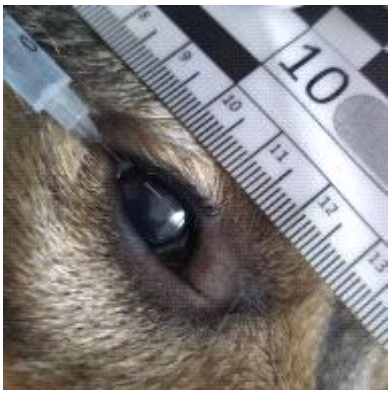


Figure 12. Pupillary reaction of a dog corpse after pharmacological stimulation with pilocarpine hydrochloride.

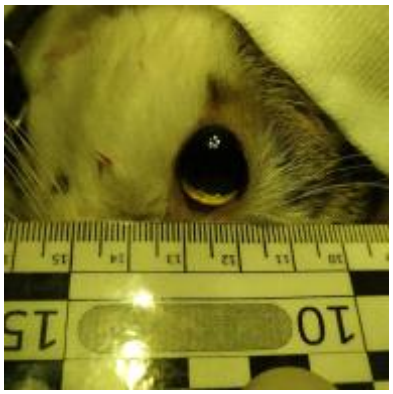


Figure 13. 'Pupillary' reaction of a cat corpse after pharmacological stimulation with atropine sulfate.

As for the cytomorphologic examination of the blood of cats of Group 1, in 6 h after their death, the formative elements look morphologically typical for the taxonomic species of animals. No structural changes were visualized in erythrocytes and segmented neutrophils (Fig. 14).

12 h after the onset of death, the erythrocyte membrane remains unchanged. However, changes are observed in the structure of leukocytes. Thus, some of them show changes in the shape of the nuclei in the form of karyopyknosis and condensation of nuclear chromatin (Fig. 15).

18 h after the death of the cats, the nuclear membrane of the erythrocytes remained unchanged. The number of altered leukocytes visually increased compared to the previous observation time (Fig. 16).

Twenty-four hours after the onset of death, all identified leukocytes were morphologically altered. The destruction of the leukocyte nucleus in the form of karyolysis enhanced cytoplasmic degranulation. The tinctorial properties inherent in the granules also change, which looks like an atypical staining according to the standard method. The nuclear membrane of erythrocytes remains unchanged (Fig. 17).

In the postmortem interval of 24–30 h after the death of cats, there are pronounced processes of destruction of blood cells, which break down into fragments, and debris is formed, which becomes a nutrient medium for the colonization of saprotrophic bacteria (Fig. 18).

36 h after the death of cats, the number of cellular debris visually increases, and rod-shaped bacteria form colonies (Fig. 19).

Blood cytograms of cat corpses obtained in the interval of 42–48 h after death do not visually differ from each other (Figs 20, 21) and are represented by patterns of residual decomposition of formative elements and colonies of polymorphic bacteria.

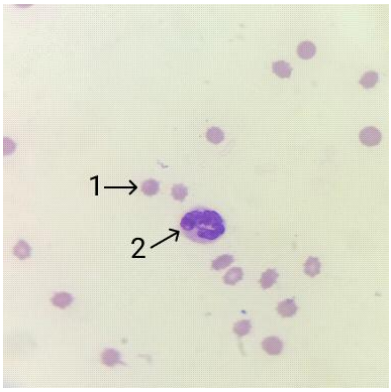


Figure 14. Blood cytogram of a dead cat 6 h after death; ×1000, Romanovski–Giemsa staining. 1 — erythrocyte without noticeable changes in shape; 2 — segmented neutrophil.

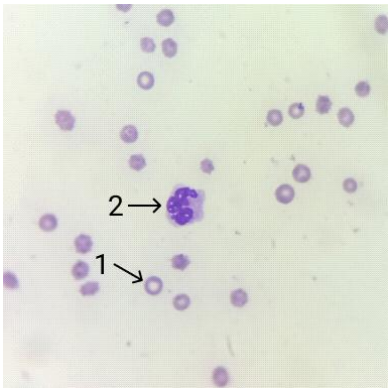


Figure 15. Blood cytogram of a dead cat 12 h after death; ×1000, Romanovski–Giemsa staining. 1 — erythrocyte without noticeable changes in shape; 2 — leukocyte with an altered nucleus.

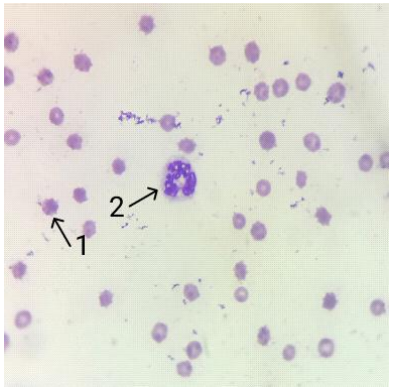


Figure 16. Blood cytogram of a cat corpse 18 h after death; ×1000, Romanovski–Giemsa staining. 1 — erythrocyte without noticeable changes in shape; 2 — leukocyte with changes in the nucleus.

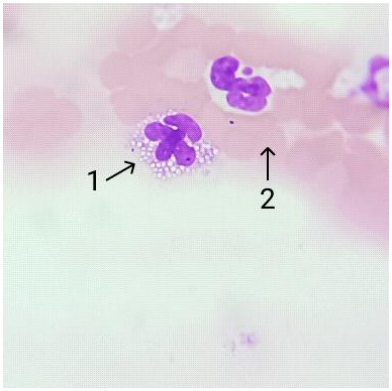


Figure 17. Blood cytogram of a dead cat 24 h after death; ×1000, Romanovski–Giemsa staining. 1 — a leukocyte with changes in the tintorial properties of cytoplasmic granules; 2 — accumulation of red blood cells without noticeable changes.

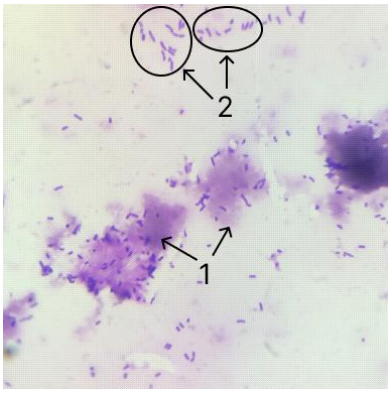


Figure 18. Blood cytogram of a cat corpse 30 h after death; ×1000, Romanovski–Giemsa staining. 1 — cellular debris; 2 — accumulation of rod-shaped bacteria.

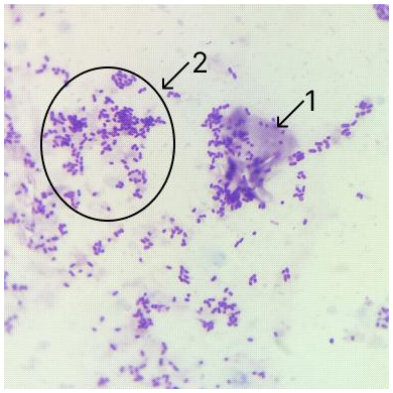


Figure 19. Blood cytogram of a cat corpse 36 h after death; ×1000, Romanovski–Giemsa staining. 1 — cellular debris; 2 — accumulation of rod-shaped bacteria.

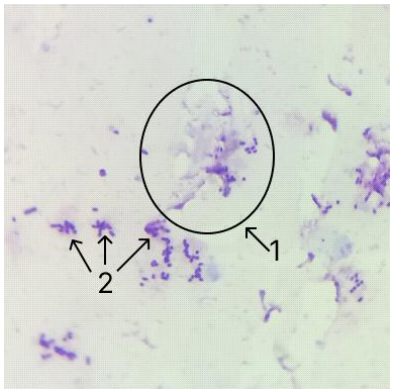


Figure 20. Blood cytogram of a dead cat 42 h after death; ×1000, Romanovski–Giemsa staining. 1 — cellular debris; 2 — accumulation of polymorphic bacteria.

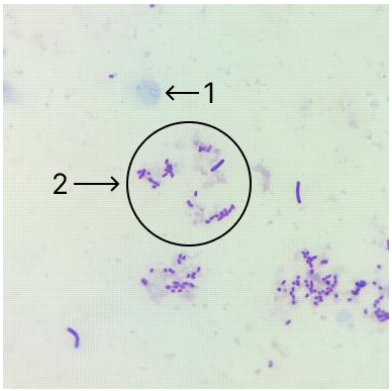


Figure 21. Blood cytogram of a cat corpse 48 h after death; ×1000, Romanovski–Giemsa staining. 1 — cellular debris; 2 — accumulation of polymorphic bacteria.

On blood cytograms of corpses of dogs of Group 1 in 6 h after the onset of death, the blood cells look morphologically typical. There are no visual changes in thrombocytes, segmented neutrophils, and monocytes (Fig. 22). 12 h after the onset of death, structural changes in white blood cells are visualized. Thus, karyopyknosis and nuclear chromatin dyscomplexity are observed in almost a quarter of the studied rod-shaped neutrophils. The nuclear membrane of erythrocytes remains without noticeable structural changes (Fig. 23).

The nuclear membrane of erythrocytes of dog corpses 18 h after death remains unchanged. However, the cytograms of white blood of dog corpses, compared to the previous period, indicate a rapid increase in the number of leukocytes with signs of nuclear destruction in the form of karyolysis (Fig. 24).

The study of the red blood picture 24 h after the onset of death of dogs showed no noticeable structural changes in erythrocytes. However, compared to the previous observation interval, all leukocytes were found to have a certain structural disorganization. Thus, some of the identified leukocytes contained nuclei in a state of rhexis. The nuclei of other white blood cells were in a state of total fragmentation (Fig. 25).

In 30 h after the death of dogs, there are pronounced processes of destruction of blood cells, they break down into fragments, forming debris, which becomes a nutrient medium for colonization by saprotrophic bacteria (Fig. 26).

In the postmortem interval of 30–36 h after the death of dogs, the number of cellular debris visually increases, and rod-shaped bacteria form colonies (Fig. 27). The blood cytograms of dog corpses obtained in the time interval of 42–48 h from the moment of death do not visually differ from each other (Figs 28, 29) and are represented by patterns of residual decomposition of cellular elements and colonies of rod-shaped and coccal forms of bacteria.

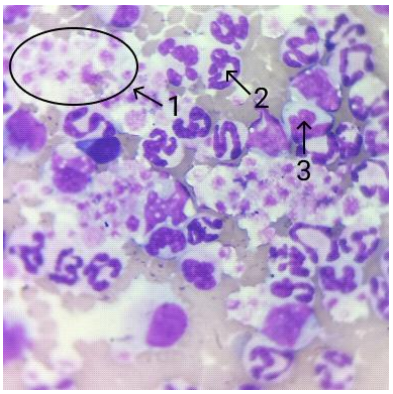


Figure 22. Blood cytogram of a dog corpse 6 h after death; ×1000, Romanovski–Giemsa staining. 1 — the shape of thrombocytes in clusters is not changed; 2 — segmented neutrophil without noticeable morphological changes; 3 — monocyte with preserved shape.

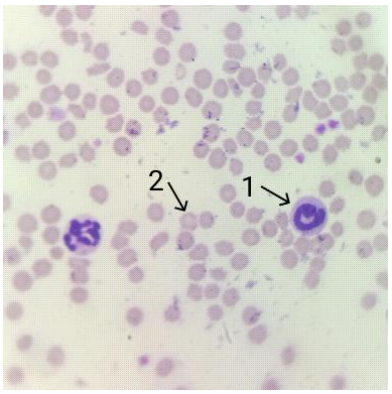


Figure 23. Blood cytogram of a dog corpse 12 h after death; ×1000, Romanovski–Giemsa staining. 1 — a rod-shaped neutrophil without noticeable changes in shape; 2 — a morphologically unchanged erythrocyte.

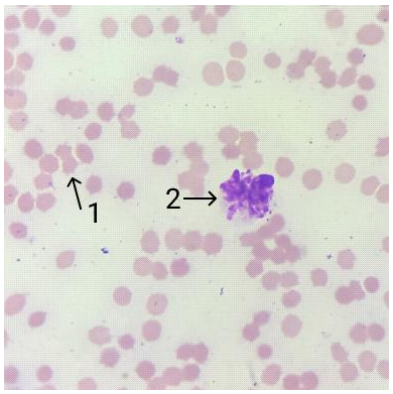


Figure 24. Blood cytogram of a dog corpse 18 h after death; ×1000, Romanovski-Gimza staining. 1 — erythrocyte without noticeable structural changes; 2 — leukocyte with changes in the nucleus.

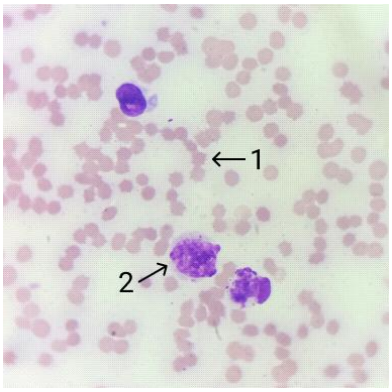


Figure 25: Blood cytogram of a dog corpse 24 h after death; ×1000, Romanovski–Giemsa staining. 1 — morphologically unchanged erythrocyte; 2 — leukocyte with changes in the nucleus.

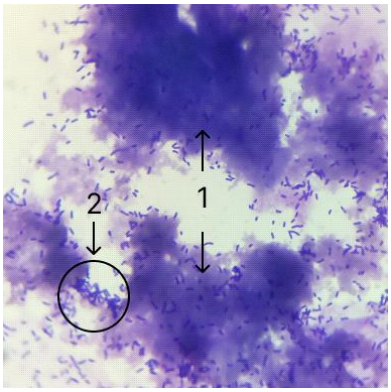


Figure 26. Blood cytogram of a dog corpse 30 h after death; ×1000, Romanovski–Giemsa staining. 1 — cellular debris; 2 — accumulation of rod-shaped bacteria.

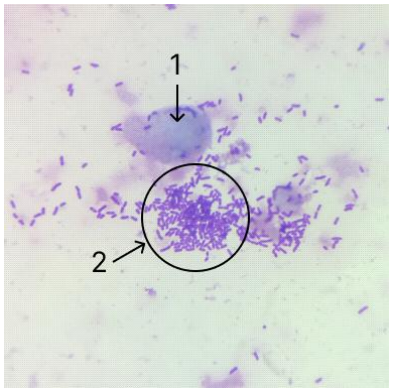


Figure 27. Blood cytogram of a dog corpse 36 h after death; ×1000, Romanovski–Giemsa staining. 1 — cellular debris; 2 — accumulation of rod-shaped bacteria.

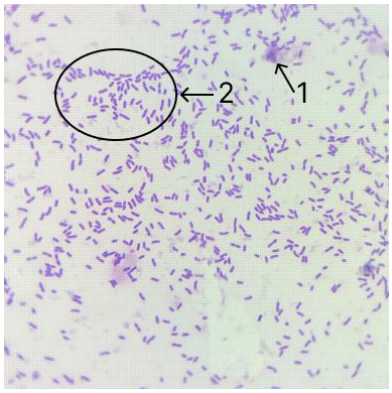


Figure 28. Blood cytogram of a dog corpse 40 h after death; ×1000, Romanovski–Giemsa staining. 1 — cellular debris; 2 — accumulation of rod-shaped bacteria.

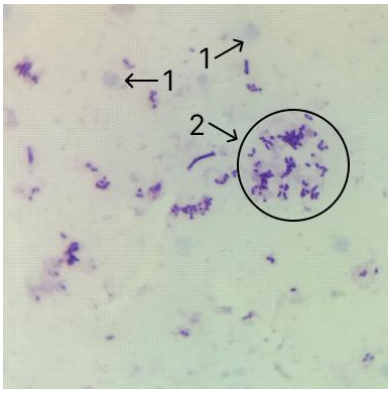


Figure 29. Blood cytogram of a dog corpse 48 h after death; ×1000, Romanovski–Giemsa staining. 1 — cellular debris; 2 — accumulation of polymorphic bacteria.

It has been established that cadaveric autolysis begins within a day after the onset of animal death and is further observed simultaneously with late cadaveric changes, and therefore probably occupies an intermediate position between early and late postmortem phenomena, catalyzing the development of the latter. Postmortem tissue autolysis, for example, in the pancreas, causes its enzymatic melting, which macroscopically looks like pancreatic necrosis, autolysis of the convoluted tubule epithelium — like necronephrosis, and myocardial autolysis — like intramural infarct.

It has been empirically proven that late cadaveric phenomena include those that lead to pronounced changes in appearance, in particular, a characteristic feature of late biotransformation is a change in the color of the animal's corpse. The skin acquires a dark, 'metallic' luster. Such cadaveric pigmentation begins with the transformation of primary cadaveric spots, at an average daily temperature above 18.0°C and after 48–72 h at a temperature of 10.0°C, and then, in the abdomen, in places where the intestines come into contact with the abdominal wall, a 'putrid venous network' of dirty green color is formed over time. The skin, due to the uneven drying of its parts due to the presence of a hair coat, becomes dense to the touch and looks like parchment.

Let's highlight the late postmortem phenomena: putrefaction, skeletalization, and fragmentation. It is known that putrefaction is the process of decomposition of protein substances in the corpse under the influence of the vital activity of various microorganisms. Along with the color change, the corpse's volume increased due to the formation and distribution of gases in certain body cavities, confirmed by the relevant metric studies of the dog (Fig. 30) and cat corpses (Fig. 31).

Microscopically, putrefaction of animal corpses is characterized by disorganization of the parenchyma, destruction of stromal elements, formation of cavities of different diameters filled with gases, focal accumulation of bacterial colonies, which are visualized around the vessels during light microscopy, confirming the vascular mechanism of microflora dissemination.

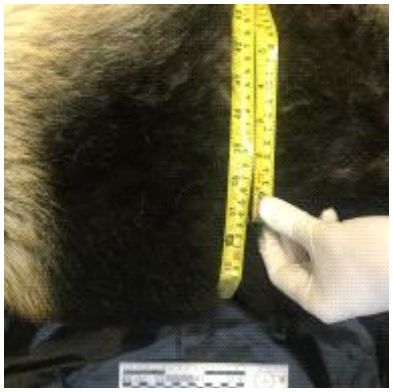


Figure 30. Girth measurement of the chest of a dog corpse.

