ISSN 2411-0388

NATIONAL ACADEMY OF AGRARIAN
SCIENCES OF UKRAINE

NATIONAL SCIENTIFIC CENTER
**'INSTITUTE OF EXPERIMENTAL
AND CLINICAL VETERINARY MEDICINE'**

JOURNAL FOR VETERINARY MEDICINE, BIOTECHNOLOGY AND BIOSAFETY

Volume 10
Issue 1

KHARKIV
2024

The Journal for Veterinary Medicine, Biotechnology and Biosafety is included **in the 'List of Scientific Special Serial Publications' of Ukraine (category 'B', specialities: 091** — Biology, 211 — Veterinary Medicine, 212 — Veterinary Hygiene, Sanitation and Expertise) that can publish the results of Ph.D. and Dr.Habil. theses in biological and veterinary sciences (orders of the Ministry of Education and Science of Ukraine: № 1328, December 21, 2015**;** № 515, May 16, 2016; № 886, July 2, 2020)

Materials approved for publication and to spread via the Internet by the Scientific Council of the National Scientific **Center 'Institute of Experimental and Clinical Veterinary Medicine'** (protocol No. 4 of March 28, 2023)

The full text of articles available at jvmbbs.kharkov.ua. JVMBBS covered in the abstract and citation databases Google Scholar (scholar.google.com), Index Copernicus (indexcopernicus.com), and CrossRef (crossref.org)

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

Certificate of state registration:
KB No. 21398-11198P of June 25, 2015

Part 1. Veterinary medicine

UDC 619:616.379-008.64:577.122:636.1 DOI 10.36016/JVMBBS-2024-10-1-1

THE ROLE OF ACUTE PHASE INFLAMMATORY PROTEINS IN THE PATHOGENESIS OF METABOLIC SYNDROME IN OBESE HORSES

Borovkov S. B., Boiko V. S.

National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Kharkiv, Ukraine, e-mail: serg_b78@ukr.net

Summary. Metabolic syndrome and obesity in horses are closely related processes that are accompanied by chronic inflammation. Our research aimed to establish the normative indicators of acute phase inflammation proteins in obese horses. We conducted a study on twenty horses, ten of which were in the control group and ten were in the experimental group, showing signs of obesity. The Henneke scoring system was used to assess the body condition. This system ranks animals from one to nine, with one being exhausted and nine being very fat. Serum concentrations of circulating immune complexes, seromucoids, C-reactive protein, haptoglobin, total protein, and its fractions were determined. It was found that significant changes in protein metabolism occur in animals with obesity, namely: the content of circulating immune complexes, seromucoids, C-reactive protein, haptoglobin, and globulins significantly increase, indicating the development of inflammatory processes in horses due to obesity. The localization of these processes is associated both with obesity in animals and possibly with laminitis, the development of which is one of the pathogenetic links of metabolic syndrome

Keywords: laminitis, blood serum, protein metabolism

Introduction. Obesity in horses against the background of metabolic syndrome is characterized by metabolic disorders, which can lead to serious consequences for animal health, especially in case of a sedentary lifestyle or overeating (Carter et al., 2010).

According to studies, a certain proportion of horses are overweight, which increases the risk of developing obesity and metabolic syndrome. Some researchers believe that the etiology and pathogenesis of obesity and the development of metabolic syndrome in horses are similar to humans and include insulin resistance, hyperglycemia, hyperlipidemia, and hypertension (Morgan, Keen and McGowan, 2015).

The pathogenesis of the metabolic syndrome involves a complex interaction between hormonal, metabolic, and inflammatory processes (Durham et al., 2019).

This leads to impaired carbohydrate and fat metabolism, as well as changes in the function of the endocrine system, which can lead to the development of cardiovascular disease, obesity, laminitis, and other diseases (Karikoski et al., 2011).

The role of acute phase inflammatory proteins in the context of equine obesity has been little studied, although it is known that equine obesity is often associated with chronic inflammation, which plays a key role in its pathogenesis. Acute phase inflammatory proteins are important mediators of inflammation and may be important indicators of the degree of inflammatory response in obesity. Studying the role of acute-phase inflammatory proteins will allow us to understand the mechanisms by which obesity affects the metabolism and health of horses. They may be important biomarkers for diagnosing and assessing the severity of obesity in horses, as well as monitoring the effectiveness of therapy (Menzies-Gow, Harris and Elliott, 2017).

Acute phase inflammatory proteins are a group of proteins that are actively synthesized in response to various stressors such as trauma, infection, tumors, and other pathological conditions (Bilous and Kovalchuk, 2015).

They perform many essential functions, such as regulating the immune response, correcting metabolism, protecting against infection and shaping the response to tissue remodeling, antiviral protection, phagocytosis, regulation of apoptosis, and other processes related to the body's defense against harmful effects (Slivinska, Maksymovych and Shcherbatyy, 2017).