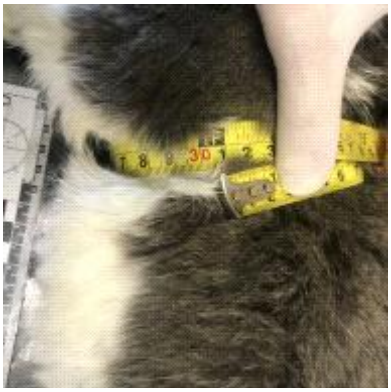


Figure 31. Girth measurement of the chest of a cat corpse.

The most representative phenomena of putrefactive biotransformation were found in the corpses of animals of Group 2. After digging up the animal corpses from the soil a week after the onset of death (Fig. 32), a pungent putrefactive odor was immediately felt, which was released through natural openings.

After the corpses were excavated a week later, macroscopic examination revealed localized 'saponification' in the head area of the cat (Fig. 33) and dog (Fig. 34) and dirty green in the abdomen, apparently as a result of the formation of sulfhemoglobin in it; then a putrid venous network is formed, which may be caused by the contamination of blood vessels by bacteria from the liquid fraction of blood; cadaveric emphysema, which occurs as a result of the release of gases by anaerobes with the formation of blisters in the hypodermis and internal organs, which is most pronounced in the liver.

The external examination of the dog's corpse revealed its unnatural position (Fig. 35), and an X-ray examination of the abdominal organs revealed the presence of a foreign body with a 'metallic' density (Fig. 36). The forensic veterinary autopsy illustrated the total blood imbibition of soft tissues and internal organs, and due to explosive gas production by anaerobes, the intestinal loops were visually distended (Fig. 37). As a result of putrefactive emphysema, gases accumulated in the subcutaneous fatty tissue and swelled it. It was noted that simultaneously with the bloating of the corpse, the epidermis of the skin was raised by gases, blisters filled with dirty bloody liquid were formed.

An X-ray examination of the cat's corpse revealed fragmentary pulmonary atelectasis and accumulation of free fluid (Fig. 38), which was further confirmed by a forensic veterinary autopsy (Fig. 39).

It is shown that the methods for estimating the postmortem interval based on histological changes in the animal corpse demonstrate the possibility of their application in the late postmortem interval, when most of the currently used methods cannot be informative. We have noted the possibility of determining such changes 72 h after the onset of death. At the same time, we emphasize that autolysis and progression of biotransformation of cadaveric material cause uninformative histomorphological examination in late postmortem intervals. However, despite the impossibility of using this type of research in every forensic case, in early cadaveric changes, such additional studies contribute to the identification of microscopic lesions in the absence of visible macroscopic patterns and narrow the range of differential diagnoses.

Figure 32. A container with sand in which animal corpses were buried.

Figure 33. A fragment of a cat's corpse after being dug up a week later.

Figure 34. A fragment of a dog's corpse after being dug up a week later.

Figure 35. Appearance of a dog corpse after excavation 7 days after death.

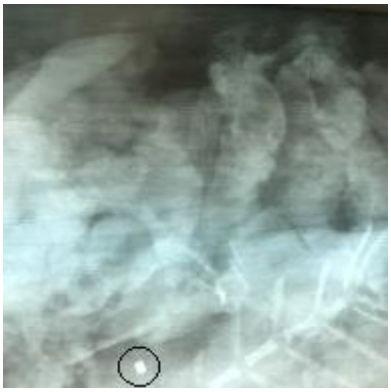


Figure 36. Overview X-ray in LL projection of the abdominal organs of a dog corpse excavated from the ground 7 days after death (a foreign object with a 'metallic' density is outlined).

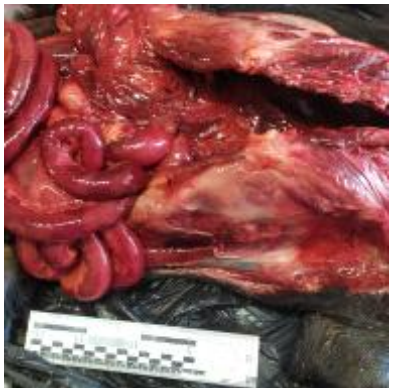


Figure 37. Internal examination of a dog corpse excavated from the soil 7 days after death.

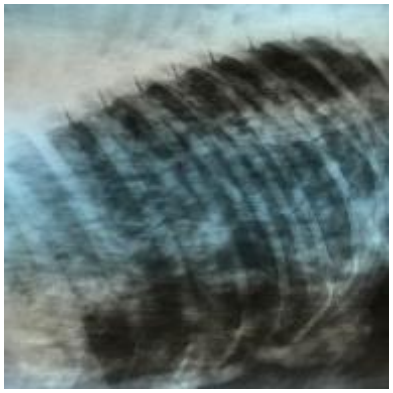


Figure 38. Overview X-ray in RL projection of the chest organs of a cat corpse excavated from the ground 7 days after death.

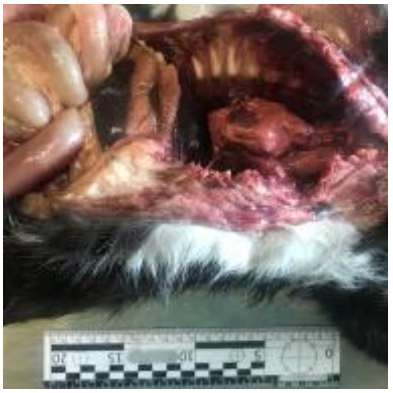
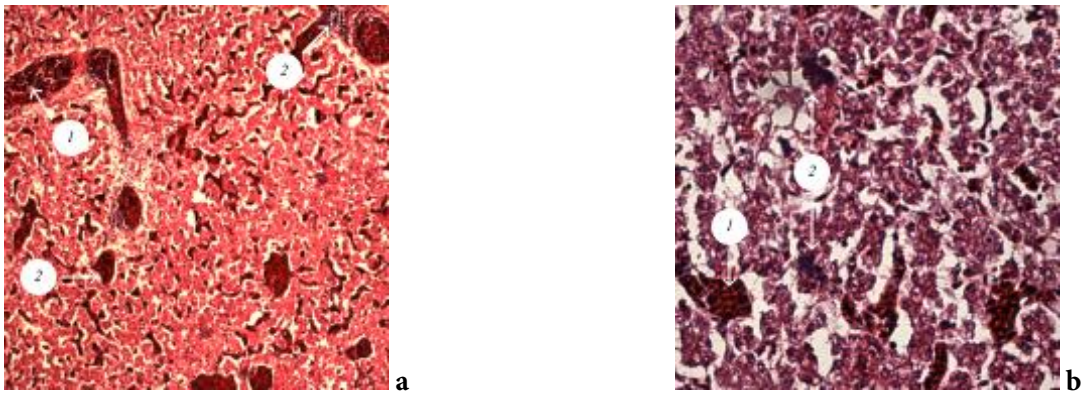


Figure 39. Internal examination of a cat corpse excavated from the soil 7 days after death.

Thus, to establish the presence and assessment of pathological changes in organs and tissues caused by violent acts and/or diseases, to determine the vitality and prescription of bodily injury, and to resolve other issues related to the determination of the microscopic structure of animal organs and tissues, we consider it necessary to conduct additional cytological/histological studies using special staining that is consistent with the principles of evidence-based veterinary medicine. It is advisable to carry them out taking into account the goal set during the forensic veterinary examination of a corpse or its fragments, and the results must be taken into account when drawing up an expert opinion, which traces the logical connection between the analytical and synthesizing parts.

We suggest that the text describing the pathological changes should be placed in the research part of the examination, the detected microscopic changes should be described in the state language, systematized into a forensic veterinary histological diagnosis according to the pathogenetic principle using the syntax of pathomorphological nomenclature, and histotopograms illustrating the conclusion should be attached in the form of photo tables. For example: 'Examination of liver histopreparations of a cat and dog corpse was performed in light transmitted under a Granum R50 microscope with an image magnification of ×400. Using a ToupCam UCMOS03100KPA digital camera installed on the microscope and the Photo Frame Studio 3.0 software, 2 histotopograms (2 samples, 2 sections) were made, reproductions of which with appropriate markings and explanatory captions are shown in the photo table (Fig. 40). Forensic-veterinary histologic diagnosis: (a) smoothed histoarchitectonics of the liver, (b) hydropic dystrophy of hepatocytes, (c) capillary hyperemia, (d) bacterial colonies'. Late changes in the corpse actually appear immediately after death, but they progress more slowly, appear later and lead to the destruction of the corpse.

It was found that after 7 days the corpses of animals putrefy — a kind of rotting in conditions of sufficient air and moderate humidity. The process of decomposition is more intensive than ordinary decomposition, with more complete oxidation, and is accompanied by the formation of a relatively small amount of gases with an unpleasant odor.



a b

Figure 40. Histotopogram of the liver 72 h after animal death: a — cat corpse, b — dog corpse; ×400, hematoxylin and eosin staining; 1 — hyperemia of hepatic hemocapillaries, 2 — bacterial colonies.

In our opinion, the general drying of the corpse is an important prerequisite for its 'conservation', which depends on a set of certain natural conditions, in particular, humidity, temperature, mineral composition of the soil, etc.

Corpse decomposition processes are related to the vital activity of bacteria, fungi, plants, and animals, particularly insects, and can be caused by insects, rodents, and predators.

Seasonal dynamics of carcass decomposition under the influence of entomofauna were observed. In the second half of May, the carcasses were massively colonized by blue flesh flies, and in June by green flies. The predominant localization of larvae in the oral cavity is the mucous membrane of the tongue (Fig. 41a) and the inner surface of the cheeks. At the same time, the mucosa becomes darker due to desiccation (Fig. 41b), and the number of larvae gradually decreases. It was found that dipterans were the first to inhabit the carcass, and as the number of their larvae increased, predatory species of beetles that destroy fly larvae appeared. Larvae of beetles and adult beetles appeared on the corpse and accumulated mainly in its location. Representatives of Hymenoptera — ants were found on the corpses from the first days and throughout the observation period. The first egg-laying flies on such carcasses appeared later. On the carcasses of the animals of Group 1, egg-laying flies appeared on the 4th day of observation. However, in the summer, dipteran oviposition was much more frequent.

As for dog carcasses, the predominant localization of blue and green fly larvae is the mucous membrane of the tongue (Fig. 43a) and the inner surface of the cheeks. At the same time, the mucous membrane also gradually changes color to darker due to drying (Fig. 43b), and the number of larvae gradually decreases.

The succession of necrophilous insects had similarities and significant differences. The common feature was that two-winged flesh flies were the first to discover the carcasses. Obligate species of necrophagous beetles — the notched-wing scavenger and the red-breasted carrion beetle — were observed at similar times. The differences found in the keratophagous group under normal decomposition conditions appeared on the corpses on days 10–14 of the observation (Figs 42, 44).

By studying the intervals of decomposition of dog and cat corpses in different states, no differences in the timing of the onset of biotransformation phenomena were found. Thus, the process of biotransformation of the animal corpse occurs through a period of microbial decomposition, which occurs from the moment of death to the development of putrefactive emphysema (from 1–2 days to 1–5 weeks): (a) 'fresh' corpse — up to 2 h; (b) early cadaveric changes (up to 2–3 days); (c) early putrefactive changes — the appearance of cadaveric green and putrefactive venous network; (d) putrefactive gigantism of the corpse. Then comes the period of active destruction of the corpse by insects (from 15–20 days to 2 months): (a) early destruction of soft tissues, mainly due to fly larvae; (b) late destruction of soft tissues, during the activity of necrophage beetle larvae (developmental duration — 30–45 days) and predator beetles (developmental duration 45–65 days), which ends with almost complete destruction of soft tissues. At the end of this interval, which we propose to consider the 'plateau' period, all corpses acquired the same appearance.

It has been empirically proven that incomplete skeletonization of a corpse (Fig. 45) lasts until the end of the warm season and can continue into the next year in winter. As for the complete skeletonization of the corpse, it has been shown that it can last for years and end in fragmentation and complete destruction of the organic and mineral bone base (Fig. 46).

We believe that the forensic veterinary examination of a skeletal corpse under certain conditions allows us to determine the possible cause of death in case of severe bone trauma, the approximate time of death. At the same time, it is necessary to clearly differentiate the signs of biotransformation from bodily injuries that occurred during the movement and transportation of the corpse to the autopsy hall of a specialized expert institution, fragmentation of the body caused by accidents during transportation trauma, dismemberment of the corpse by predators, etc.


a


b

Figure 41. A fragment of a cat's corpse on the 4th day after death: a — drying of the mucous membrane of the tongue; b — pigmentation of the scalp.

Figure 42. The corpse of a cat 10 days after death.


a


b

Figure 43. Fragment of a dog corpse on the 4th day after death: a — drying of the tongue mucosa; b — pigmentation of the scalp.

Figure 44. Dog corpse 10 days after death.

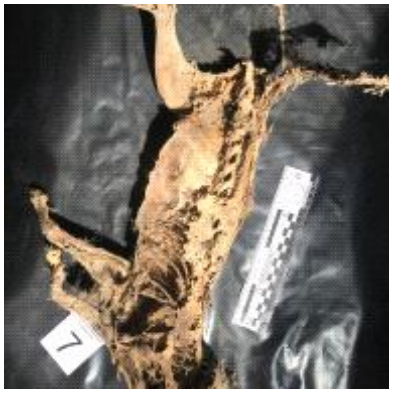


Figure 45. Skeletonization and fragmentation of a cat corpse 6 months after death.

Figure 46. Skeletonization and fragmentation of cat and dog corpses 12 months after death.

Conclusions. Morphological signs of early phenomena of biotransformation of animal corpses were detected. Postmortem joint contracture started in the direction of contraction from the masticatory muscles to the skeletal muscles of the trunk with a peak 12 h after death, then gradually disappeared in a similar sequence and did not recover after 72 h of postmortem interval. 6 h after the death of the animals, the formation of irregular triangular spots of grayish-brown color (Larsche's sign) was observed on the cornea. A certain sequential stage in the development of cadaveric spots was established: hypostasis, stasis, imbibition, and their time ranges were identified. The cooling of the corpse took place in the form of a gradual decrease of the temperature in the first hour after death by 3.0°C, then, 2–3 h after death, the temperature level decreased by about 1.0°C, in the range of 3–18 h — from 0.5°C to 1.0°C, and in the following hours — by 0.1°C every hour

until the end of the observation period. The 'idiomuscular' roll was pronouncedly high in the first 2 h, appeared and disappeared quickly, in the period of 2–6 h it was low, appeared and disappeared more slowly, and in 6–8 h it appeared only in the form of focal thickening at the site of mechanical irritation. Pupillary reflexes were preserved for 24 h after death, but the duration of reactions slowed down every 6 h. The dynamics of putrefactive disorganization of venous blood of dog and cat corpses within 48 h after death was determined.

Cadaveric autolysis begins within a day after the onset of animal death and is observed simultaneously with late cadaveric changes. It has been shown that the decomposition of animal corpses is macroscopically characterized by specific pigmentation, blood imbibition of soft tissues, explosive gas formation of internal organs, and putrefactive emphysema. Microscopic patterns of disorganization of the liver of animal corpses are characterized by the destruction of the parenchyma, hydropic degeneration, and bacterial dissemination. The sequence of cadaveric succession by entomofauna is shown: blue flies, green flies, ants, and necrophagous beetles. Incomplete skeletonization of animal carcasses lasts until the end of the warm season, followed by complete skeletonization, which ends with fragmentation and destruction of bones.

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THE CURRENT SITUATION REGARDING BOVINE LEUKEMIA IN LIVESTOCK PRODUCTION AND A STRATEGIC APPROACH TO ANTI-EPIZOOTIC MEASURES IN THE POST-WAR CONTEXT

Gorbatenko S. K., Kornieikova O. B., Stegniy B. T., Kuznetsova O. V., Miahkykh N. V.

National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Kharkiv, Ukraine, e-mail: st.gorbatenko@gmail.com

Summary. The current, conditionally safe, epizootic state of livestock in Ukraine regarding bovine leukemia has been determined. The lack of comprehensive anti-leukemia health programs in the detection of single animals infected with the leukemia virus in almost every region, except for Zakarpattia and Lviv regions, is due to violations of the regulations and the scope of diagnostic tests for leukemia and measures to prevent recurrence of the epizootic. In the postwar period, it would be strategic to develop and implement regulations for serological control of livestock on each farm, depending on specific epizootic circumstances

Keywords: immunosuppression, immunodiffusion, ELISA, seroprevalence, serological monitoring

Introduction. Bovine leukemia, classified as a slow infection, is one of the most common viral infectious diseases of cattle (Constable et al., 2017; Scobie et al., 2001; Straub and Levy, 1999; Meas et al., 2002; Amborski, Lo and Seger, 1989). The financial impact of this disease can be attributed to several factors. Firstly, there are the direct costs associated with the loss of animals, which is relatively minimal given the long clinical course of the disease. Secondly, there is the loss of the gene pool, which has an impact on the future breeding potential of the livestock population. Thirdly, there are indirect costs related to the quality of dairy products, which may be affected by the disease.

The current legislation prohibits the use of milk from clinically sick animals, even after heat treatment (Jacobs, Jefferson and Suarez, 1998; Hachiya et al., 2018; SCVMU, 2007). Additionally, immunosuppression in animals infected with the leukemia virus makes it impossible to obtain a positive effect when using therapeutic and prophylactic agents. In addition, the causative agent of cattle leukemia poses a potentially dangerous medical and social threat, as it is structurally similar to the causative agents of AIDS and human T-cell leukemia (Supotnitskiy, 2009).

The implementation of government programs has resulted in the eradication of bovine leukemia in most European countries. Ukrainian livestock production is now at the final stage of recovery. In recent years, the number of locations unsafe for the above disease, which are collective livestock farms, has decreased. According to the statistical reports of the Main Department of the State Service of Ukraine for Food Safety and Consumer Protection, the number of livestock farms affected by the disease has been between 8 units and 15 units, although isolated cases of animals infected with the leukemia virus have also been recorded. The majority of cases have been identified on small farms and in private households across the country, except for the Zakarpattia and Lviv regions. This is according to the findings of research conducted by regional laboratories of the State Service of Ukraine for Food Safety and Consumer Protection.

The results of scientific research and monitoring control of veterinary medicine laboratories of the State Service of Ukraine for Food Safety and Consumer Protection have proven that violations of regulations on the scope of serological research in the presence of risks of isolated cases of leukemia virus-infected animals in previously sanitized areas inevitably lead to the recurrence of the disease epizootic (Dombrovskyi et al., 2003; Gorbatenko et al., 2014; Bashchenko et al., 2016; Kornieikov et al., 2019). In the context of military aggression, the livestock industry is facing challenges due to the implementation of martial law. These challenges include violations of anti-epizootic measures regulations, which in the case of leukemia are reflected in a decline in serological monitoring levels. This, in turn, leads to the recurrence of epizootic in previously unstable areas and the dissemination of the pathogen due to the loss of control over the epizootic status of specific animal groups.

The **aim of the study** was investigate and analyze the current situation regarding bovine leukemia in livestock of Ukraine and features of anti-epizootic measures in the post-war context.

Materials and methods. Two approaches were used to determine the epizootic status of the Ukrainian livestock sector concerning bovine leukemia: specialists of the Laboratory of Leukosis Study of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' conducted serological monitoring in livestock farms, most of which were conditionally safe, in six regions, namely, Kharkiv, Poltava, Kirovohrad, Chernihiv, Sumy, and Cherkasy regions.

At the same time, the results of serological monitoring of regional laboratories of the State Service of Ukraine on Food Safety and Consumer Protection were analyzed in terms of the volume and effectiveness of research in recent years. In both cases, serological tests for leukemia were performed using the immunodiffusion (ID) and enzyme-linked immunosorbent assay (ELISA).

The reliability of the data on the epizootic situation in livestock production in Ukraine was determined by the

seroprevalence index, which is the ratio of the number of animals infected with the leukemia virus to the number of susceptible animals during the survey period, per hundred animals (%). The number of susceptible animals in farms with different types of ownership was taken into account, as well as the number of serological tests performed.

Results. It is well known that ELISA has significant advantages in assessing the epizootic status of cattle with regard to leukemia. In contrast to the ID, the ELISA method allows the detection of animals infected with the leukemia virus in the early stages of the infectious process, so that the use of this test guarantees higher efficiency in the implementation of measures to cleanse the herd of virus carriers. As mentioned above, both diagnostic tests were used by veterinary laboratories in the serological monitoring system, although from year to year the ELISA method was preferred. According to the results of the analysis, there was a decrease in the use of ID tests and an increase in the role of ELISA in disease control measures during 2019–2024 (Fig. 1).

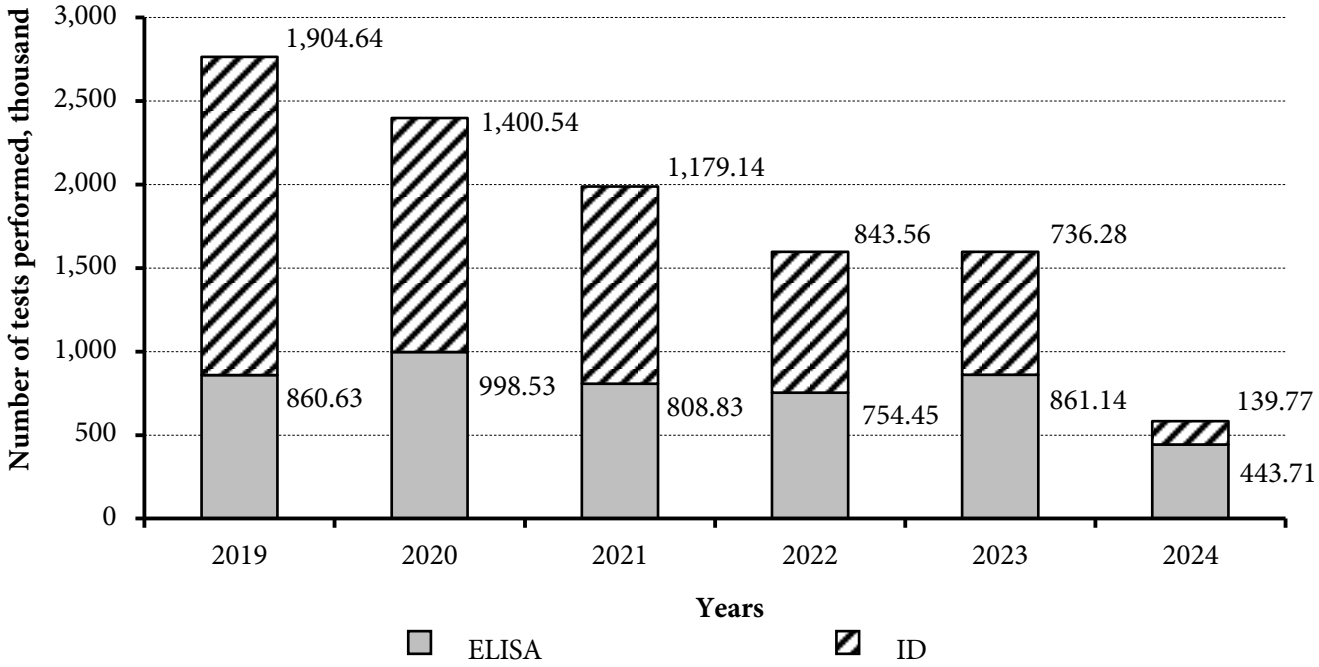

Figure 1. The ratio of serological tests for leukemia using ELISA and ID tests in Ukraine (according to the State Service of Ukraine on Food Safety and Consumer Protection).

In analyzing the data, it was noted that the examination of cattle for leukemia in cattle-breeding enterprises and large farms is primarily conducted using ELISA, while the examination of animals in small farms and households in Ukraine is predominantly done using ID tests. In recent years, there has been a notable increase in the number of cattle in enterprises and farms compared to the same indicator in private households in Ukraine.

The analysis revealed that from 2019 to 2021, the primary method for leukemia diagnosis in Ukraine was the ID test. In 2022, there was a notable shift towards a more balanced approach, with a near-equilibrium between these diagnostic methods. Subsequently, there was a notable increase in the use of enzyme-linked immunosorbent assays for cattle leukemia diagnosis.

In terms of the efficacy of these serological diagnostic methods for cattle leukemia and their influence on the prevalence of the disease in Ukraine, it can be argued that with the widespread implementation of the ELISA method into the leukemia control system in cattle breeding in Ukraine, there is initially an increase in the level of seroprevalence to the leukemia virus, followed by a gradual decrease in this indicator in subsequent years (Fig. 2).

Fig. 2 illustrates that the seroprevalence rate for BLV has remained relatively stable over the past five years. This is in contrast to the significant increase observed in the first year of ELISA implementation, which has since shown a consistent downward trend. This is due to the ID test having a much lower threshold of sensitivity than ELISA. This allows for the detection of BLV-infected cattle much earlier, reducing the risk of infection in the herd. Some discrepancy in the data occurred in 2023, which is explained by the most active phase of hostilities in Ukraine. This resulted in a decrease in the number of animals studied and the quality of leukemia control measures in Ukraine.

In terms of the overall data on the prevalence of animals with bovine leukemia virus, it can be argued that there has been a positive shift in the epizootic situation with leukemia, including due to the introduction of ELISA. During the study period, this indicator decreased from 0.65% (2020) to 0.29% (2024).

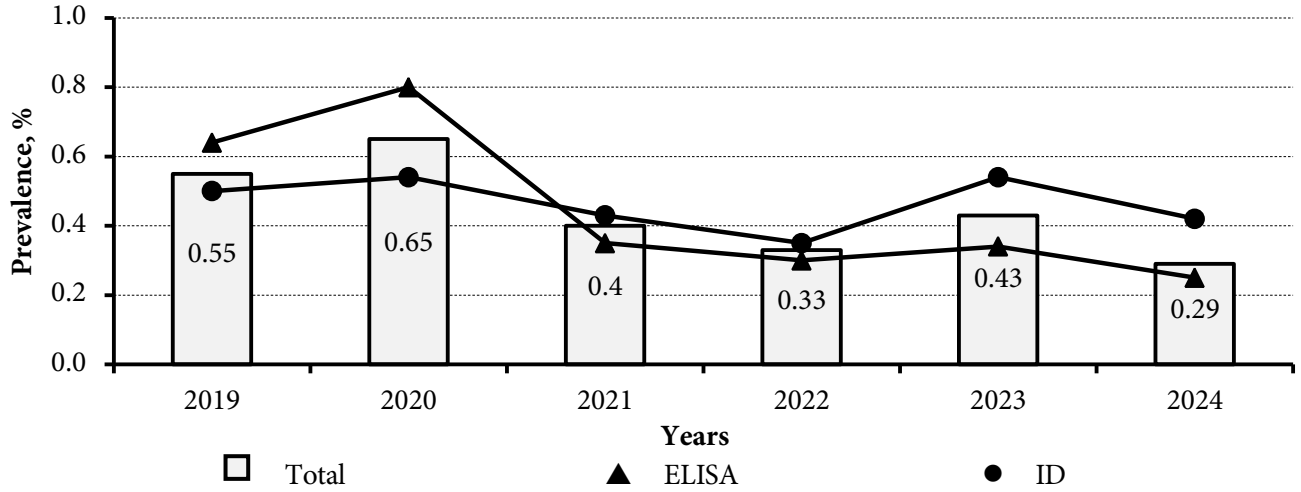

Figure 2. Seroprevalence to bovine leukemia virus determined by ELISA and ID methods in Ukrainian farms of different forms of ownership.

A review of the data from the State Service of Ukraine for Food Safety and Consumer Protection, specifically the leukemia test results from regional veterinary laboratories in Ukraine (Table 1), revealed that infected animals were not identified in the Zakarpattia and Lviv regions during the 2023–2024 period. It is not possible to consider the negative results obtained from the territories of the Donetsk, Luhansk, and Kherson regions reliable due to the temporary occupation of these regions due to the russian federation's military aggression.

Table 1 — Results of serological testing of blood serum from cattle in Ukraine, using different methods

Region	2023				2024 (first quarter)			
	ELISA		ID		ELISA		ID	
	Number of tests performed	Positive reactions	Number of tests performed	Positive reactions	Number of tests performed	Positive reactions	Number of tests performed	Positive reactions
Vinnytsia	71,201	147	57,313	194	27,903	40	34,827	86
Volyn	53,857	9	50,575	232	18,848	54	18,001	103
Dnipropetrovsk	25,866	65	52,731	440	10,243	95	4,147	40
Donetsk	2,563	0	2,394	6	0	0	162	0
Zhytomyr	43,158	62	40,767	161	30,846	25	9,326	47
Zakarpattia	920	0	34,620	0	600	0	5,704	0
Zaporizhzhia	1,421	3	4,198	1	622	0	203	0
Ivano Frankivsk	14,615	0	28,717	0	8,186	0	2,470	0
Kyiv	89,489	243	47,978	214	39,747	39	3,781	2
Kirovohrad	22,286	159	25,469	59	11,823	8	948	2
Luhansk	0	0	0	0	0	0	0	0
Lviv	19,437	0	69,098	0	4,362	0	10,982	0
Mykolaiv	13,474	23	23,880	23	1,192	27	1,989	2
Odesa	10,431	601	38,210	274	3,103	30	7,064	57
Poltava	107,045	108	26,427	249	61,540	72	0	0
Rivne	19,764	115	35,891	868	12,174	25	11,406	191
Sumy	54,200	39	15,839	213	33,714	0	820	4
Ternopil	30,806	20	42,648	13	14,860	0	8,046	1
Kharkiv	35,399	743	11,046	359	15,755	367	703	12
Kherson	475	0	5,498	0	462	0	161	0
Khmelnytskyi	67,257	5	64,804	23	48,586	7	15,051	6
Cherkasy	93,898	47	10,307	61	46,787	56	374	1
Chernivtsi	6,630	9	21,149	3	4,390	15	1,989	0
Chernihiv	76,950	512	26,700	604	47,968	266	1,618	35
Kyiv City	0	0	19	2	0	0	0	0
Total	**861,142**	**2,910**	**736,278**	**3,999**	**443,711**	**1,126**	**139,772**	**589**

As illustrated in Table 1, the presence of infected animals was confirmed in each region of Ukraine. This was determined through serological examinations, including the ID test and ELISA. The results of leukemia tests for the first quarter of 2024 were not included in the analysis as they represented only a portion of the spring animal examination. However, the overall trend continued.

The number of animals studied and the level of seropositivity in different regions of Ukraine varied depending on the epizootic situation in the region, proximity to the combat zone, and the level of anti-leukemia health measures in previous years. In the Kharkiv region, located in the combat zone, 46,445 animals out of 79,200 cattle were tested. Of these, 1,102 heads of cattle were found to be infected with BLV, 743 by ELISA, and 359 by ID test. This equates to a prevalence rate of 2.37%. It should be noted that the infection rate of animals in a particular region may not be entirely accurate, as not all susceptible livestock are tested, and the trend is downward every year. For instance, in Kharkiv Region, the number of cattle was 59.0% compared to 2022. A similar situation is observed every year in other regions of Ukraine and the country as a whole (Fig. 3).

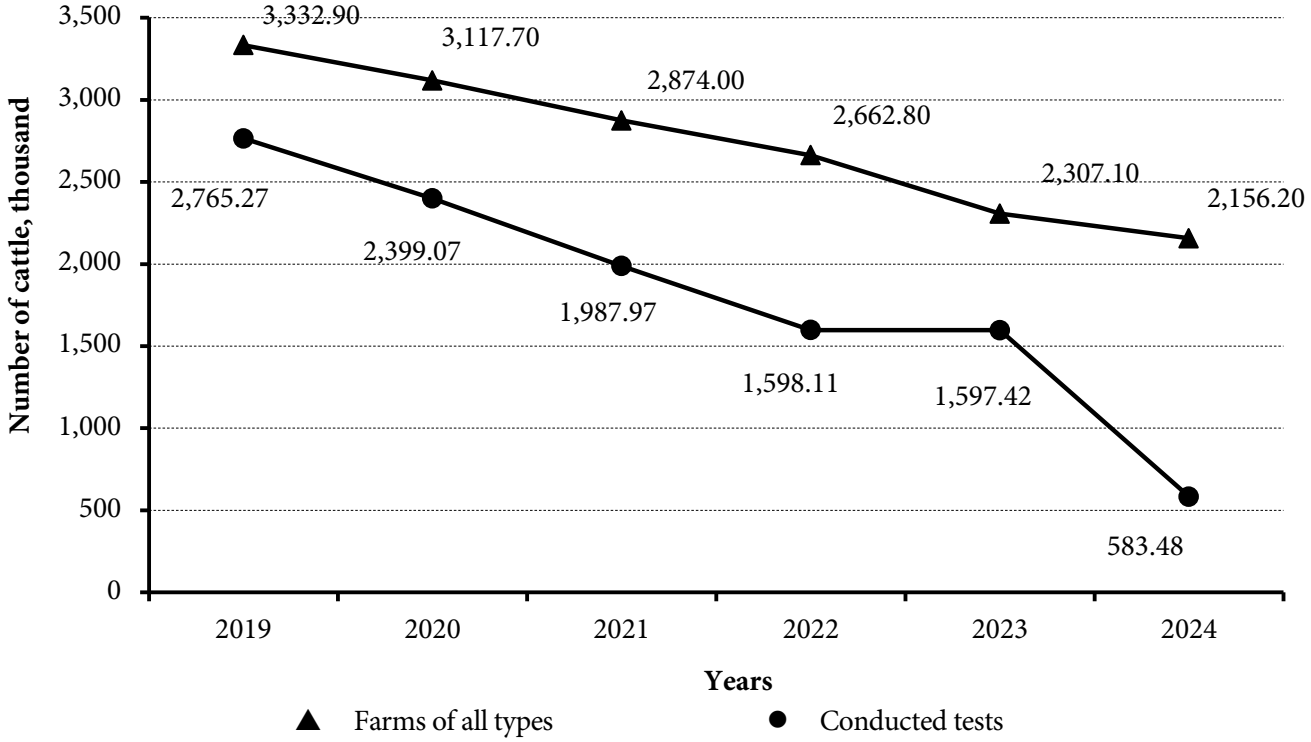

Figure 3. The ratio of the number of cattle in farms of all forms of ownership in Ukraine and the number of animals tested for leukemia (according to the State Service of Ukraine for Food Safety and Consumer Protection and the State Statistics Service of Ukraine).

Fig. 3 illustrates a clear correlation between the decline in serological tests for leukemia in Ukraine and the number of cattle over the same period. However, by calculating the percentage of livestock tested for leukemia during 2019–2024 and the number of cattle kept in livestock farms of all forms of ownership in Ukraine, we can conclude that this indicator is declining annually in Ukraine. In 2019, 82.9% of animals in Ukraine were tested for leukemia. This figure declined to 76.9% in 2020, 69.0% in 2021, 60.0% in 2022, and 69.2% in 2023. While the decline in the number of studies conducted in 2022–2023 can still be attributed to the russian aggression, this cannot be done for the period 2019–2021.

It is worth noting the effectiveness of serological tests for leukemia and the level of seroprevalence within the farms of the six regions where the program was implemented by specialists from the Laboratory of Leukosis Study of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine'. The level of seroprevalence was slightly higher than the average regional laboratory indicators, with figures ranging from 0.45% to 2.7%. This is because the studies were conducted exclusively on farms where anti-leukemia health measures were implemented at various stages, primarily at the final stage.

The results of the analysis of serological monitoring conducted by regional laboratories of the State Service of Ukraine for Food Safety and Consumer Protection and a scientific institution indicate that the epizootic situation in Ukraine's livestock sector regarding bovine leukemia is currently stable and in a state of conditional

well-being. The presence of a limited number of infected animals in each region, except for Zakarpattia and Lviv, indicates that the anti-leukemia health measures program, as outlined in veterinary legislation and the current 'Instruction on the Prevention and Rehabilitation of Cattle Against Leukemia', is incomplete. Regional veterinary laboratories of the State Service of Ukraine for Food Safety and Consumer Protection have reported a downward trend in the volume of serological testing of livestock over the past five years. This is not related to a decrease in the number of cattle. In 2019, 82.9% of the total number of animals were tested for leukemia. In 2019, 0.9% of the total number of animals were tested for leukemia. This figure rose to 76.9% in 2020, 69.0% in 2021, 60.0% in 2022, and 69.2% in 2023. The lack of comprehensive serological monitoring and epizootic prevention measures in cases of isolated relapse cases allows the disease to spread in previously healed areas.