Acute phase proteins are actively involved in the body's immune and inflammatory responses and are synthesized by cells such as hepatocytes, monocytes, macrophages, and fibroblasts.

Importantly, inflammatory proteins can also participate in the formation of inflammasomes, protein complexes that play a key role in initiating the body's inflammatory cascade response.

The purpose of this study was to determine the levels of acute phase inflammatory proteins in normal and obese horses.

Materials and methods. The study was conducted on twenty horses of different breeds and sexes, from which age-matched groups were formed: clinically healthy animals (n = 10) and a control group with signs of obesity (n = 10). The horses were mostly of the Ukrainian Riding breed and partially crossbred on its basis. The body condition of each horse was scored on a 9-point Henneke scale after visual inspection by a veterinarian.

Feeding and housing conditions met the physiological needs of the animals. The diet contained the required amount of nutrients, and access to water and exercise was unrestricted. Regular clinical examination of the animals was performed, including the determination of basic physiological parameters and examination of organs and systems by general clinical methods.

The control group consisted of clinically healthy animals with normal physiological parameters.

The diagnosis of obesity was made according to the Henneke scoring system, where the first rank corresponds to emaciation and the ninth to a very fat animal, and the assessment was made by a veterinarian on the farm.

Blood was collected directly from the jugular vein into tubes for further biochemical studies. Blood was collected from the jugular vein on an empty stomach into 10 cm^3 Vacuette tubes for further serum collection.

The following biochemical parameters were determined in serum: concentration of total protein, albumin, total globulins, haptoglobin, and seroglycoids using reagent kits from PJSC 'Reagent' (Ukraine). The concentration of circulating immune complexes was determined as described Gołda et al. (2004) by precipitation of protein complexes antigen-antibody PEG-6000. Biochemical parameters were recorded using a Shimadzu UV-1800 spectrophotometer (Japan).

Experiments on animals were conducted following the recommendations of the 'European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes' (CE, 1986) and Council Directive 2010/63/EU (CEC, 2010), and 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). The research program was reviewed and approved by the Bioethics Committee of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' under the current procedure.

Statistical analysis of the data was performed using Minitab v. 19 (Minitab Inc., USA). Based on the results of statistical processing, the table show non-parametric indicators, such as: median, quartiles Q1 and Q3. A significant difference between the study groups was established based on the calculation of the Mann–Whitney test ($p < 0.05$).

Results and discussion. The detailed results of serum concentrations of circulating immune complexes, seromucoids, C-reactive protein, haptoglobin, total protein, and protein fractions are presented in Table 1. At the same time, it should be noted that there was no significant difference between the ages of animals in different groups. Blood serum is a dynamic equilibrium system, which consists of 70.0% proteins, and the specificity of their metabolism reflects the state of almost all body tissues. In the animals of the experimental group, a significant increase in the score of body condition was recorded to a median score of 7.0 ($p < 0.05$), which corresponds to the score 'well-fed animal'. Thus, significant differences between the groups were found for almost each of the studied indicators.

Table 1 — Results of acute phase protein concentration in the control and obese groups

Indicators	Age, years	Body condition, points	Circulating immune complexes, mmol/l	Haptoglobin, g/l	C-reactive protein, mg/l	Seromucoids, g/l	Total protein, g/l	Albumin, g/l	Globulins, g/l
Clinically healthy horses, n = 10									
Median	11.5	6.50	116.00	0.462	3.19	2.19	65.50	34.0	32.0
Q_1	9.75	5.75	99.50	0.429	3.08	2.06	61.75	32.0	30.5
Q_3	12.25	7.00	129.00	0.477	3.63	2.26	72.00	36.0	36.0
Obese horses, n = 10									
Median	12.0	7.00*	160.00***	0.617***	5.62***	3.21***	75.00*	35.0	39.5***
Q_1	12.0	6.75	138.75	0.581	4.98	3.11	71.50	33.3	37.8
Q_3	14.0	8.00	170.00	0.678	5.92	3.61	76.50	36.3	42.0

Notes: * — $p < 0.05$, ** — $p < 0.01$, *** — $p < 0.001$, compared to clinically healthy horses.

Circulating immune complexes (CICs) can be formed when antigens interact with antibodies in the blood. When these complexes are produced in excessive amounts or cannot be effectively cleared by the body, they can trigger an inflammatory response. Inflammation can result from the deposition of CICs in tissues, where they activate inflammatory processes, contributing to tissue damage and the further development of

inflammatory diseases (Tkaczenko et al., 2023). Thus, according to the results of our studies, the level of CICs in obese horses was increased by 37.9% and reached the level of 160.0 mmol/l ($p < 0.001$). It should also be noted that the concentration of CICs had a significant positive correlation (0.769) with the content of seromucoid, which may be associated with chronic inflammation, which is often observed in obesity. In addition, it is known that the processes caused by CICs deposition in tissues can affect metabolism, causing insulin resistance and other factors that contribute to the development of obesity (Stefaniuk-Szmukier, Piórkowska and Ropka-Molik, 2023).