The primary reason for the program's incompleteness is, as the analysis indicates, noncompliance with regulations and the scope of serological tests for leukemia on farms where even a single case of infected animals is identified or there is a risk of recurrence of the epizootic. In light of these circumstances, the strategic direction of post-war anti-epizootic measures should be the development and implementation of planned regulations on the scope and timing of serological monitoring. These regulations should take into account the epizootic circumstances in each particular farm and the timing of research, taking into account the method used. It is reasonable to conclude that the ELISA method is more effective than the ID test in regulating the epizootic status of livestock concerning leukemia. To reduce the economic burden of these measures in Ukraine, it is advisable to conduct combined studies of blood serum samples from animals. Specifically, the ID test should be used at the stages of detecting infected animals, with an interval between studies of no more than 30 days. Subsequently, if a negative result is obtained for the herd, the welfare of livestock farms should be monitored using ELISA.

Conclusions. 1. Current situation regarding bovine leukemia in Ukraine's livestock industry can be considered favorable overall. While in recent years, according to the statistical reports of the Main Department of the State Service of Ukraine for Food Safety and Consumer Protection, there have been 8-15 residual unfavorable locations annually, regional laboratories annually record isolated cases of leukemia virus-infected animals in livestock in each region, except for Zakarpattia and Lviv regions.

2. The state program of anti-leukemia health measures is incomplete due to violations of current veterinary legislation and the 'Instruction on the Prevention and Rehabilitation of Cattle Against Leukemia'. Specifically, there have been violations regarding the regulations for serological control of livestock on farms of various subordinations and measures to prevent the recurrence of the epizootic in previously treated epizootic foci.

3. The need to develop and implement regulations for the serological control of livestock production on each farm, depending on specific epizootic circumstances and measures to prevent the recurrence of epizootic, should be considered a strategic anti-epizootic direction in the post-war period.

Prospects for further use of the results obtained. Increasing the requirements for specialists of regional departments of the State Service of Ukraine for Food Safety and Consumer Protection in terms of awareness and compliance with the provisions of current legislation, guidelines for the organization and implementation of anti-epizootic measures, and emphasis on increasing the responsibility of managers and specialists of livestock farms.

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Part 2. Biotechnology

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EVALUATION OF MECHANICAL STABILITY OF DOG ERYTHROCYTES UNDER THE INFLUENCE OF CRYOPROTECTANTS

Denysova O. M.

State Biotechnological University, Kharkiv, Ukraine, e-mail: denysova78@gmail.com

Summary. The mechanical stability of erythrocytes is a critical factor in ensuring their effective functioning during storage, transportation, and cryopreservation. The objective of this study was to ascertain the impact of diverse cryoprotectants, including glycerol, sucrose, dimethyl sulfoxide (DMSO), polyethylene glycol-1500 (PEG-1500), and hydroxyethyl starch (HES), on hemolytic damage to dog erythrocytes subjected to mechanical stress. For this purpose, dog erythrocytes were incubated in varying concentrations of cryoprotectants and NaCl. The cells were subjected to mechanical stress by stirring the suspension in a container filled with plastic beads at room temperature. The resulting hemolysis was evaluated spectrophotometrically. The results demonstrated that the most pronounced stabilization of erythrocyte membranes was observed during incubation with PEG-1500 and HES, while high glycerol concentrations caused membrane destabilization. Sucrose demonstrated a dual effect: at low concentrations, it exhibited protective properties for cellular membranes, while at higher concentrations, enhanced membrane vulnerability to stress. The results demonstrated that DMSO at all studied concentrations did not significantly change the mechanical stability of erythrocytes compared to the control group. Our findings indicate that an increase in salt concentration in the extracellular medium is associated with a reduction in the mechanical stability of dog erythrocytes. The effect of cryoprotectants on the mechanical stability of erythrocytes was found to be closely related to their physicochemical properties. This highlights the importance of precise selection of cryoprotectant concentrations to improve the results of red blood cell storage and transportation. The conclusions of this study are important for further optimization of technologies for the long-term storage of canine erythrocytes, in particular in cryobanks

Keywords: mechanical stress, cryopreservation

Introduction. The mechanical stability of erythrocytes is an important parameter that determines their ability to withstand physical and osmotic stress during storage, transportation, and cryopreservation (Baskurt and Meiselman, 2013; Ugurel et al., 2017). Erythrocytes, whose main function is to transport oxygen throughout the body, must have high mechanical stability to ensure this function even after prolonged storage or freezing (Tarasev, Chakraborty and Alfano, 2015). Erythrocyte cryopreservation is one of the most effective methods of long-term blood storage, but it is accompanied by numerous negative factors: crystal formation during the transition of liquid to solid state, increase in salt concentration and osmotic pressure in the cooled liquid, dehydration of macromolecules, changes in the location and composition of membrane lipids (Gao and Critser, 2000), increased lipid peroxidation due to a decrease in superoxide dismutase activity (Alvarez and Storey, 1992), and ionic and electrical effects associated with the integration of ions into ice crystals. Osmotic stress caused by changes in the concentration of ions in the medium during the crystallization and thawing of water during cryopreservation is one of the factors that affect the mechanical stability of erythrocytes. During cryopreservation, cells are exposed to extreme temperatures, which leads to the formation of ice crystals and dehydration (Gao and Critser, 2000). To prevent these processes, penetrating and non-penetrating cryoprotectants are used to protect cells from intracellular ice formation and minimize osmotic stress (Elliott, Wang and Fuller, 2017). However, the choice of the optimal concentration of cryoprotectants is critical, as excessive concentrations can cause additional osmotic stress and damage the cell membrane. In addition, during cryopreservation, the volume of cells changes, which leads to mechanical stress that can affect their structure and function. This can be caused by the formation of extracellular ice crystals or by the interaction of cells with each other and the container walls (Ishiguro and Rubinsky, 1994; Saragusty et al., 2009). Thus, the choice of the right cryopreservation conditions, including the concentration of cryoprotectants and NaCl, is key to ensuring high mechanical stability of erythrocytes after long-term low-temperature cell storage. The optimal concentrations of these substances allow to preserve the integrity of the cell membrane, reduce the level of hemolysis, and maintain the functionality of erythrocytes during a long storage period.

This study **aimed** to evaluate the effect of cryoprotectants such as glycerol, sucrose, dimethyl sulfoxide (DMSO), polyethylene glycol-1500 (PEG-1500), and hydroxyethyl starch m. m. 200 (HES), on the development of hemolytic damage to erythrocytes under mechanical stress.

Materials and methods. The object of the study was dog erythrocytes. All animals were clinically healthy, sexually mature males of an unspecified breed, aged 2 to 10 years. Manipulations with animals were performed by veterinarians 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 in accordance with 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).

Blood was taken by venipuncture from the brachial vein following current ethical standards. No animals were harmed during the experiment.

The blood was collected in a glucose-citrate preservative and stored at 5°C for no more than 48 h before the experiments commenced. Red blood cells were isolated by centrifuging the whole blood at 750 g for five minutes, after which the plasma and leuko-platelet layer were removed. The red blood cells were then washed three times in a solution of four times the volume of isotonic saline (150 mM NaCl, 10 mM phosphate buffer, pH 7.4).

The resistance of erythrocytes to mechanical stress was assessed by the degree of hemolysis caused by the action of small beads moving in suspension (Shpakova, Orlova and Alexandrova, 2010). The washed erythrocytes (1 ml) were mixed with cryoprotectant solutions (5 ml) in 20 ml plastic cups, with a final hematocrit of approximately 15%. Solutions of glycerol, sucrose, DMSO, PEG-1500, and HES m. m. 200 were prepared based on isotonic saline at 5–20% concentrations. Then 50 plastic beads (5 mm in diameter, 1.5 g) and a magnetic stirrer were carefully added. The erythrocytes and plastic beads suspension was stirred on a magnetic stirrer MM-5 at 1,200 rpm. To evaluate hemolytic cell damage, aliquots of the erythrocyte suspension were taken, centrifuged at 1,200 g, and the supernatant was collected. Hemolysis was determined by the spectrophotometric method (device SF-46 LOMO, Russia) in a flow-through cuvette at $\lambda = 543$ nm to determine the amount of hemoglobin released from damaged cells. The level of hemolysis was expressed as a percentage relative to 100% hemolysis of erythrocytes in the presence of 0.1% Triton X-100 detergent.

Statistical results were processed using the Statgraphics software package (Manugistic Inc.; STATistical GRAPHICs system, USA). The data were presented in the format M ± SE (mean ± standard error). Differences between the experimental and control groups were evaluated using a nonparametric method, in particular, Fisher's multiple range test using the procedure of grouping samples by the least significant difference. Each series of experiments was performed at least six times.

Results and discussion. Hemolytic damage to dog erythrocytes under the influence of mechanical stress was studied for 60 min in the presence of various cryoprotectants (Fig. 1). The cryoprotectant solutions, balanced by ionic strength and pH to physiological values, had different effects on the stability of dog erythrocyte membranes. The greatest destabilization was observed when using glycerol, except for its 5% concentration, and sucrose at 20% concentration.

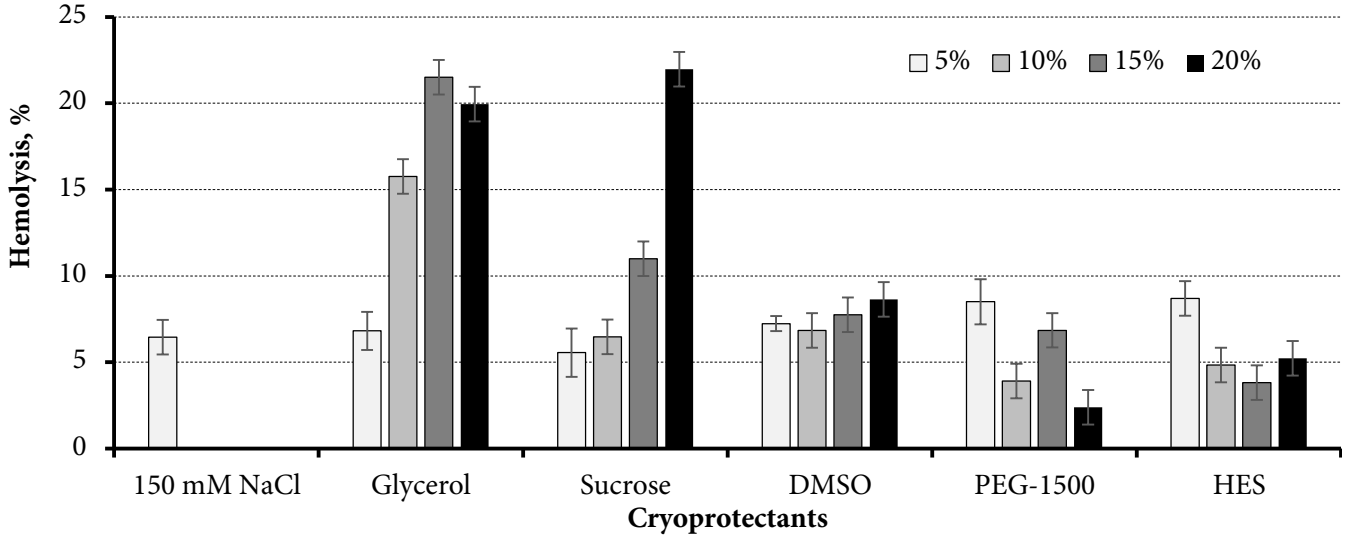

Figure 1. Hemolytic damage of dog erythrocytes under mechanical stress for 60 min in the presence of 5–20% cryoprotectants. * — decrease in hemolysis compared to control (150 mM NaCl, 10 mM phosphate buffer, pH 7.4). $P < 0.05$.

Other cryoprotectants, such as DMSO, PEG-1500, and HES, had variable effects on the mechanical stability of erythrocytes, depending on the concentration. PEG-1500 at concentrations of 10% and 20%, as well as HES at concentrations of 10%, 15%, and 20% demonstrated the ability to stabilize cells during mechanical stress. In contrast, DMSO at all studied concentrations did not demonstrate significant differences from the control group (150 mM NaCl, 10 mM phosphate buffer, pH 7.4).

The mechanical stability of erythrocytes is significantly affected by the concentration of NaCl in the medium. In a hypertonic medium, for instance, at concentrations of 400 mM or 600 mM NaCl, cells undergo dehydration, which reduces their volume and increases the risk of mechanical damage to the membrane. In contrast, a hypotonic medium, where the NaCl concentration is much lower than physiological, can result in cell swelling and, ultimately, hemolysis. Studying the mechanical stability of erythrocytes in different concentrations of cryoprotectants and NaCl allows us to gain a deeper understanding of how these factors interact with each other and affect cell integrity. For instance, the use of an optimal cryoprotectant concentration can minimize the adverse effects of osmotic stress caused by high NaCl concentration when the temperature is lowered, while an inadequate cryoprotectant concentration can exacerbate membrane damage due to osmotic stress.

Increasing the NaCl concentration to 400 mM resulted in a 6-fold increase in hemolytic damage after 30 min and a 7-fold increase after 60 min compared to the control (Fig. 2). At a NaCl concentration of 600 mM, these values increased to 8 and 9 times, respectively.

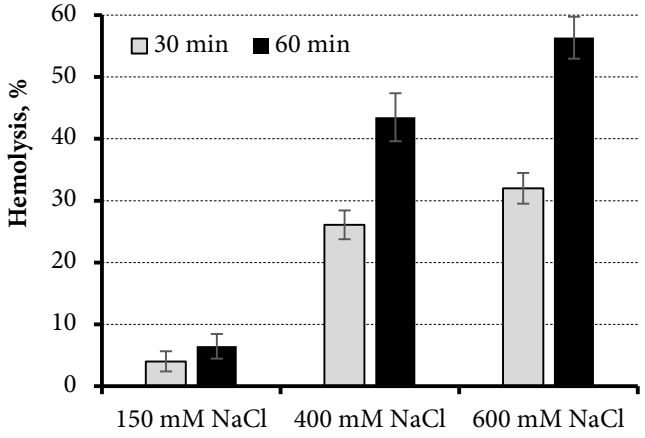

Figure 2. Hemolytic damage of dog erythrocytes under mechanical stress in NaCl solutions of different concentrations. $P < 0.05$.

The effect of cryoprotectants on the mechanical stability of dog erythrocytes during osmotic stress is closely related to their physicochemical properties and mechanism of action.

The results of the study revealed that glycerol, known for its ability to stabilize human erythrocyte membranes under mechanical stress (Zemlianskykh, 2018), destabilizes dog erythrocyte membranes under mechanical stress. These differences may be related to the different structures of membrane lipids and proteins in these two species. In particular, dog erythrocyte membranes have a higher content of cholesterol and saturated phospholipids, which increases membrane stiffness and reduces its ability to adapt during stress (Zhegunov and Denisova, 2010). It has also been noted (Shpakova et al., 2015) that the higher the content of phosphatidylethanolamine (PEA), the less sensitive the cells are to mechanical stress. The percentage of PEA in human erythrocytes is 27.2% of the total phospholipid content, while in dog erythrocytes it is 22.4%. At the same time, glycerol can cause an increase in osmotic pressure within the cell, which, when combined with a stiffer membrane, leads to an elevated susceptibility to hemolysis. Furthermore, the protein composition of canine erythrocyte membranes differs from that of human erythrocytes (Zemlyanskikh and Denisova, 2009). Dog erythrocyte membranes contain higher levels of spectrin and ankyrin, proteins that support the membrane skeleton. In conditions of elevated osmotic pressure, these proteins can activate mechanisms that lead to a decrease in membrane flexibility, contributing to mechanical damage during stress.

Sucrose demonstrated a twofold effect. At high concentrations (15% and 20%), it increased the sensitivity to mechanical stress, which led to membrane damage. At low concentrations (5% and 10%), however, this cryoprotectant helped to maintain membrane stability. Based on the results of this study, sucrose can be used as a cryopreservative component in low concentrations, as it contributes to the preservation of membrane stability. This makes it a promising addition to cryoprotective solutions where minimizing cell damage during cryopreservation is a priority.

PEG-1500 and HES have been shown to effectively reduce cell sensitivity to mechanical stress. This is due to their ability to form a protective barrier around the cell membrane, which reduces osmotic pressure and increases resistance to mechanical damage (Elliott, Wang and Fuller, 2017). High concentrations of these substances have been shown to have a positive effect on the mechanical stability of cells, which highlights their potential as effective cryoprotectants in the context of mechanical stress. Research (Kameneva et al., 2003) indicates that PEG can alter the physicochemical properties of surfaces through adsorption on beads, enhancing biocompatibility, and through adsorption on cells, reducing membrane sensitivity to mechanical stress. However, this does not fully explain the effectiveness of HES in stabilizing dog erythrocytes under mechanical stress.

In contrast to non-penetrating cryoprotectants such as PEG-1500 and HES, which previous studies (Denysova and Zhegunov, 2021; Zhegunov, Denysova and Zhegunova, 2022) have shown to cause membrane damage after freezing and thawing, this study did not reveal any significant changes in the mechanical stability of erythrocytes when using DMSO. The results demonstrated the effectiveness of this cryoprotectant in maintaining the physicochemical properties of membranes during cryopreservation.

The mechanical stability of erythrocytes was found to be significantly affected by the concentration of NaCl. High concentrations of NaCl, such as 400 mM and 600 mM, were observed to significantly increase hemolytic damage, indicating an increase in the

membranes sensitivity to osmotic stress. This effect can be explained by an increase in osmotic pressure, which leads to cell dehydration and increased mechanical stress on the membrane (Gao and Critser, 2000).

Conclusions. The studied cryoprotectants have a significant effect on the mechanical stability of dog erythrocytes. Stabilization of cell membranes was observed during incubation with PEG-1500 and HES, while glycerol in high concentrations destabilized the membranes. Sucrose showed a dual effect: low concentrations provided protection, while high concentrations increased sensitivity to mechanical stress. Unlike other cryoprotectants, the use of DMSO in all concentrations studied did not lead to significant differences in the mechanical stability of erythrocytes compared to the control group. This indicates the potential safety and efficacy of DMSO as a cryoprotectant for preserving the mechanical stability of erythrocytes after cryopreservation. Changes in the mechanical stability of erythrocytes under the influence of cryoprotectants may be related to their effect on the physicochemical properties of cell membranes, which is important for optimizing the conditions of storage and transportation of erythrocytes.

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POTENTIATION OF ACARICIDAL DRUGS WITH THE HELP OF A PHYTOCOMPLEX THAT UNDERGOES CRYODESTRUCTION

Kiptenko A. V., Dunaiev Yu. K., Paliy A. P., Bogach M. V., Keleberda M. I.

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

Summary. The objective of this study was to develop a novel natural veterinary pharmaceutical agent for the treatment of tick and mite infections, with the aim of experimentally confirming its efficacy *in vivo*. The dogs selected for the experiment were divided into three groups (n = 27) according to the type of tick infection, with each group divided into three subgroups (n = 9). The first experimental group of dogs was affected by ixodid ticks, the second by sarcoptic mites, and the third by thrombidiform mites. The groups were then subdivided into three smaller groups. Group I received treatment with 'AnimAll VetLine' antiparasitic tablets for dogs and cats, Group II received treatment with 'Acaro Spectra' antiparasitic tablets for dogs, and Group III served as the control group, whose condition was monitored without treatment. The antiparasitic tablets, designated 'Acaro Spectra', demonstrated efficacy within 24 hours. The test results indicated that the ticks were removed within the same timeframe, and no new ticks attached. Additionally, the administration of 'Acaro Spectra' did not elicit any allergic reactions or signs of distress in the animals. In the treatment of dogs affected by ixodid ticks, sarcoptic, and thrombidiform mites, the drug 'Acaro Spectra' antiparasitic tablets for dogs showed 100% effectiveness. The results obtained allow us to recommend the drug 'Acaro Spectra' antiparasitic tablets for dogs for the treatment and prevention of ixodid ticks (*Ixodes ricinus*, *Rhipicephalus sanguineus*, *Dermacentor reticulatus*), sarcoptic mites (*Otodectes cynotis*, *Notoedres cati*, *Sarcoptes canis*), thrombidiform mites (*Demodex* spp., *Cheyletiella* spp.)

Keywords: ixodid ticks, sarcoptic mites, thrombidiform mites, treatment

Introduction. External and internal parasites can cause serious diseases in domestic animals, which is a concern for their owners. Antiparasitic treatment is challenging due to the large number of available products and the limited understanding of parasiticides among dog owners (Bebrysz et al., 2021; Paliy et al., 2021). Some owners do not protect their dogs against all parasites, while others use effective doses less frequently than recommended (Boost et al., 2017; Lavan et al., 2018). The most common types of ticks and mites that parasitize dogs and cats are ixodid ticks (*Ixodes ricinus*, *Rhipicephalus sanguineus*, *Dermacentor reticulatus*), sarcoptic mites (*Otodectes cynotis*, *Notoedres cati*, *Sarcoptes canis*), thrombidiform mites (*Demodex* spp., *Cheyletiella* spp.) (Kruchynenko, 2020).

Ticks and mites can transmit various viruses, bacteria or parasites that can cause serious infections or diseases in humans and animals. Tick-borne diseases are becoming a growing and serious problem in Europe and worldwide (Beck et al., 2006; Heyman et al., 2010).

Rhipicephalus sanguineus, the brown dog tick, is adapted to colonize both human and dog habitats and can be found in areas near human habitation (Aguilar-Meraz et al., 2024).

Several species of fleas and ticks are established in different bioclimatic zones (Estrada-Pena et al., 2017). Protecting domestic animals from the adverse effects of ectoparasites, including ectoparasite-induced blood loss, skin diseases, and vector-borne pathogens, may require long-term protection against ticks and fleas (Lavan et al., 2018). Ectoparasites present a persistent challenge to veterinarians and pet owners throughout the year (Paliy et al., 2023; Tishyn, Yuskiv and Yuskiv, 2024).

The successful control of ectoparasites in dogs and cats is possible with the availability of highly effective antiparasitic drugs (Matos et al., 2015; Paliy et al., 2024; Giannelli et al., 2024).

The advent of new technologies has facilitated the development of a superior antiparasitic drug that is more natural and efficacious. There are ongoing endeavors to create environmentally benign and cost-effective products. It is imperative to control the damage caused to the skin by infection and to minimize the re-infection by pathogens, such as ticks and fleas.

The objective of this study is to develop a novel natural veterinary drug for the treatment of tick and mite infections. The aim is to establish and subsequently confirm in vivo acaricidal efficacy of the drug.

Materials and methods. To develop a new antiparasitic drug with the addition of a phytocomplex, we studied the composition of 'AnimAll VetLine' and 'Quadro Tab' antiparasitic tablets for dogs and cats.

'AnimAll VetLine' was selected for potentiation based on its superior efficacy indicators. The drug's composition is as follows: 100 mg of the drug contains the active substances imidacloprid (2.4 mg), lufenuron (20.0 mg), and milbemycin oxime (0.6 mg). The excipients are lactose, starch, artificial flavor, aerosil, and calcium stearate.

To create our drug 'Acaro Spectra' antiparasitic tablets for dogs with the addition of a phytocomplex, we used the same composition of chemicals and excipients as in 'AnimAll VetLine'. After studying the different types of plant action, we chose the following composition of the phytocomplex for cryodestruction and subsequent addition to the drug (wt. %): laminaria (*Laminaria*) —

1.5–2.4%; neem leaves (*Azadirachta indica*) — 2.8–3.2%; garlic (*Allium sativum*) — 0.9–1.8%; lemon balm (*Melissa officinalis*) — 1.4–2.6%, which work together to repel common parasites, helping to protect the animal from ticks and fleas (Schmahl et al., 2010; Bharadwaj, Hayes and Stafford, 2015).

The selected phytocomplex has anti-allergic properties, provides soothing relief from itching, and is rapidly absorbed by the animal body.

The equipment utilized in the cryodestruction of plant raw materials plays a pivotal role in the process. In this instance, a cryogenic vibratory chopper (CVC-3) is employed, which provides continuous cooling with liquid nitrogen through a built-in cooling system both prior to and during grinding (https://www.hd-grinder.com/info/advantages-of-cryogenic-grinders-in-processing-95091427.html).

One of the active substances of 'Acaro Spectra', imidacloprid, is subjected to cryo-grinding in conjunction with the phytocomplex.

The phytocomplex, along with imidacloprid, a component of the veterinary drug, is manufactured using cryo-grinding technology (cryodestruction). This process offers a significant advantage in that the biologically active compounds in plants are not subjected to harsh processing and are preserved in their natural forms and proportions. This technology enables the concentration of the primary attributes of plant materials in a limited volume, and it is currently regarded as the most effective of all existing technologies.

The use of cryotechnology on plant raw materials in conjunction with the active ingredient imidacloprid results in the production of fine and ultrafine powders with a considerable specific surface area. This has an impact on the rate of biochemical reactions within the animal's body. Additionally, the drug comprises a micro-structured combination of plant fibers and an insecticide belonging to the neonicotinoid class. This unique formulation confers the drug with efficacious ectoparasiticidal, insecticidal, and repellent properties, which collectively repel common parasites and protect the animal from ticks, particularly the ixodid ticks (*Dermacentor* spp., *Rhipicephalus* spp., *Ixodes* spp.), sarcoptic mites (*Otodectes cynotis*, *Notoedres cati*, *Sarcoptes canis*), thrombidiform mites (*Demodex* spp., *Cheyletiella* spp.).

Thus, we have developed a tick control tablet for dogs that contains the following active ingredients imidacloprid (a neonicotinoid belonging to the group of chloronicotinyl compounds), lufenuron (a compound of the benzoylphenylurea group), milbemycinoxime (a second-generation macrocyclic lactone of milbemycins, a compound of biosynthetic products of the species *Streptomyces hygroscopicus* subsp. *aureolacrimosus*), and a phytocomplex, which is included as an excipient. The phytocomplex contains a mixture of fully active bioavailable ingredients, also as excipients: lactose, starch, artificial flavor, aerosil, calcium stearate, etc.

To ascertain the efficacy of the pharmaceutical agent, a series of tests were conducted to substantiate or refute the purported beneficial impact of the pharmaceutical preparation designated as 'Acaro Spectra'. It is our contention that the phytocomplex amplifies the efficacy of the drug. To substantiate this assertion, we conducted a series of tests and comparisons between 'AnimAll VetLine' tablets for dogs and cats and 'Acaro Spectra' antiparasitic tablets for dogs.

The study involved 81 canines aged between two months and seven years with a live weight from 2 kg to 32 kg. The animals were housed in standardised cages at an air temperature of 24 ± 1.5°C, relative humidity of 40–70%, and air movement of 0.2–0.5 m/s. The animals were fed according to the diet approved by the Municipal Enterprise 'Animal Treatment Center'.

The experimental animals were selected based on a clinical evaluation of their natural infection status and the presence of dermatological lesions, including dermatitis, alopecia, and the overall condition of the skin. A total of 81 canines presenting with dermatological abnormalities were selected. The study population consisted of 76 adult dogs and five puppies. The animals were crossbreeds (Labrador, German Shepherd, Australian Shepherd, Pomeranian Spitz, etc.) and outbreds (OB). The objective was to test the effectiveness of the developed drug, 'Acaro Spectra' antiparasitic tablets for dogs, and to compare its effect with that of the drug 'AnimAll VetLine' antiparasitic tablets for dogs and cats.

The clinical examination of the affected animals revealed the presence of ixodid ticks (*Ixodes* spp., *Dermacentor* spp., *Rhipicephalus* spp.), sarcoptic mites (*Otodectes cynotis*, *Notoedres cati*, *Sarcoptes canis*), and thrombidiform mites (*Demodex* spp., *Cheyletiella* spp.). The presence of flea allergic dermatitis was documented, and the examination revealed the presence of *Ctenocephalides* sp. fleas. Among the naturally affected experimental animals, a mixed course of infection was observed.

The canines selected for the experiment were divided into three groups (n = 27) according to the type of tick infection, with each group further divided into three subgroups (n = 9). The experimental canines were maintained under identical conditions. All data and animal condition were recorded in the observation log.

The first experimental group of dogs was infected with ixodid ticks, the second — with sarcoptic mites, and the third — with thrombidiform mites. Each group was divided into three subgroups: I — treatment with 'AnimAll VetLine' antiparasitic tablets for dogs and cats; II — treatment with 'Acaro Spectra' antiparasitic tablets for dogs; III — control group, to monitor the condition of animals without treatment.

The experimental animals were administered the antiparasitic tablets 'AnimAll VetLine' and 'Acaro Spectra' in oral tablet (*per os*) form. The tablets are palatable, as they also contain a flavoring, and were readily consumed by most animals. In some cases, the

animal did not take the tablet voluntarily; in these instances, it was administered with food or directly into the oral cavity. The study was conducted for 21 days.

Results and discussion. In accordance with the established protocols, parasitological examinations of canines were conducted through visual inspection and sampling for subsequent laboratory analysis. This was done in order to detect the presence of ectoparasites, in accordance with the standards set forth by the Good Clinical Practice (GCP) guidelines, as well as in alignment with the guidelines set forth by the European Union (EU) and the World Association for the Advancement of Veterinary Parasitology (WAAVP) for the evaluation of the efficacy of antiparasitic substances in the treatment and prevention of tick infection. The identification of ectoparasite pathogens was conducted through microscopic examination, in accordance with the established practical guidelines (Jacobs et al., 1994; Geurden et al., 2022).

In the first group of dogs affected by ixodid ticks, a visual examination of the animals, brushing and collection of ticks were performed. Five ticks were left on each animal to observe the effect of the drugs.

On the first day, the dogs treated with 'AnimAll VetLine' antiparasitic tablets for dogs and cats and 'Acaro Spectra' antiparasitic tablets for dogs were given the drugs in the form of tablets according to the dosage by weight of the experimental animals. The control group did not receive the drugs.

On the second day, the dogs were subjected to a visual examination and brushing. Subsequently, the aforementioned procedures were repeated on days 7, 14, and 21. The results of the examinations are presented in Table 1.

Table 1 — Effectiveness of treatment of dogs affected by ixodid ticks

Group	Number of ticks				
	1st day	2nd day	7th day	14th day	21st day
'AnimAll VetLine' antiparasitic tablets for dogs and cats	45	1	0	0	0
'Acaro Spectra' antiparasitic tablets for dogs	45	0	0	0	0
Control group (no treatment)	45	45	42	55	48

The control group of dogs exhibited a range of symptoms, including weakness, increased sleeping, redness of the skin, allergic reactions, a poor appetite, and fever. The dogs in the experimental group, which were treated with 'AnimAll VetLine' antiparasitic tablets for dogs and cats and 'Acaro Spectra' antiparasitic tablets for dogs, exhibited a notable improvement in their condition. The administration of the pharmaceutical agents resulted in the crawling of ticks on the dogs' fur, yet none of the ticks proceeded to bite.

Thus, the antiparasitic tablets 'Acaro Spectra' for dogs resulted in the death of all ticks on the second day, which were subsequently captured. The antiparasitic tablets 'AnimAll VetLine' for dogs and cats led to the death of 44 out of 45 ticks on the second day, with no fixed ticks observed on the seventh day. Notably, the ticks that were initially planted on the dogs did not become fixed, and there was no evidence of re-infection.

In the control group, we noticed that only those ticks that had already drunk blood were sucked out, and in a few days, they would bite the dogs again. The majority of the ticks that had been implanted remained attached to the dogs and proceeded to bite them.

In the second group of dogs affected by sarcoptic mites, a visual examination of the animals was conducted on control days. Additionally, smears and scrapings were taken from the ears and skin, examined under a microscope, and the hair follicles were examined.

The animals exhibited a range of symptoms, including lethargy, an unwillingness to eat, and a constant scratching behavior. Additionally, they displayed partial hair loss, reddened skin, self-inflicted wounds, and a tendency to shake their heads.

To enhance the effect of the drug and to speed up healing and soothe the skin, remove itching and redness, the damaged areas were treated twice a day with an antibacterial spray with chlorhexidine and ketonazole (Vitomax, 'Pet Skin Spray'). The auricles were treated and cleaned every day with 'Auricap' to remove mite vital secretions in the animal's auricle as quickly as possible. The results of the examinations are presented in Table 2.

Table 2 — Effectiveness of treatment of dogs affected by sarcoptic mites

Group	Intensity of skin infection in percentage, %				
	1st day	2nd day	7th day	14th day	21st day
'AnimAll VetLine' antiparasitic tablets for dogs and cats	30	30	25	18	12
'Acaro Spectra' antiparasitic tablets for dogs	30	30	26	15	10
Control group (no treatment)	30	30	40	45	52

On the first day, when examining the scrapings under a microscope, a considerable number of mites was identified in all animals.

On the second day, a notable reduction in the number of mites was evident in the groups of animals treated with 'AnimAll VetLine' antiparasitic tablets for

dogs and cats and 'Acaro Spectra' antiparasitic tablets for dogs. By the seventh day, no mites were observed under the microscope.

The animals treated with 'AnimAll VetLine' antiparasitic tablets for dogs and cats and 'Acaro Spectra' antiparasitic tablets for dogs exhibited notable improvements in health. The animals displayed enhanced physical condition, with wool growth on exposed skin, absence of skin redness and itching, and favorable changes in appetite and mood. Additionally, the dogs exhibited increased activity levels and a cessation of ear shaking.

In contrast, the control group exhibited a decline in the animals' condition, accompanied by a notable intensification of the aforementioned symptoms, and three animals displayed a complete refusal to eat.

In the third group of dogs affected by sarcoptic mites on the control days, a comprehensive examination was conducted, including a visual assessment and skin scrapings, which were subsequently examined under a microscope. The clinical manifestations of sarcoptic mite infection are highly analogous to those observed in thrombidiform mite infection. Consequently, the examination and treatment are nearly identical.

Dogs display a consistent tendency to scratch, resulting in partial hair loss, cutaneous redness, and self-inflicted wounds caused by itching.

To optimize the efficacy of the medication and facilitate the healing process, the affected areas were treated twice daily with an antibacterial spray containing chlorhexidine and ketonazole (Vitomax, 'Pet Skin Spray'). The findings of the examinations are presented in Table 3.

Table 3 — Effectiveness of treatment of dogs affected by thrombidiform mites

Group	Intensity of skin infection in percentage, %				
	1st day	2nd day	7th day	14th day	21st day
'AnimAll VetLine' antiparasitic tablets for dogs and cats	45	45	40	30	10
'Acaro Spectra' antiparasitic tablets for dogs	45	45	41	28	8
Control group (no treatment)	44	44	56	60	68

On the initial examination of the scrapings under a microscope, a considerable number of mites were recorded. On the second day, the number of mites exhibited a notable decline in dogs treated with 'AnimAll VetLine' antiparasitic tablets for dogs and cats and 'Acaro Spectra' antiparasitic tablets for dogs. On the seventh day, no mites were observed under the microscope. Following treatment, the dogs exhibited improved physical condition, with improved skin appearance, hair growth on exposed skin, absence of redness, itching, and enhanced appetite.

The healing of the skin was observed to occur at a rate 2% faster when the dogs were administered the antiparasitic tablets 'Acaro Spectra' than when they were administered the antiparasitic tablets 'AnimAll VetLine' for dogs and cats.

In the control group, the condition of the dogs worsened. This was evidenced by an increase in the number of areas of skin devoid of hair, an increase in the frequency of scratching, a change in the color of the skin to a reddish hue, and the presence of wounds that were actively licked by animals.

A comparison was conducted between 'AnimAll VetLine' antiparasitic tablets for dogs and cats, which are similar in effect and chemical composition, and 'Acaro Spectra' antiparasitic tablets for dogs, which were developed by our team. The results demonstrated that both products exhibited 100% effectiveness against ixodid ticks starting from the second day. No reinfection with ticks was observed until the 21st day of observation. The weekly attachment rates are an important parameter for understanding the role of the drug in controlling reinfection. All animals were successfully cured of the tick infection.

In the fight against ixodid ticks, thrombidiform mites and sarcoptic mites, both drugs proved to be effective, and the number of ticks decreased significantly on the second day. The results of skin healing were 2% faster after administration of 'Acaro Spectra' antiparasitic tablets for dogs.