Other important proteins in the acute phase of inflammation are haptoglobin and C-reactive protein (CRP). Haptoglobin is known to be elevated in horses with peritonitis or after surgery, can be produced by adipocytes, and is considered a marker of obesity (Johnson, 2002). In this study, we found a 33.5% increase in haptoglobin levels to 0.617 g/l ($p < 0.001$) compared to the control group. The haptoglobin level was also directly correlated with the age of the animals (0.771) and the level of CRP (0.781). It is known that CRP levels in horses increase several times during inflammation and obesity (Girardi et al., 2019).

CRP levels are increased in enteritis, pneumonia, and arthritis in adult horses and foals. In addition, CRP levels have been shown to correlate with inflammatory markers and to increase during experimentally induced laminitis; however, others have shown no difference in CRP levels in obese horses (Johnson et al., 2010). We found that CRP levels increased by 76.2% ($p < 0.001$) to a level of 5.62 mg/l, which is in full agreement with previous authors and may indicate both the development of animal obesity and the subclinical course of laminitis, which requires further research (Reynolds et al., 2019) (Zak et al., 2020). In addition, the development of the inflammatory process in horses of the experimental group is evidenced by an increase in the level of globulins by 25.8% ($p < 0.001$) against the background of an increase in the level of total protein by 14.3% ($p < 0.05$). It should be noted that the level of albumin did not undergo significant changes.

The role of seromucoids in distinguishing between acute and chronic inflammation is debated: they are indicated because they are a rather sensitive marker of inflammatory processes, during which their level increases several times. An increase in the level of seromucoids has been described in many inflammatory processes of bacterial and viral etiology, while other data suggest that seromucoids should be considered as a marker of chronic rather than acute inflammation (Galatyuk et al., 2018).

The results obtained in our study indicate that the level of seromucoids increased significantly by 47.2% ($p < 0.001$) to the level of 3.21 g/l in comparison with the control group. In addition, seromucoids are normal constituents of connective tissue, so when it is destroyed, they enter the bloodstream in significant amounts and are therefore considered markers of destructive and degenerative processes (Witkowska-Piłaszewicz et al., 2019). This is supported by correlation data on the direct dependence of seromucoid levels on body condition, circulating immune complexes, and haptoglobin and CRP. According to some reports, seromucoids also correlate with body mass index and adipose tissue content (Henneke et al., 1983) and adiponectin levels (Gołda et al., 2004). Therefore, in our opinion, seromucoid content can be used as an integral indicator of proteins in the acute phase of inflammation in obese horses.

Conclusions. Obesity in horses leads to a significant increase in acute phase inflammatory proteins. Determination of seromucoid concentration can be used as an integral indicator of acute phase inflammatory proteins in obese horses.

References

Bilous, L. L. and Kovalchuk, N. A. (2015) 'Immune status different lines of Russian Trotting horses' [Imunnyi status konei rosiiskoi rysystoi porody riznykh linii], *The Animal Biology [Biolohiia tvaryn]*, 17(4), pp. 162. Available at: http://nbuv.gov.ua/UJRN/bitv_2015_17_4_28. [in Ukrainian].

Carter, R. A., McCutcheon, L. J., Valle, E., Meilahn, E. N. and Geor, R. J. (2010) 'Effects of exercise training on adiposity, insulin sensitivity, and plasma hormone and lipid concentrations in overweight or obese, insulin-resistant horses', *American Journal of Veterinary Research*, 71(3), pp. 314–321. doi: 10.2460/ajvr.71.3.314.

CE (The Council of Europe). (1986) *European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes*. (European Treaty Series, No. 123). Strasbourg: The Council of Europe. Available at: https://conventions.coe.int/treaty/en/treaties/html/123.htm.

CEC (The Council of the European Communities) (2010) 'Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes', *The Official Journal of the European Communities*, L 276, pp. 33–79. Available at: http://data.europa.eu/eli/dir/2010/63/oj.

Durham, A. E., Frank, N., McGowan, C. M., Menzies-Gow, N. J., Roelfsema, E., Vervuert, I., Feige, K. and Fey, K. (2019) 'ECEIM consensus statement on Equine metabolic syndrome', *Journal of Veterinary Internal Medicine*, 33(2), pp. 335–349. doi: 10.1111/jvim.15423.

Galatyuk, O. Ye., Begas, V. L., Antonyuk, A. A. and Kalnaus, O. R. (2018) 'Indicators of blood and cell metabolism for the current course of Leptospyrosis and Herpesvirus infections of first and second types in the concept' [Pokaznyky krovi ta klitynnoho metabolizmu za sumisnoho perebihu leptospirozu i herpesvirusnykh infektsii pershoho ta druhoho

typiv u konei], *News of Dnipropetrovsk State Agrarian and Economic University [Visnyk Dnipropetrovskoho derzhavnoho ahrarno-ekonomichnoho universytetu],* 1–2, pp. 75–79. Available at: http://nbuv.gov.ua/UJRN/vddau_2018_1-2_15. [in Ukrainian].