'AnimAll VetLine' antiparasitic tablets for dogs and cats showed its effectiveness within 21 days against thrombidiform and sarcoptic mites.

The efficacy of 'Acaro Spectra' antiparasitic tablets for dogs was demonstrated within 21 days against thrombidiform and sarcoptic mites, and ixodid ticks. The healing of skin lesions and the occurrence of allergic reactions in animals were observed to be slightly faster in comparison to the antiparasitic tablets 'AnimAll VetLine' for dogs and cats. It is postulated that the drug 'Acaro Spectra' is straightforward to use, highly efficacious, and more effective than other pharmaceutical agents.

Conclusions. 1. The developed drug 'Acaro Spectra' antiparasitic tablets for dogs showed that the acaricidal effect is manifested after 24 hours. The test results proved that ticks fall off after 24 hours, and new ones do not attach.

2. The administration of 'Acaro Spectra' antiparasitic tablets for dogs has been observed to elicit no allergic reactions or signs of anxiety in the animal.

3. The drug 'Acaro Spectra' antiparasitic tablets for dogs has been demonstrated to be 100% effective in the treatment of dogs affected by ixodid ticks, sarcoptic mites, and thrombidiform mites.

4. The results obtained allow us to recommend the drug 'Acaro Spectra' antiparasitic tablets for dogs for the treatment and prevention of infection with ixodid ticks (*Ixodes ricinus*, *Rhipicephalus sanguineus*, *Dermacentor reticulatus*), sarcoptic mites (*Otodectes cynotis*, *Notoedres cati*, *Sarcoptes canis*), thrombidiform mites (*Demodex* spp., *Cheyletiella* spp.).

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Part 3. Biosafety

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THE ROLE OF THE GREATER WHITE-FRONTED GOOSE, *ANSER ALBIFRONS* (SCOPOLI, 1769) (ANSERIFORMES: ANATIDAE) IN MAINTAINING THE NATURAL CIRCULATION OF THE INFLUENZA A VIRUS IN THE NORTHWESTERN PART OF BLACK SEA REGION (UKRAINE)

Haidash O. M. [1, 2], **Muzyka D. V.** [1, 3, 4]

[1] H. S. Skovoroda Kharkiv National Pedagogical University, Kharkiv, Ukraine, e-mail: alexgaidash@gmail.com
[2] Danube Biosphere Reserve, Vylkove, Ukraine.
[3] National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Kharkiv, Ukraine
[4] Linnaeus University, Kalmar, Sweden

Summary. The publication combines the results of ornithological observations of the greater white-fronted goose, *Anser albifrons* (Scopoli, 1769) during migration and wintering in the south-western part of Odesa Region of Ukraine (Bilhorod-Dnistrovskyi, Izmail, and Bolhrad districts) and the results of laboratory tests of biological samples of this species for the presence of the influenza A pathogen. The study was conducted in 2017–2019. A total of 1,591 samples of biological material (feces) of the greater white-fronted goose, collected in 48 locations in Odesa Region, were examined using polymerase chain reaction (PCR). The influenza A virus genome was detected in 23 samples. The total annual prevalence was 1.44%, in winter — 1.44%, in spring — 1.81%. During the fall migration, no influenza A virus was detected in the genome samples, which determines the wintering period as the most important stage of integration of the greater white-fronted goose in maintaining the natural circulation of influenza A in the study area. By year, the prevalence was distributed as follows: in 2017 — 1.75%, in 2018 — 1.97%, in 2019 — 0.78%. All cases of detection of influenza A virus genome in fecal samples of the greater white-fronted geese concerned areas near large bodies of water in the coastal part of the Black Sea (Sasyk Lagoon and Tuzly Lagoons). Among the factors that may determine the high involvement of the greater white-fronted goose in the circulation of influenza A in these areas are the presence of other sources of infection: numerous dense multi-species aggregations of birds in the wetland complex and favorable conditions for pathogen transfer

Keywords: epizootological monitoring, wild birds, prevalence, Odesa Region, polymerase chain reaction

Introduction. Today, studying and controlling the circulation of hazardous pathogens in a natural reservoir is vital. One of these pathogens is the influenza A virus, which threatens the health of mammals, birds, and humans. Today, this pathogen is considered to be one of those that could cause the next pandemic. Wild waterfowl are known to be the main natural reservoir of the influenza A virus in the world (Swayne, Suarez and Sims, 2013). Studies in Ukraine have also revealed widespread circulation of this pathogen among wild birds (Muzyka et al., 2012; Stegniy et al., 2018). At the same time, the role of certain species of waterfowl and shorebirds has not been sufficiently studied, especially in Ukraine. One of these species is the greater white-fronted goose, *Anser albifrons* (Scopoli, 1769).

The greater white-fronted goose is a migratory, wintering species of the fauna of Ukraine. The wintering areas of the species are located in Azov-Black Sea Region and the lower part of the Dnipro (Fesenko and Bokotej, 2007). There are two main migratory routes of the greater white-fronted geese in Ukraine: northern and southern. Both routes are latitudinal, i. e. the general direction of flight is from east to west in the fall and from west to east in the spring (Poluda, 2009). During the migration period, geese nesting in northern Eastern Europe, Western Siberia, and Taymyr are found in Ukraine. Birds migrating through Ukraine spend the winter in Western Europe (the Netherlands, Belgium, Great Britain), Central Europe (Middle Danube Plain), and Black Sea Region (Balkan Peninsula, Southern Ukraine — Odesa, Mykolaiv, Kherson, Zaporizhzhya regions, and AR Crimea) (Poluda, 2009). According to some ornithologists, the total number of the greater white-fronted geese migrating through Ukraine may reach more than one million individuals (Poluda, 2009).

An important ecological feature of this species is that during migration and wintering in southern Ukraine, birds form numerous aggregations in feeding and resting areas (large reservoirs, agricultural landscapes), where they come into contact both with other groups of their species and with other species of birds in the wetland complex. Such dense aggregations create optimal conditions for the exchange of pathogens, including the influenza A virus.

The number of wintering the greater white-fronted geese in Ukraine decreased from 82,953 birds in 2005 to 3,116 birds in 2017, according to the results of annual January censuses in 2005–2017 (Andryushchenko et al.,

2019). However, according to the results of similar population censuses in 2018–2021, the number of the species wintering in the mainland of Ukraine was 22,038 birds in 2018, 17,011 birds — in 2019, 17,440 birds — in 2020, 15,823 birds — in 2021, and 26,970 birds — in 2022 (Results …, 2023).

Thus, taking into account the wide circulation of influenza A virus in the natural reservoir in Ukraine, as well as the peculiarities of the migratory behavior of the greater white-fronted goose, its migratory connections, its number during migration and wintering, this **study aimed** to determine the role of the greater white-fronted goose as one of the natural reservoirs in maintaining the natural circulation of influenza A virus in Ukraine based on the results of ornithological and virological monitoring.

Materials and methods. Ornithological studies were conducted in 2017–2019. Field material was collected during field visits to the territory of the south-western part of Odesa Region (Bilhorod-Dnistrovskyi, Izmail, and Bolhrad districts). The territory was surveyed by car, boat and on foot. During the survey of the study area, absolute bird counts were made of the wetland complex within the waters and adjacent areas of large water bodies (the Danube lakes, Tuzly Lagoons, Sasyk Lagoon, and the Danube Delta). In cases where absolute counts were not possible, the method of relative (point) counts was used. Counts were conducted during daylight hours, starting 30 minutes after sunrise and ending 30 minutes before sunset. During bird counts, geographical location, species composition and number were recorded. During the collection of field material, ×10 binoculars, ×20–60 telescopes, cameras with 300–500 mm lenses, GPS navigators and drones were used. The collected data were recorded in a field diary and an electronic database. The field data were processed using MS Excel and QGIS software.

The collection of biological material (feces) for laboratory tests to detect the genome of the avian influenza virus was carried out in 2017–2019 in the south-western part of Odesa Region (Bilhorod-Dnistrovskyi, Izmail, and Bolhrad districts) within the framework of monitoring studies on the circulation of particularly dangerous infections in wild birds in Ukraine under the state scientific research of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' and the international partner project UP4 'Analysis of risks associated with certain particularly dangerous pathogens that can be transmitted by migratory birds in Ukraine'.

Fecal samples were collected by standard methods using sterile applicators (FLOQSwabs, Copan Flocked Swabs). Brain Heart Infusion Broth (BHIB), manufactured by Sigma-Aldrich (USA) in a mixture with antibiotics, pH (7.4 ± 0.2) was used as a transport medium (Spackman et al., 2013). After field collection, samples were immediately placed in liquid nitrogen in a Dewar vessel (−196°C). Before molecular biological studies, biological samples were stored in liquid nitrogen at −196°C (in the field) and −80°C (in the laboratory). Sampling, transport, and storage were performed under biosafety requirements.

The RNA was extracted from fecal samples using QIAamp Pathogen Mini Kit (Qiagen). Positive AIV diagnosis was based on real-time RT PCR (qRT-PCR) using universal primers targeting the Matrix Protein (MP) gene (Spackman, 2014).

Results. ***Ornithological studies of the greater white-fronted goose.*** During 2017–2019, 106 flocks of the greater white-fronted geese with a total number of 76,053 birds were recorded during field surveys (Table 1, Fig. 1). During the observation period, flocks of the greater white-fronted geese were recorded from September to April. The highest number of flocks was recorded in winter — 47.2% (n = 50). The total number of recorded flocks was 39,903 birds in winter and 34,139 birds in spring, which is 52.5% and 44.9%, respectively. The highest number of birds was recorded in 2018. Flocks were recorded in agricultural landscapes (mainly winter wheat and rapeseed fields), wetlands, and in the air. In 55.7% of the cases, accompanying bird species were present in the flocks of the greater white-fronted geese or near the flocks. The localization of concentrations of the greater white-fronted geese has been linked to large bodies of water: the Danube lakes (China Lake, Katlabukh Lake, Kugurlui Lake, Yalpug Lake, Kartal Lake, Kagul Lake, etc.), Sasyk Lagoon and the group of Tuzly Lagoons (Shahany, Alibey, Burnas, etc.) (Haidash and Yakovliev, 2024)

During migration and wintering, most concentrations of the species prefer areas bordering the Black Sea. In areas far from the sea, birds were recorded mainly during migratory movements (Fig. 1).

The birds fed mainly on winter crops and rapeseed fields, which are the dominant crops in the region.

Molecular biological studies. The results presented in this article are part of a wider study of wild birds in Ukraine. Thus, from December 2016 to January 2020, 14,502 samples of biological material from 53 species of wild birds of the wetland complex were collected in the northern and southern parts of the country. Of these, 3,072 samples were collected from the greater white-fronted geese at 91 sites. In particular, in the south-western part of Odesa Region from January 2017 to December 2019 (our target region and the research period in this article) 1,591 samples were collected in 48 locations (Table 1).

The results of laboratory screening indicate the presence of influenza A pathogen among migratory and wintering groups of the greater white-fronted geese in the northwestern part of Black Sea Region. The circulation of the pathogen was recorded in winter and spring within the territories located along the Black Sea coast (Fig. 1). In total, the influenza A virus genome was detected in 23 samples of biological material from the greater white-fronted goose in the south-western part of Odesa Region (Table 2). Circulation of the influenza A virus was confirmed in 11 out of 48 flocks of this species.

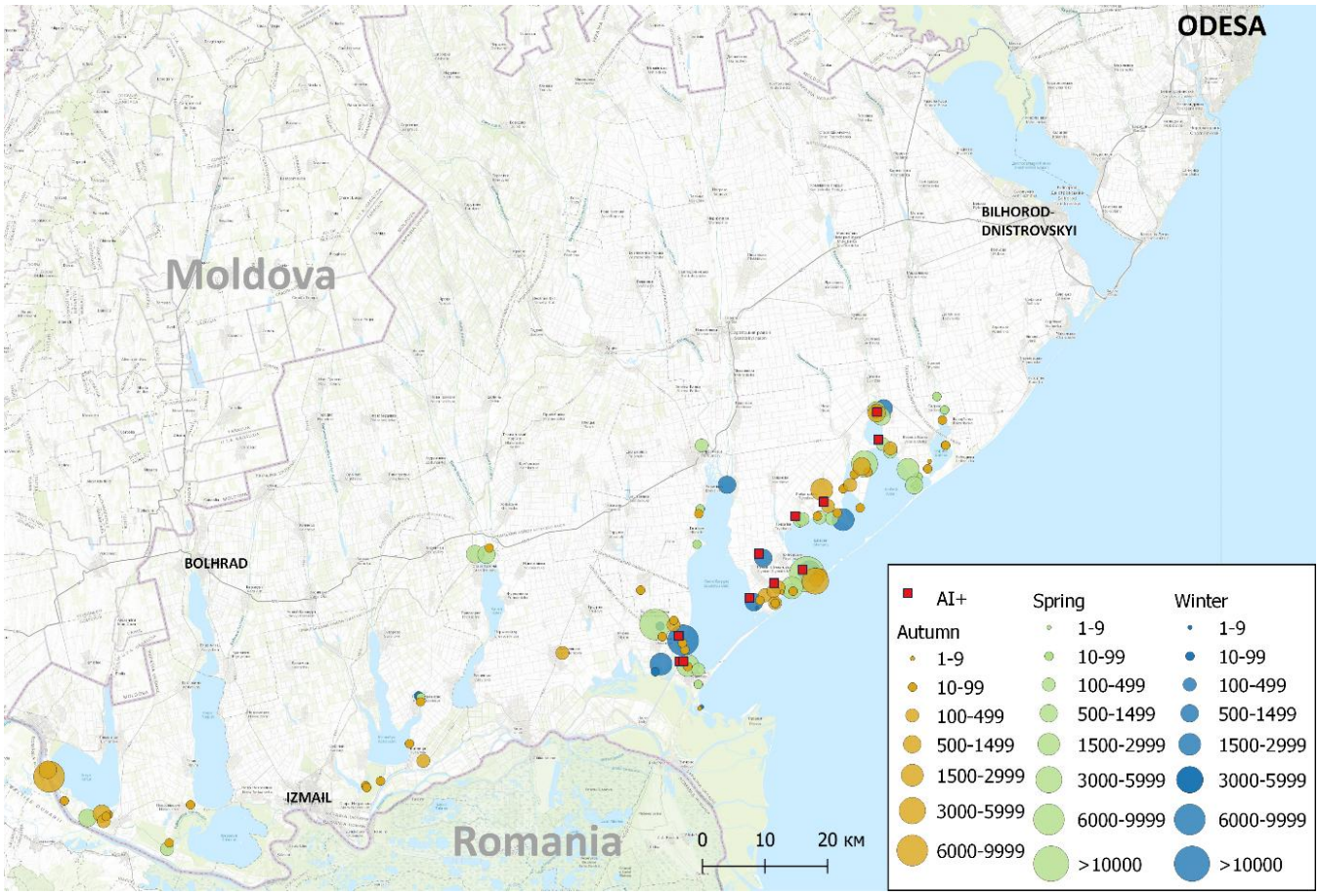

Figure 1. Location and number of flocks of the greater white-fronted goose, *Anser albifrons* (Scopoli, 1769) in the south-western part of Odesa Region in 2017–2019 and sampling sites where the influenza A virus gene was detected by PCR.

The prevalence in flocks in which the presence of influenza A virus was detected ranged from 2.00% to 13.33%. In one case the prevalence was 50.00%, but the study included biological material from only 2 geese, one of which was positive for influenza A. The average prevalence was 1.44%. The prevalence was 1.44% in winter and 1.81% in spring. No infected the greater white-fronted geese were detected in the fall period. The prevalence was distributed by years as follows: in 2017 — 1.75%, in 2018 — 1.97%, in 2019 — 0.78%.

Table 1 — Summary of the results of the registration of the greater white-fronted goose, *Anser albifrons* (Scopoli, 1769) in the south-western part of Odesa Region in 2017-2019 and the sampling of biological material

Parameters	Year			Total
	2017	2018	2019	
Identified flocks	48	38	20	106
Total number of birds counted	26,680	43,700	5,673	76,053
Flocks sampled	20	13	15	48
The total number of samples collected	398	558	635	1,591

Discussion. The results of molecular biological studies and the detection of the influenza A virus genome in the populations of the greater white-fronted geese migrating and wintering in the south-western part of Odesa Region of Ukraine indicate the involvement of the species in the process of influenza A circulation.

The absence of pathogen detection during the fall migration may indicate both insufficient sampling (101 fecal samples collected during the fall period were examined) and the fact that most birds acquire the pathogen during the wintering period, in contact with other bird species in the wetland complex in feeding, resting and roosting areas. This hypothesis is supported by some other studies (Yin et al., 2017; Ely et al., 2013).

During similar studies on the circulation of influenza A pathogen in flocks of the greater white-fronted geese in southern Ukraine in 2010–2011 during the fall migration of the virus among birds, no virus was detected, while 9 isolates were obtained from wintering birds (Muzyka et al., 2012). Similarly, a highly pathogenic avian influenza virus was isolated from the greater white-fronted geese in the Askania Nova reserve in 2016 during the wintering period (Stegniy et al., 2018). The role of the greater white-fronted goose as a vector of infection in the wintering grounds during fall migration needs to be clarified.

Table 2 — The results of molecular genetic studies of biological samples from the greater white-fronted goose, *Anser albifrons* (Scopoli, 1769) for the presence of the influenza A virus genome in the south-western part of Odesa Region

Date	The nearest settlement to the sampling site/habitat	Number of samples collected / number of positive samples / prevalence
28.01. 2017	Prymorske village, Izmail district/winter crops field	2 / 1 / 50.00%
09.03. 2017	Balabanka village, Bilhorod-Dnistrovskyi district/winter crops field	15 / 1 / 6.66%
09.03. 2017	Prymorske village, Bilhorod-Dnistrovskyi district/winter crops field	50 / 1/ 2.00%
10.03. 2017	Prymorske village, Izmail district/winter crops field	15 / 2 / 13.33%
16.12. 2017	Lyman village, Bilhorod-Dnistrovskyi district/winter crop field	30 / 2 / 6.66%
25.02. 2018	Prymorske village, Izmail district/winter crops field	120 / 3 / 2.50%
31.03. 2018	Zhovtyi Yar, Bilhorod-Dnistrovskyi district/wetlands	35 / 1 / 2.85%
01.04. 2018	Vesela Balka village, Bilhorod-Dnistrovskyi district/winter crops field	40 / 2 / 5.00%
01.12. 2018	Balabanka village, Bilhorod-Dnistrovskyi district/winter crops field	40 / 5 / 12.50%
14.01. 2019	Prymorske village, Bilhorod-Dnistrovskyi district/winter crops field	80 / 3 / 3.75%
14.01. 2019	Lyman village, Bilhorod-Dnistrovskyi district/winter crop field	55 / 2 / 3.63%

The absence of pathogen fixation among flocks of the species during fall migration may indicate a much lower prevalence than during wintering and spring migration.

The value of wintering grounds and stopover sites during migration for the greater white-fronted geese is determined by the availability of a trophic base (mainly winter wheat and rapeseed seedlings, grain residues, corn, sunflower, and soybeans), as well as the availability of watering and resting places with minimal impact of avian disturbance factors (Andryushchenko et al., 2019). In the south-western part of Odessa Region, such places are concentrated near large bodies of water. Firstly, fodder crops are concentrated along the banks of these reservoirs, while the reservoirs themselves are safe places for watering, resting, and roosting.

Large reservoirs in the northwestern part of Black Sea Region of Ukraine are concentrated in the lower reaches of the Danube and the Dniester. Within our study area (the south-western part of Odesa Region) such reservoirs can be divided into 3 groups:

(i) The Danube lakes are freshwater lakes located at the mouth of the Danube, far from the sea (Lake Kagul, Lake Kurgului, Lake Kartal, Lake Kotlabukh, etc.).

They are large and rather deep lakes. The total water surface of the Danube lakes is about 468.7 km^2, with an average depth of 0.8–2.6 m and a maximum depth of 2–7 m, depending on the level of the Danube (Shvebs and Igoshin, 2003). During severe winters, the lakes can be completely covered with ice;

(ii) The Danube Delta (Ukrainian part) is an extensive system of the Danubian freshwater channels, canals, straits, inland freshwater lakes of various sizes and depths, bays, floodplain ecosystems, and the sea coast. The total area of the Danube Delta is 2500 km^2, but most of the delta is located in Romania (Klymenko, 2010). During low winter temperatures, a large part of the delta's water surface freezes;

(iii) Seaside lakes include the system of salt lakes Shahany–Alibey–Burnas and others (Tuzly Lagoons), and the desalinated Sasyk Lagoon. These are large lakes, most of which are shallow: the area of Sasyk Lagoon is 210 km^2, with an average depth of 2 m and a maximum depth of 3 m (Shvebs and Igoshin, 2003). The total area of the Tuzly Lagoons is more than 208 km^2, with an average depth of 0.6–1.2 m and a maximum depth of 1.0–2.5 m (Shvebs and Igoshin, 2003). The Sasyk reservoir is desalinated and connected to the Danube. Canals connect the Dzhantshey and Malyi Sasyk lakes to both Sasyk Lagoon and the saline Tuzly Lagoons, so the salinity of these lakes can vary considerably. The Sasyk reservoir freezes in severe winters, while other lakes can be ice-free due to their high salinity.

The epizootological potential for influenza A and other infections circulating in wild bird populations within these three groups of water bodies is determined, in particular, by the species richness and number of birds using these water bodies.

The analysis of the long-term monitoring of the wintering bird fauna within these groups of water bodies within the framework of the Regional Ornithological Monitoring Program shows that the Danube and the sea lakes are equivalent in terms of the number of species and the number of birds, while these indicators are higher in the Danube Delta (Table 3).

The largest number of bird species of the water and wetland complex and their abundance in the study area is usually concentrated in the Danube delta, which creates ample opportunities for the exchange of infections in places of congregation (Table 3). However, the number of the greater white-fronted geese in this area is small and varies from 1 to 264 birds in winter (Results …, 2011, 2017, 2023). Quantitative indicators of wintering avifauna are primarily influenced by the ice regime of the water bodies in the Delta.

Table 3 — Comparative characteristics of the qualitative and quantitative composition of the avifauna during the wintering period in different types of water bodies in the south-western part of Odesa Region (Results …, 2011, 2017, 2023)

Group of water bodies	Period, year	Number of birds			Number of species		
		max	min	average	max	min	average
The Danube lakes	2006, 2018–2022	28,431	35	12,494.6	28	4	19.2
The Danube Delta	2006, 2009, 2011–2016, 2018–2022	50,045	3,807	19,073.1	43	13	30.3
Seaside lakes	2005–2010, 2014, 2015, 2017–2022	24,332	523	13,191.9	32	7	18.9

Species diversity and abundance of wintering avifauna within the Danube lakes also depend largely on the ice regime of the water bodies. According to our observations, wintering species in this area are usually dispersed over water surfaces, and multi-species aggregations are formed in shallow areas (coastal parts of water bodies, fish hatcheries, etc.).

First of all, the distribution of the birds in this area is determined by the large area of the Danube lakes and the homogeneity of the ecosystems. The greater white-fronted goose does not form winter aggregations here every year but regularly uses the reservoirs and surrounding agrocenoses during migration (Fig. 1, 2). The largest overnight aggregation of the species we recorded during the fall migration on Lake Kahul in 2011 consisted of about 15,000 individuals. According to our observations, the formation of winter aggregations of the species is directly related to temperature, snow, and ice conditions in the area. For example, wintering aggregations of the greater white-fronted geese have been regularly recorded in the area since 2019. During the same period, the region was virtually free of severe winters with ice cover and heavy snowfall.

The most numerous and regular wintering of the greater white-fronted geese within the study area is recorded in the Seaside Lakes (Fig. 2, Tabl. 3). This area unites the most diverse saltwater and freshwater wetland ecosystems in the northwestern part of Black Sea Region. A significant part of the water area in this area does not freeze even in severe winters due to its high salinity. The distribution of birds in the waters of these lakes is not uniform. Due to the concentration of birds in shallow waters and on the spits within the lakes, many thousands of dense multi-species aggregations of birds of the wetland complex are recorded. All this creates conditions for the formation of stable centers of pathogen transfer.

Taking into account our own and retrospective data of ornithological studies in the south-western part of Odessa Region, as well as the results of virological studies, we can conclude that the territory of the seaside estuaries (Tuzly Lagoons and Sasyk Lagoon) is both the most important area for the greater white-fronted goose during migration and wintering, and a place where the species is involved in the process of influenza A circulation.

In our opinion, the main factors that determine the ecological role of this area for the greater white-fronted goose can be the following: availability of food resources, large non-freezing water bodies — places for watering, resting and overnighting, low disturbance factor, which is determined by the conservation status of the area.

The epizootological role of seaside estuaries for the species can be determined by the presence of a source of infection, primarily regular, numerous and multi-species wintering aggregations of birds in the wetland complex, which are the main reservoir of infection (Swayne, Suarez and Sims, 2013). First of all, these are the mallard, *Anas platyrhynchos* Linnaeus, 1758 and the Eurasian duck, *Tadorna tadorna* Linnaeus, 1758, among which the circulation of influenza A viruses has been confirmed in the southern regions of Ukraine (Muzyka et al., 2012; Stegniy et al., 2018). Both species make up the majority of wintering ducks in the sea estuaries: mallard — from 10 to 4,992 birds, an average of 1,905 birds, Eurasian duck — from 0 to 10,468 birds, an average of 2,564 birds, according to the results of traditional winter bird counts (Results …, 2011, 2017, 2023).

Transmission of the virus between birds occurs through direct contact between infected and susceptible birds and indirect contact via aerosolized droplets and fomites, primarily virus-contaminated droppings (Swayne, Suarez and Sims, 2013).

Therefore, of particular importance for the spread of the virus are large multispecies flocks of birds that remain in one place for long periods of time, shed the virus, and exchange infections. Such aggregations are recorded annually in the Primorsky estuaries, mainly in the shallow areas and spits of Sasyk Lagoon and Malyi Sasyk, Dzhantshey, and Shagany lagoons. Such aggregations can play a special role in winter, when the birds stay in the wintering area for a long time, having daily contact at resting, sleeping and feeding places, exchanging infections.

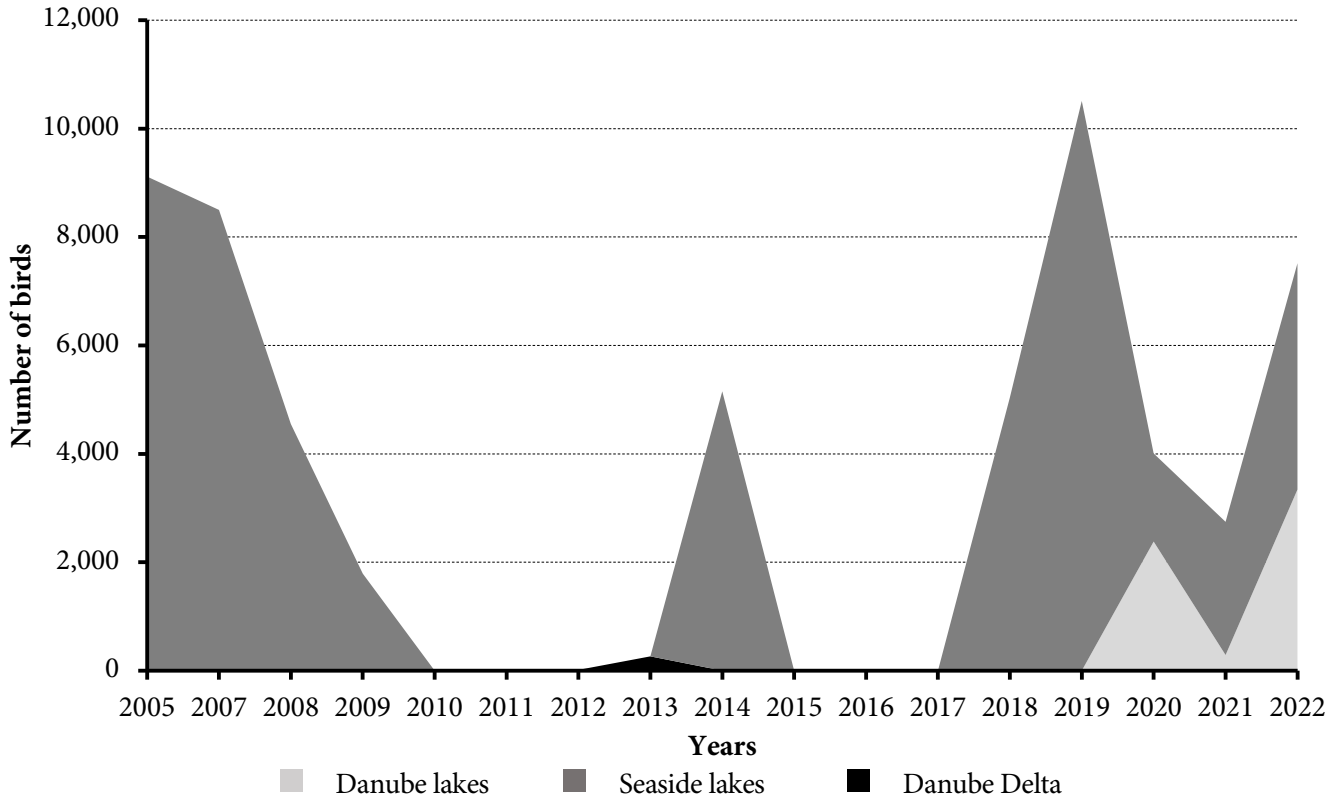

Figure 2. Wintering of the greater white-fronted goose, *Anser albifrons* (Scopoli, 1769) in the south-western part of Odesa Region according to the results of the Regional Ornithological Monitoring (Results …, 2011, 2017, 2023).

Conclusions. Thus, the results of our research indicate that the greater white-fronted goose is involved in maintaining the natural circulation of influenza A virus in the natural reservoir in Odesa Region. The degree of involvement is likely to be most influenced by seasonality and the nature of intra- and interspecific interactions between birds in feeding, resting, and roosting areas. All positive samples were collected within the Seaside estuaries (Sasyk Lagoon and Tuzly Lagoons), which are characterized by the presence of large dense polygynous aggregations of birds localized in shallow waters, spits, etc. Such places contain valuable trophic habitats and, at the same time, watering and resting places for birds of the wetland complex. At the same time, they can be important sites for the transfer of pathogens. For the greater white-fronted goose, these are traditional wintering and migratory stopover sites for at least the last 15 years.

Our data complete the ecological picture of the avian influenza virus circulation in the greater white-fronted geese populations. At the same time, questions remain about the contact of this species with the source of infection during seasonal migrations, especially in the fall. The continuation of these studies is an important element in studying the ecology of the influenza A virus as one of the unpredictable pathogens, especially in a natural reservoir.

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BACTERIOLOGICAL STUDIES OF PROBE SWABS WITH NASOPHARYNGEAL SECRETIONS FROM CANINES DIAGNOSED WITH BORDETELLOSIS

Hadzevych D. V.

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

Summary. At the present work was developed the greatest effectiveness of bacteriological investigations and detection of clinical isolates of *Bordetella bronchiseptica* from nasopharyngeal secretions was observed when the casein-charcoal agar (CCA) was frozen with 5% blood and cephalexin. Thus, during the first trial and sampling of animals, 12 (66.67%) positive results were recovered, and with repeated sampling and sampling of animals after 24 h and 48 h, 15 (83.33%) positive results were recovered from the total number of animals with bordetheliosis based on the results of PCR. The addition of cephalexin in the form of a selective component in nutrient media allows increasing the effectiveness and speed of bacteriological investigations by 2–4 doses per month, suppressing the growth of third-party nasopharyngeal microflora. This is a simple vision of pure culture. Repeated sampling and sampling of nasopharyngeal secretions from sick animals at a short interval of 24 h and 48 h allows us to obtain 16.66% more positive results from clinical isolates of *B. bronchiseptica* using the bacteriological method of investigation

Keywords: *Bordetella bronchiseptica*, tracheobronchitis, canine infectious respiratory disease complex

Introduction. Bordetellosis is a highly contagious infectious disease of farm and small domestic animals widespread in many countries. It is characterized by general malaise, dry, painful cough, conjunctivitis, rhinitis, tracheobronchitis, vomiting, progressive weight loss, and animal death (Rampelotto et al., 2016; Prüller et al., 2015). Bordetellosis is caused by *Bordetella bronchiseptica* (Stępniewska et al., 2014; Carbonetti et al., 2016; MHU, 2005).

In the natural environment, *B. bronchiseptica* most commonly infects dogs, causing kennel cough, and cats and pigs, causing atrophic rhinitis. Animals of all ages are susceptible to the disease, but the highest incidence is in dogs and cats under one year and pigs aged under 4 months. In dogs, *B. bronchiseptica* is a major cause of canine infectious respiratory disease complex (CIRDC). The severity of the disease is associated with the presence of *B. bronchiseptica* pathogenicity factors: adhesins (filamentous hemagglutinin, pertactin, and fimbriae) and toxins (dermanecrotic, adenylate cyclase hemolysin and lipopolysaccharide) (Carbonetti et al., 2016; MHU, 2005).

B. bronchiseptica is a causative agent of chronic and often asymptomatic respiratory infections in animals, making diagnosis, prevention, and treatment of the disease very difficult (Rampelotto et al., 2016).

According to the scientific literature, researchers and specialists in diagnostic laboratories are currently focusing on developing and applying modern molecular genetic methods to detect *B. bronchiseptica* for diagnosing bordetellosis in animals. It is important to note that the polymerase chain reaction (PCR) has a sensitivity of several bacteria in a sample and a specificity of about 100%. In cases of negative bacteriological tests, PCR is positive in 71% of cases (MHU, 2005; Miguelena Chamorro et al., 2023; Goodnow, 1980; Mattoo et al., 2001; Goto et al., 2023).

Some authors have noted that the bacteriological method of laboratory diagnosis of bordetellosis is practically retrospective in nature, with the final result obtained at least a week after the start of the study. The diagnostic efficiency of the test in clinical practice usually does not exceed 10.0–38.0% (Prüller et al., 2015; Fastrès et al., 2020). The duration and low efficiency of the study are due to contamination of the test material with other microorganisms, imperfect formulation of nutrient media, and unsatisfactory selection of selective components (Stępniewska et al., 2014; MHU, 2005; Coutinho et al., 2009). Some authors recommend adding penicillin to the culture medium to suppress the unwanted growth of extraneous nasopharyngeal microflora. However, some of the associated microflora remains due to penicillin resistance and interferes with bacteriological studies (Prüller et al., 2015). Methicillin and cefsulodin (12 mg/l) exhibit superior inhibitory effects on nasopharyngeal microflora compared to penicillin, and cephalexin demonstrates superiority over methicillin (Chambers et al., 2019; Rodriguez and Berliner, 2023; Stępniewska et al., 2014; MHU, 2007). Additionally, cephalexin-rich casein-charcoal agar (CCA) can serve as an effective selective medium (Carbonetti et al., 2016).

Bacteriologic studies for diagnostic purposes are recommended to be performed early in the course of the disease (but not later than week 3), because later the inoculability of the pathogen decreases sharply. It is necessary to take samples for research twice, repeatedly at short intervals every day or every other day, and for culturing the material it is necessary to use Bordet–Gengou medium or casein-charcoal agar with the addition of blood and penicillin or cephalexin as selective components (MHU, 2005).

Our work **aimed** to determine the efficiency of isolating clinical isolates of *B. bronchiseptica* by

bacteriologic examination of nasopharyngeal secretion swabs from dogs with bordetellosis. To determine the effect of the frequency of sampling of nasopharyngeal secretions during repeated examinations of sick animals at short intervals, twice daily or every other day. Also, to determine whether Bordet–Gengou medium or casein-charcoal agar with blood and penicillin or cephalexin as the selective component is best for primary isolating of clinical isolates of *B. bronchiseptica* by bacteriologic examination of nasopharyngeal secretions from dogs with bordetellosis.