Girardi, F. M., Da Fonseca, L. A., Ribeiro Filho, J. D., Souto, P. C., Ferreira, D. A. C., Dornelas, L. R. S. M., Bento, L. D. and De Carvalho Filho, W. P. (2019) 'Influence of obesity on serum concentrations of acute-phase proteins in horses', *Journal of Equine Veterinary Science*, 83, p. 102810. doi: 10.1016/j.jevs.2019.102810.

Gołda, R., Wolski, Z., Wyszomirska-Gołda, M., Madaliński, K. and Michałkiewicz, J. (2004) 'The presence and structure of circulating immune complexes in patients with Prostate tumors', *Medical Science Monitor*, 10(3), pp. 123-127. PMID: 14976452.

Henneke, D. R., Potter, G. D., Kreider, J. L. and Yeates, B. F. (1983) 'Relationship between condition score, physical measurements and body fat percentage in mares', *Equine Veterinary Journal*, 15(4), pp. 371–372. doi: 10.1111/j.2042-3306.1983.tb01826.x.

Johnson, P. J. (2002) 'The equine metabolic syndrome', *Veterinary Clinics of North America: Equine Practice*, 18(2), pp. 271–293. doi: 10.1016/S0749-0739(02)00006-8.

Johnson, P. J., Wiedmeyer, C. E., LaCarrubba, A., (Seshu) Ganjam, V. K. and Messer, N. T. (2010) 'Laminitis and the Equine metabolic syndrome', *Veterinary Clinics of North America: Equine Practice*, 26(2), pp. 239–255. doi: 10.1016/j.cveq.2010.04.004.

Karikoski, N. P., Horn, I., McGowan, T. W. and McGowan, C. M. (2011) 'The prevalence of Endocrinopathic laminitis among horses presented for laminitis at a first-opinion/referral equine hospital', *Domestic Animal Endocrinology*, 41(3), pp. 111–117. doi: 10.1016/j.domaniend.2011.05.004.

Menzies-Gow, N. J., Harris, P. A. and Elliott, J. (2017) 'Prospective cohort study evaluating risk factors for the development of Pasture-associated laminitis in the United Kingdom', *Equine Veterinary Journal*, 49(3), pp. 300–306. doi: 10.1111/evj.12606.

Morgan, R., Keen, J. and McGowan, C. (2015) 'Equine metabolic syndrome', *Veterinary Record*, 177(7), pp. 173–179. doi: 10.1136/vr.103226.

Reynolds, A., Keen, J. A., Fordham, T. and Morgan, R. A. (2019) 'Adipose tissue dysfunction in obese horses with Equine metabolic syndrome', *Equine Veterinary Journal*, 51(6), pp. 760–766. doi: 10.1111/evj.13097.

Simmonds, R. C. (2017) 'Chapter 4. Bioethics and animal use in programs of research, teaching, and testing', in Weichbrod, R. H., Thompson, G. A. and Norton, J. N. (eds.) *Management of Animal Care and Use Programs in Research, Education, and Testing*. 2nd ed. Boca Raton: CRC Press, pp. 35–62. doi: 10.1201/9781315152189-4.

Slivinska, L. G., Maksymovych, I. A. and Shcherbatyy, A. R. (2017) 'Indicators of hematopoiesis in working horses' [Pokaznyky hemopoezu v robochykh konei], *Scientific and Technical Bulletin of the Livestock Farming Institute of NAAS [Naukovo-tekhnichnyi biuleten Instytutu tvarynnytstva NAAN]*, 117, pp. 152–162. Available at: http://nbuv.gov.ua/UJRN/Ntb_2017_117_27. [in Ukrainian].

Stefaniuk-Szmukier, M., Piórkowska, K. and Ropka-Molik, K. (2023) 'Equine metabolic syndrome: A complex disease influenced by multifactorial genetic factors', *Genes*, 14(8), p. 1544. doi: 10.3390/genes14081544.

Tkaczenko, H., Aksonov, I., Tkachova, I. and Kurhaluk, N. (2023) 'Exercise-induced changes in lipid and protein oxidation in the blood of mares and stallions of English Half-Breed horses living in the Pomeranian Region (Northern Poland)', *Scientific Bulletin of Natural Sciences (Biological Sciences) [Pryrodnychyi almanakh (biolohichni nauky)],* 35, pp. 46–59. doi: ksu2524-0838/2023-35-4.

VRU (Verkhovna Rada Ukrainy) (2006) 'Law of Ukraine No. 3447-IV of 21.02.2006 'About protection of animals from cruel treatment'' [Zakon Ukrainy № 3447-IV vid 21.02.2006 'Pro zakhyst tvaryn vid zhorstokoho povodzhennia'], *News of the Verkhovna Rada of Ukraine [Vidomosti Verkhovnoi Rady Ukrainy]*, 27, art. 230. Available at: https://zakon.rada.gov.ua/laws/3447-15. [in Ukrainian].

Witkowska-Piłaszewicz, O. D., Żmigrodzka, M., Winnicka, A., Miśkiewicz, A., Strzelec, K. and Cywińska, A. (2019) 'Serum amyloid A in equine health and disease', *Equine Veterinary Journal*, 51(3), pp. 293–298. doi: 10.1111/evj.13062.

Zak, A., Siwinska, N., Elzinga, S., Barker, V. D., Stefaniak, T., Schanbacher, B. J., Place, N. J., Niedzwiedz, A. and Adams, A. A. (2020) 'Effects of Equine metabolic syndrome on inflammation and acute-phase markers in horses', *Domestic Animal Endocrinology*, 72, p. 106448. doi: 10.1016/j.domaniend.2020.106448.

UDC 591.43:611.3:636.596 DOI 10.36016/JVMBBS-2024-10-1-2

MORPHOLOGICAL FEATURES OF THE FERAL PIGEON'S (*COLUMBA LIVIA* F. *URBANA*) DIGESTIVE SYSTEM

Kolomak I. O.

Poltava State Agrarian University, Poltava, Ukraine, e-mail: kolomak-ihor@ukr.net

Summary. Peculiarities of the morphological structure of the feral pigeon's (*Columba livia f. urbana*) digestive system have been established. In the process of evolution, the digestive system has acquired certain anatomical changes that perform adaptive mechanisms for flying and egg-laying. In pigeons, the rostral part of the skull is represented by a beak consisting of an upper bill (maxilla) and gnathotheca (mandible) part. Due to the absence of a palatal fold, the digestive tract begins with the oropharynx, which lacks lips, teeth, and gums. The gorge is present in both sexes, consists of the right and left parts, and performs the function of accumulating fodder and secreting gorge milk, which is fed to the young through regurgitation. The mucous membrane of the esophagus forms longitudinal folds. There is a large number of digestive glands in the proventriculus. The cavity of the muscular stomach contains gastroliths, which act as teeth, ensuring grinding of food mass. The small intestine consists of the duodenum and ileum, which are joined by the mesentery and form three loops. The mucous membrane is developed, represented by numerous crypts that ensure the absorption of nutrients. The large intestine is represented by the paired rectal cecum and the rectum. The mucous membrane of the thick intestine is represented by leaf-like villi. The muscle layer is the most developed, myocytes are located in circular and longitudinal directions, forming folds

Keywords: anatomy, histology, synanthropic pigeons

Introduction. The feral pigeons (*Columba livia f. urbana*) are descendants of domestic pigeons (*Columba livia* f. *domestica*) that have returned to the wild. The domestic pigeon was originally bred from the wild rock dove (*Columba livia* Gmelin, 1789), which naturally inhabits sea cliffs and mountains, and its closest relative is the hill pigeon (*Columba rupestris* Pallas, 1811). Domestic pigeons are bred for sports (racing breeds), exhibitions (refined breeds), and food (meat-type breeds) and used as objects for scientific research (Adil and Magray, 2012; Bailey et al., 1997; Kolomak and Kruchynenko, 2017; Santos et al., 2020).

The study of the birds' morphological structure deepens and supplements the data on physiological processes that occur during digestion in pigeons. Considering the longtime of evolution, depending on the habitat, pigeons adapt to external conditions. With the processes of adaptation, there are changes in the anatomical structures of the digestive system, which affect the physiological mechanisms of digestion (Bindari and Gerber, 2022; Scanes and Dridi, 2021; Klasing, 1999).

Birds tend to consume food frequently, which is associated with a shorter gut, compared to mammals. The time for digestion and absorption of nutrients is reduced, despite significant energy requirements, particularly during flying. However, the physiological mechanisms of digestion that occur in the digestive system of birds are compensated by fast enzymatic processes that ensure a high rate of substrates' decomposition and active transport of nutrients (Gugołek, Jastrzębska and Strychalski, 2016; McWhorter, Caviedes-Vidal and Karasov, 2009; Wang et al., 2020). The performed studies established the correlation features of the digestive tract's length which indicates that the birds had a 51% smaller nominal surface area of the small intestine and a 32% smaller volume of the large intestine. The short small intestine in birds reduces the time of digestion and absorption of nutrients, which is compensated by the species-specific composition of digestive enzymes. In birds, there is no compensation for the decrease in digestive and absorption capacity due to the longer retention time of food in the intestine. Partial compensatory mechanisms may be attributed to increased mucosal surface area and villous area, although this is not sufficient to compensate for the reduced nominal intestinal surface area (Hamoda and Farag, 2018; Lavin et al., 2008).

According to Price et al. (2015), birds have smaller intestines and shorter digestion time. Partial compensation of the smaller intestine occurs due to increased paracellular absorption of nutrients (Price et al., 2015; Oakley et al., 2014).