Materials and methods. The process of isolating clinical isolates of *B. bronchiseptica* from nasopharyngeal secretions consisted of three steps:

The first stage is the collection and inoculation of biological material (nasopharyngeal secretion) on nutrient media. Sampling of nasopharyngeal secretions for *Bordetella* isolating was performed using a sterile disposable probe-tampon from the mucous membrane of the tonsillar and peripheral areas. For this purpose, the probe-tampon was applied to the posterior pharyngeal wall with a spatula after firm fixation of the studied animals. After touching the mucous membrane of the tonsillar and peripheral pharyngeal areas, the probe tampon was carefully removed from the mouth without touching the tongue and cheeks, and the tampon was rotated around its axis, The nasopharyngeal secretion was rubbed in the center of the Petri dish in a circular motion with a Z-shaped stroke and in the periphery in the form of 4–5 areas with CCA (casein-charcoal agar with 5% blood) and Bordet–Gengou medium (potato-glycerol agar with 20% blood). In addition, the biological material was simultaneously inoculated on other nutrient media (MPA, MPB, blood agar, Endo, McConkey, Sabouraud, etc.) to isolate and identify other types of microorganisms-associates. This is due to the fact that different types of microorganisms can be found in biological material and it is necessary for diagnostics to grow as many as possible (Carbonetti et al., 2016). Inoculation was performed in such a way as to obtain separate isolated colonies (MHU, 2005). The cultures were placed in a thermostat at a temperature of 35.5 ± 0.5°C for 48 h, and to detect Candida fungi, the cultures were placed in a thermostat at a temperature of 28.0 ± 0.5°C for 5 days. The growth of microorganisms on the media was evaluated daily. The morphology of the colonies was examined under an MBS-9 microscope.

In parallel, they were inoculated on CCA Petri dishes with 5% blood and Bordet–Gengou with 20% blood, spread with a thin layer 1.5–2.0 mm to evaluate the hemolytic properties of clinical isolates of *B. bronchiseptica*. And to find out the effect of selective components on the inhibition of foreign microflora during the isolating of clinical isolates of *B. bronchiseptica*, samples of nasopharyngeal secretions were also inoculated on Bordet–Gengou medium and CCA with blood and penicillin (50 IU/100 ml) and cephalexin (4 mg/100 ml) by adding antibiotics to the media, as recommended in the guidelines for the microbiological diagnosis of pertussis and parapertussis (MHU, 2005). Sampling of nasopharyngeal secretions for isolating of *Bordetella* was performed in 24 1.5–3-month-old dogs (n = 24) with bordetellosis (according to anamnesis, clinical picture and detection of *B. bronchiseptica* genetic material by PCR). At the time of biological sampling, the dogs had a specific characteristic dry painful cough, rhinitis, conjunctivitis and signs of tracheobronchitis. No antibacterial drugs were used to treat the animals at the time of biological sampling. Samples from sick animals were taken at an early stage of the disease (but not later than week 3) because, as scientists point out (MHU, 2005), susceptibility to pathogens decreases sharply at a later stage. In order to obtain objective results for the isolating of clinical isolates of *B. bronchiseptica*, 36 samples of nasopharyngeal secretions from the mucous membrane of the tonsillar and peripheral regions were collected from each dog using a separate sterile disposable probe-tampon for each sample (Fig. 1).

Figure 1. Sterile disposable probe swabs for sampling nasopharyngeal secretions from the mucous membrane of the tonsillar and around the pharyngeal areas of animals for isolating of bordetella.

Samples of nasopharyngeal secretions from the same sick dogs were collected three times in a row during repeated examinations of the animals at short intervals of 24 h. The selected samples were inoculated on CCA with 5% blood, on CCA with 5% blood and penicillin, on CCA with 5% blood and cephalexin, on Bordet–Gengou with 20% blood, on Bordet–Gengou with 20% blood and penicillin, on Bordet–Gengou with 20% blood and cephalexin. The inoculation of the selected biological material was performed in such a way that two samples from each experimental dog were necessarily inoculated on two Petri dishes with each type of medium, including media with the addition of selective components. Media and specific nutrients were used from HiMedia Laboratories Pvt. Limited, India and Pharmaktiv LLC, Ukraine.

The second step is to obtain a pure culture of a clinical isolate of B. bronchiseptica. The cultures were examined on nutrient media. The appearance of the

colonies (size, color, shape), certain rapid tests and light microscopy after appropriate staining with aniline dyes determined the possible type of microorganisms and their significance in a particular case (Stępniewska et al., 2014; Carbonetti et al., 2016; MHU, 2005). Selected colonies were inoculated onto nutrient media to accumulate a pure culture of microorganisms. In the case of growth of colonies of only one species, the identification and determination of antibiotic sensitivity was performed without the accumulation stage of pure culture.

The third step is the identification of microorganisms. Depending on the type of microorganism, its identification (determination of biochemical and antigenic properties) was performed using a fixed kit of substrates, diagnostic selective media, and sera. The substrates are carbohydrates, amino acids, polyhydric alcohols, and other complex compounds. The results were used to determine the genus and species of the microorganism (Stępniewska et al., 2014; Carbonetti et al., 2016; MHU, 2005). To study the biochemical properties of microorganisms, we used selective and diagnostic-selective media and specific components manufactured by HiMedia Laboratories Pvt. Limited, India and Pharmaktiv LLC, Ukraine. Hiss medium with sugars and polyhydric alcohols, acetate agar, nitrate agar for determining the reduction of nitrates to nitrites, gelatinase medium, Pizou medium, Simmons citrate agar, etc. were used.

To determine the catalytic activity of clinical isolates of *B. bronchiseptica*, a microbial mass was looped onto a slide, and a drop of 3% hydrogen peroxide solution was immediately added to it. The release of oxygen bubbles indicated the presence of catalase in the microbes.

Antibiotic susceptibility was determined by the disc diffusion method by studying the zones of inhibition of microbial growth around a paper disc impregnated with an antibiotic. The determination of sensitivity to antibacterial drugs was performed according to the guidelines 'Determination of the sensitivity of microorganisms to antibacterial drugs' approved by the Ministry of Health of Ukraine (MHU, 2007). Depending on the zones of growth retardation, resistant, moderately resistant, and sensitive strains of microorganisms to a particular antibiotic were determined. The pathogenicity of the isolated clinical isolates was determined by bioassay on white mice (Goodnow, 1980; Tizolova et al., 2014).

Results. According to the results of bacteriologic studies of nasopharyngeal secretion samples, clinical isolates of *B. bronchiseptica* were isolated from 15 dogs (n = 15) using CCA with 5% blood and cephalexin, which is 83.33% of the total number of animals with bordetellosis by PCR.

The isolated clinical isolates of *B. bronchiseptica* had morphological and biochemical characteristics typical of the species. They looked like small gram-negative ovoid rods 1–1.2 μm long and 0.3–0.5 μm wide. They were motile by peritrichial flagella and had no spores or capsules. They were weakly stained with aniline dyes, more intensely at the poles.

Biochemically, the isolated clinical *B. bronchiseptica* were inactive. They did not degrade proteins and carbohydrates, but secreted catalase (Fig. 2). They were oxidase-positive, urease-positive, and nitrate-positive without gas formation. They did not produce hydrogen sulfide on iron trisugar agar. *B. bronchiseptica* are resistant aerobes which are sensitive to growth conditions, so we used Bordet–Gengou medium with 20% blood and CCA with 5% blood for primary isolating. After primary isolating, clinical isolates of *B. bronchiseptica* were well cultured on blood agar, MPA, MPB, McConkey, Endo, and SS agar.

B. bronchiseptica on CCA with 5% blood and Bordet–Gengou with 20% blood grew as small, smooth, shiny, round, convex, almost transparent colonies. The growth of *B. bronchiseptica* colonies was observed after 18–24 h of cultivation at a temperature of 35.5 ± 0.5°C. Their diameter ranged from 1.0 mm to 1.5 mm. After 48 h of cultivation, the diameter of *B. bronchiseptica* colonies was 1.0–2.0 mm. The growth of bronchoseptic microbes on CCA medium with 5% blood and Bordet–Gengou with 20% blood was not accompanied by a change in their color. A small zone of β-hemolysis was observed around the colonies on Petri dishes with CCA with 5% blood and Bordet–Gengou with 20% blood spread in a thin layer 1.5–2.0 mm to evaluate the hemolytic properties of clinical isolates of *B. bronchiseptica*.

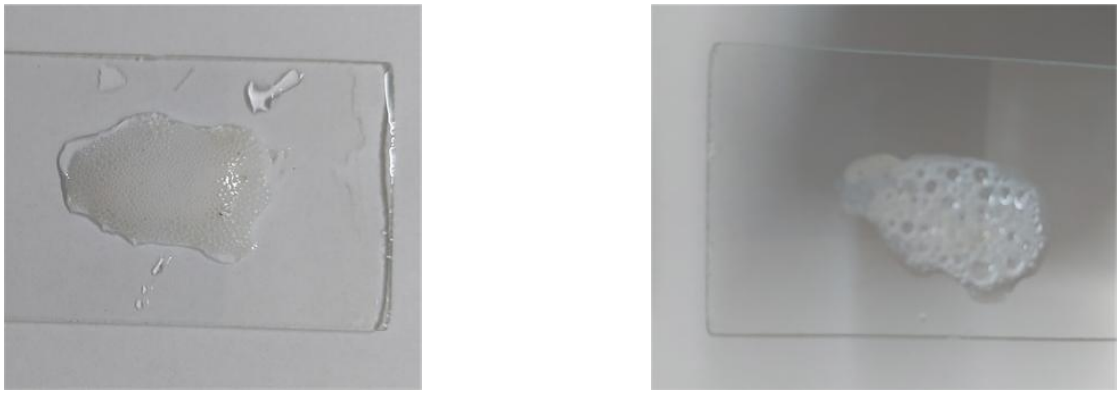

Figure 2. Release of oxygen bubbles during the determination of the catalytic activity of clinical isolates of *B. bronchiseptica*.

In blood broth and MPB, the isolated clinical isolates of *B. bronchiseptica* formed uniform turbidity, light sediment, and a wall ring. On semi-liquid media (0.7% MPA), growth was observed over the entire surface of the medium. On blood agar, colonies were surrounded by small zones of β-hemolysis.

As shown in Table 1, the highest efficiency of bacteriologic studies and isolating of clinical isolates of *B. bronchiseptica* from nasopharyngeal secretions was observed when using CCA with 5% blood and cephalexin. Thus, 12 (66.67%) positive results were obtained during the initial examination and sampling of animals, and 15 (83.33%) positive results were obtained during the repeated examination and sampling of animals after 24 h and 48 h.

The lowest result for the isolating of clinical isolates of *B. bronchiseptica* was obtained using Bordet–Gengou medium with 20% blood without the addition of selective components. During the initial examination and sampling of sick dogs, 5 (22.78%) positive results were obtained, which is 43.89% less compared to the isolating of clinical isolates of *B. bronchiseptica* on CCA with 5% blood and cephalexin. The use of Bordet–Gengou medium with 20% blood with the addition of cephalexin allowed to obtain 10 (55.56%) positive results during the re-examination and sampling of animals after 48 h, which is 27.77% less compared to the result of isolating clinical isolates of *B. bronchiseptica* on CCA with 5% blood and cephalexin.

Table 1 — Results of bacteriological studies of probe swabs with nasopharyngeal secretions obtained from dogs with bordetellosis

Parameters		Type of medium used for primary isolating clinical isolates of *B. bronchiseptica*					
		CCA with 5% blood	**CCA with 5% blood and penicillin**	**CCA with 5% blood and cephalexin**	**Bordet–Gengou with 20% blood**	**Bordet–Gengou with 20% blood and penicillin**	**Bordet–Gengou with 20% blood and cephalexin**
Results of isolating clinical isolates of *B. bronchiseptica* during the first examination and sampling of animals							
The number of animals with bordetellosis (according to PCR results) from which nasopharyngeal secretions were collected for bacteriological studies		18	18	18	18	18	18
The number of positive samples	n	7	10	12	5	7	6
	%	38.89	55.56	66.67	22.78	38.89	33.33
Results of isolating clinical isolates of *B. bronchiseptica* during the repeated examination and sampling of animals after 24 hours							
The number of animals with bordetellosis (according to PCR results) from which nasopharyngeal secretions were collected for bacteriological studies		18	18	18	18	18	18
The number of positive samples	n	10	11	15	7	8	9
	%	55.56	61.11	83.33	38.89	44.44	50.00
Results of isolating clinical isolates of *B. bronchiseptica* during the repeated examination and sampling of animals after 48 hours							
The number of animals with bordetellosis (according to PCR results) from which nasopharyngeal secretions were collected for bacteriological studies		18	18	18	18	18	18
The number of positive samples	n	10	11	15	7	8	10
	%	55.56	61.11	83.33	38.89	44.44	55.56

The use of penicillin as a selective component allowed only a slight increase in the results of isolating clinical isolates of *B. bronchiseptica* due to the inhibition of extraneous nasopharyngeal microflora. Thus, when re-examining and sampling from sick dogs after 24 h and 48 h using CCA with 5% blood and penicillin, 11 (61.11%) positive results were obtained, and using Bordet–Gengou with 20% blood and penicillin, 8 (44.44%) positive results were obtained, which is 22.22% and 38.89% less than the result of isolating of clinical isolates of *B. bronchiseptica* on CCA with 5% blood and cephalexin. The results of Table 1 indicate that repeated examination and sampling of nasopharyngeal secretions from sick animals at short intervals of 24 h and 48 h allows more positive results in isolating clinical isolates of *B. bronchiseptica* by bacteriological examination.

Thus, using CCA with 5% blood and cephalexin, 12 (66.67%) positive results were obtained at the initial examination and sampling of nasopharyngeal secretions from animals, and 15 (83.33%) positive results were obtained at the repeated examination and sampling of animals after 24 h and 48 h. That is, repeated examination and sampling of nasopharyngeal secretions from animals after 24 h and 48 h allowed us to obtain 16.66% more positive results of isolating clinical isolates of *B. bronchiseptica*. In our opinion, it is the most expedient to repeat the examination and sampling of nasopharyngeal secretions from animals after 48 h, when the preliminary result of the first inoculation of biological material has already been obtained and it is possible not to repeat samples with a positive result.

As shown in Table 2, the duration of isolating and identification of clinical isolates of *B. bronchiseptica* was due to contamination of the test material with concomitant foreign nasopharyngeal microflora. The studies were delayed by 2–4 days due to additional inoculations for isolating pure culture.

Thus, the duration of isolation and identification of clinical isolates of *B. bronchiseptica* on CCA medium with 5% blood and cephalexin was 5–7 days. When using CCA or Bordet–Gengou medium without selective components, clinical isolates of *B. bronchiseptica* were isolated and identified within 6–9 days. The study results indicate that it is necessary to use a selective component of cephalexin in the culture media to inhibit the growth of extraneous nasopharyngeal microflora.

In our opinion, as pointed out by Kennedy et al. (2024) and Dong et al. (2022), to reduce the time for isolation and identification of clinical isolates of *B. bronchiseptica* in clinical practice, it is necessary to improve the formulation of nutrient media using selective-elective components, as well as to optimize the use of selective diagnostic media. This will significantly reduce the time for isolation and identification of clinical *B. bronchiseptica* isolates.

It should be emphasized that the duration and efficiency of bacteriological examination of nasopharyngeal secretion samples for isolating clinical isolates of *B. bronchiseptica* are greatly affected by untimely and incomplete examination of animals, violation of rules for collection and transport of biomaterial, insufficient number of examinations, low qualification of diagnosticians, as well as use of antibiotics before the analysis.

Table 2 — Duration of isolation and identification of clinical isolates of B. bronchiseptica on different media

Parameters	Type of medium used for primary isolating clinical isolates of *B. bronchiseptica*					
	CCA with 5% blood	CCA with 5% blood and penicillin	CCA with 5% blood and cephalexin	Bordet–Gengou with 20% blood	Bordet–Gengou with 20% blood and penicillin	Bordet–Gengou with 20% blood and cephalexin
Number of days required to obtain primary growth of microorganisms on the medium	1–2	1–2	1–2	1–2	1–2	1–2
Number of days required to obtain a pure culture of a clinical isolate of *B. bronchiseptica*	2–4	2–4	1–2	2–4	2–4	1–2
Number of days required to identify a clinical isolate of *B. bronchiseptica* by biochemical properties	3	3	3	3	3	3
Duration of isolation and identification of clinical isolates of *B. bronchiseptica*, days	6–9	6–9	5–7	6–9	6–9	6–9

These factors require further study and careful analysis to avoid false-positive and false-negative bacteriologic test results in the future. It is also important to optimize and standardize the conditions and steps of the methods for isolating and identification of clinical *B. bronchiseptica* isolates by bacteriological testing. First of all, it is necessary to analyze the currently available nutrient media for isolating *B. bronchiseptica* and the selective components used to suppress the associated nasopharyngeal microflora.

Conclusions. 1. The highest efficiency of bacteriologic examinations and isolating of clinical isolates of *B. bronchiseptica* from nasopharyngeal secretions was observed when using CCA with 5% blood and cephalexin. Thus, during the initial examination and sampling of animals, 12 (66.67%) positive results were obtained, and during the repeated examination and sampling of animals after 24 h and 48 h, 15 (83.33%) positive results were obtained from the total number of animals with bordetellosis by PCR.

2. Repeated examination and sampling of nasopharyngeal secretions from sick animals with a short interval of 24 h and 48 h allowed us to obtain 16.66% more positive results of isolating clinical isolates of *B. bronchiseptica* by bacteriological examination.

3. The use of cephalexin as a selective component in culture media can increase the efficiency and reduce the duration of bacteriological studies by 2–4 days by inhibiting the growth of extraneous nasopharyngeal microflora and significantly simplify the isolating of pure culture.

Prospects for further research. The disadvantage of the bacteriological method of laboratory diagnosis of bordetellosis is the complexity and duration of the research. The final result can be obtained at least 5–9 days after the start of the test. However, the advantage is that it is available and traditional for most domestic laboratories and specialists and remains the 'gold standard' for bordetellosis laboratory diagnosis in our country. The results of the study indicate that to reduce the time for isolating and identification of clinical isolates of *B. bronchiseptica* in clinical practice, it is necessary to improve the formulation of nutrient media using selective-elective components, as well as to optimize the use of selective diagnostic media. This will significantly reduce the time for isolation and identification of clinical *B. bronchiseptica* isolates.

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