Taking into account the existing data of the digestive system's studies, it should be noted that the study of digestion in pigeons has not been investigated enough. Thus, a review of the digestive system's anatomical structure will provide a comprehensive representation of the physiological processes occurring in the body, and a histological analysis of the digestive system organs' structure will complement the existing morphological data.

The aim of the study was investigate morphological features of the feral pigeon's digestive system.

Material and methods. The morphological study of 25 feral pigeons caught in the city of Poltava involved the pathoanatomical study of cadavers, performed according to the Shore method, as well as the selection of

pathological material (small and large intestines, liver) for the manufacture of histologic specimens (Scanes and Pierzchala-Koziec, 2014).

Manufacturing of histologic specimens was carried out of selected organs from the digestive tract of pigeons. Fixation of the material was carried out in a 10% aqueous solution of neutral formalin. At the next stage, samples were washed in distilled water, and dehydrated, and the pathological material was embedded in paraffin. Histological sections with a thickness of 7–10 μm were made of the produced compacted paraffin blocks on the MPS-2 type sledge microtome.

Staining of histologic specimens was carried out with hematoxylin and eosin, which involved the process of deparaffinization in xylene, washing in distilled water, staining with Ehrlich's hematoxylin, washing in distilled water, dehydrating, staining with a 1% aqueous solution of eosin. Afterward, the histologic specimen was clarified in xylene and covered with a coverslip glass.

Stained histological sections were examined using a MICROmed XS-5520 light microscope with magnifications of ×40, ×100, ×400, and ×800. The material for the illustrations was photographed using a MICROmed microscope with 5 Mpix attachment.

Experiments on animals were conducted following the recommendations of the 'European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes' (CE, 1986) and Council Directive 2010/63/EU (CEC, 2010), and 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). The research program was reviewed and approved by the Bioethics Committee of the Poltava State Agrarian University under the current procedure.

Results. In the process of evolution, the anatomical structure of the pigeon's digestive tract has acquired adaptive changes associated with the ability to fly. The digestive system includes the intestines (small and large), stomach (glandular and muscular), and a system of digestive glands (Fig. 1).

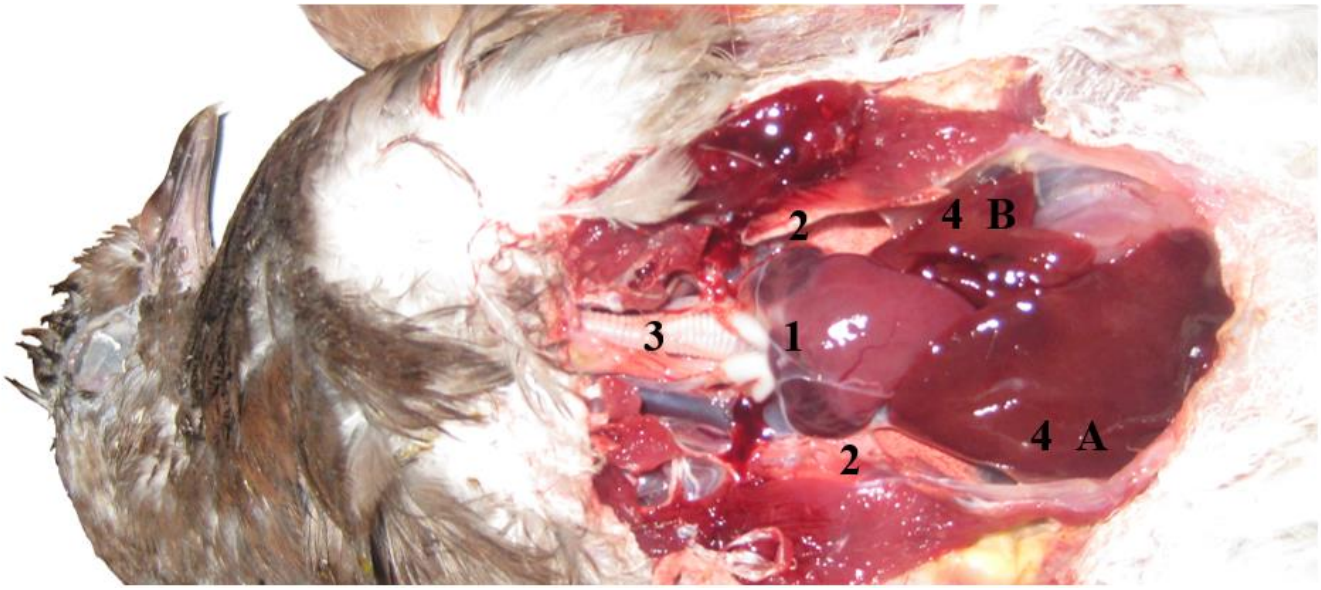

Figure 1. General view of the thoracic-abdominal cavity internal organs of the feral pigeon: 1 — heart, 2 — lungs, 3 — trachea, 4 — liver (A — right part, B — left part).

The digestive system begins with the oropharynx since the palatal fold separating the oral cavity and pharynx is absent in pigeons. The oropharynx lacks lips, teeth, and gums. The rostral part of the skull is represented by the beak, which provides the entrance to the oropharynx and consists of two parts: maxillar and mandibular. The distal part of the oropharynx is represented by the buccal cavity, which passes into the pharynx. At the bottom of the buccal cavity, there is the tongue.

The submucosal layer of the oropharynx's mucous membrane contains numerous salivary glands that secrete saliva through ducts. Depending on their localization, they can be divided into sublingual, maxillar, and mandibular. Saliva permeates the feed lump and provides ramollissement during swallowing, which is accomplished through rostrocaudal movements.

The alimentary canal is built according to the general type of tubular organs' structure, which determines the presence of mucous, muscular, and serous membranes.

The pharynx passes into the esophagus, which forms an ampoule-shaped diverticulum — a goiter, in which food accumulates, is processed by digestive enzymes, and the initial stages of enzymatic digestion take place. In pigeons, the goiter is a highly differentiated organ consisting of two lateral parts.

In both sexes, a secret is formed — 'bird's milk', which is fed to the young with the help of regurgitation. Depending on the section of the esophagus, the cervical part will be covered with adventitia, unlike the thoracic part, where the outer layer is represented by a serous membrane.

Analyzing the histological structure of different alimentary canal sections, each of the membranes, depending on the localization, will have certain variations in the structure due to the functional significance of the small and large intestine sections.

Histological examination of the esophagus established that the mucous membrane forms longitudinal folds, the apical part of which is covered with a multilayered flat partially keratinized epithelium. The submucous base is represented by an irregular connective tissue in which there are blood, lymphatic vessels, and nerve fibers. A large number of glands and secretory goblet cells producing mucus were found. The muscle membrane is represented by several layers of myocytes, located in a circular and longitudinal orientation. The muscle layer of the esophagus cranial part, to the diverticulum, is more developed, due to constant rostrocaudal movements during swallowing. Anatomically, the goiter divides the esophagus into a cervical part, up to the entrance into the oesophagus, directly into the oesophagus, and the thoracic part, which starts from the exit from the oesophagus and ends at the entrance to the glandular stomach. From the esophagus, the feed mass enters the differentiated stomach. In case when the stomach is full, food accumulates in the goiter, in the absence of fodder mass in the stomach, the fodder lump is directed directly to the stomach. The stomach of pigeons is highly differentiated and consists of glandular and muscular parts, between which there is an isthmus. The glandular stomach, which is located more cranially than the muscular stomach, contains a large number of digestive glands, the secretion of which permeates the fodder lump and provides enzymatic digestion. The muscular stomach is represented by highly developed muscle tissue, which provides mechanical digestion of feed.

The cavity of the muscle stomach is covered with cuticles and has cranial and caudal diverticulum-like extensions that form the blind gastric pouches. Exit from the stomach is carried out through the pyloric sphincter.

Between the proventriculus and the stomach, on the right side, there is a spleen, which has an elongated and rounded shape.

The liver consists of the right and left parts (Figs 1 and 2A), where the right part is much larger. The left part is not divided, and the gall bladder is absent. Bile moves along the paired hepatic-intestinal duct, the right duct opens into the ascending part of the duodenum, and the left hepatic-intestinal duct, together with the pancreatic duct, opens into the proximal part of the duodenum.

The liver parenchyma is represented by hepatic lobes consisting of hepatocytes (Fig. 2B), which are located radially from the central hepatic vein. Sinusoidal spaces stand out between the beams of hepatocytes. The connective tissue separating the liver lobes is weakly expressed.

The mucous membrane of the glandular stomach is overlayed with a single-layered prismatic glandular epithelium containing mucocytes that produce a mucous secretion. The transition zone leading to the muscle stomach does not have glands and separates the glandular and muscle stomach.

The cavity of the muscular stomach contains gastroliths involved in the mechanical grinding of fodder clods (cereal crops), acting as teeth, that are absent. The cuticle lining the muscular stomach is a product of the digestive glands of the gastric mucosa and performs a protective function for the mucous and muscular membranes.

From the pyloric part of the stomach, through the pyloric sphincter, the small intestine opens, consisting of the duodenum and ileum. The small intestine of an adult pigeon forms three loops, where the first and the third ones are short, having a cone-like shape with outer centripetal and inner centrifugal parts, collected by the mesentery. The small intestine contains numerous protrusions that can be traced macroscopically (Fig. 3), which increase the absorption area.

Duodenum and ileum are characterized by a developed mucous membrane (Fig. 4), which contains numerous villi (crypts), which are processes of the mucous membrane. The central part of the crypts is formed by connective tissue.

The proper mucous plate of the mucous membrane contains blood and lymphatic capillaries, as well as individual lymphoid clusters that form Peyer's plaques (Fig. 5).

On the outside, crypts are covered with a single-layer prismatic epithelium with numerous inclusions of goblet cells. The muscle layer is represented by myocytes oriented in the circular and longitudinal direction, in some areas, a morphological connection between the muscle layer and myocytes located at the base of the crypts can be traced.

The large intestine is represented by paired rectal appendages (Fig. 6), located on the border of the ileum and rectum.

In the mucous membrane of the large intestine, circular folds are replaced by simple tubular crypts (Fig. 7). The mucous membrane of the rectum has branched leaf-like villi (Fig. 8), which ensure reabsorption. The apical surface is covered with a single-layer prismatic epithelium alternating with glandular goblet cells. The lamina of the mucous membrane is represented by a layer of collagen fibers and a system of lymphatic vessels.

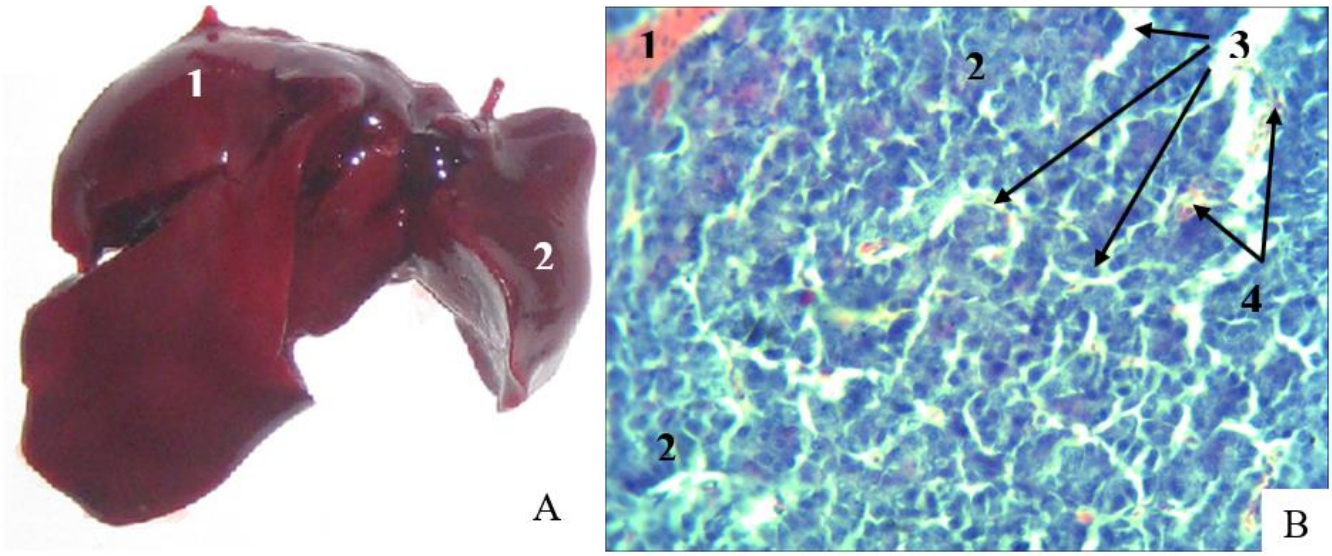

Figure 2. Macroscopic structure of pigeon liver: A1 — right part, A2 — left part; B — fragment of the liver histological structure: 1 — blood vessels, 2 — hepatocytes, 3 — sinusoidal spaces, 4 — bile ducts. Hematoxylin and eosin, × 400.

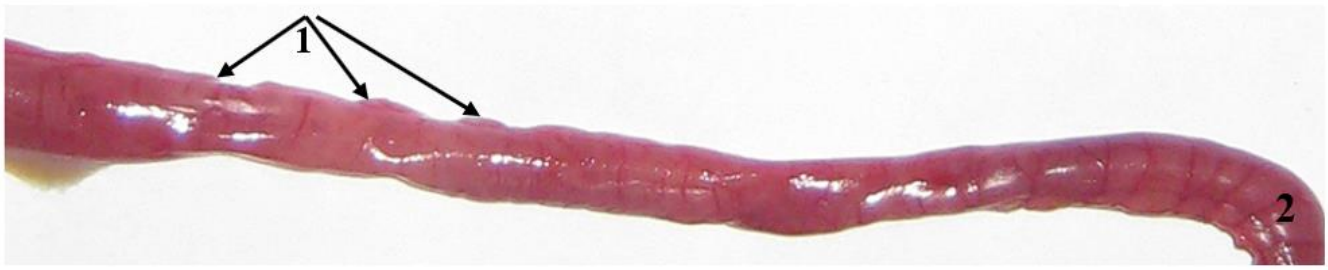

Figure 3. Fragment of the pigeon duodenum: 1 — protrusion of the duodenum, 2 — flexure of the duodenum.

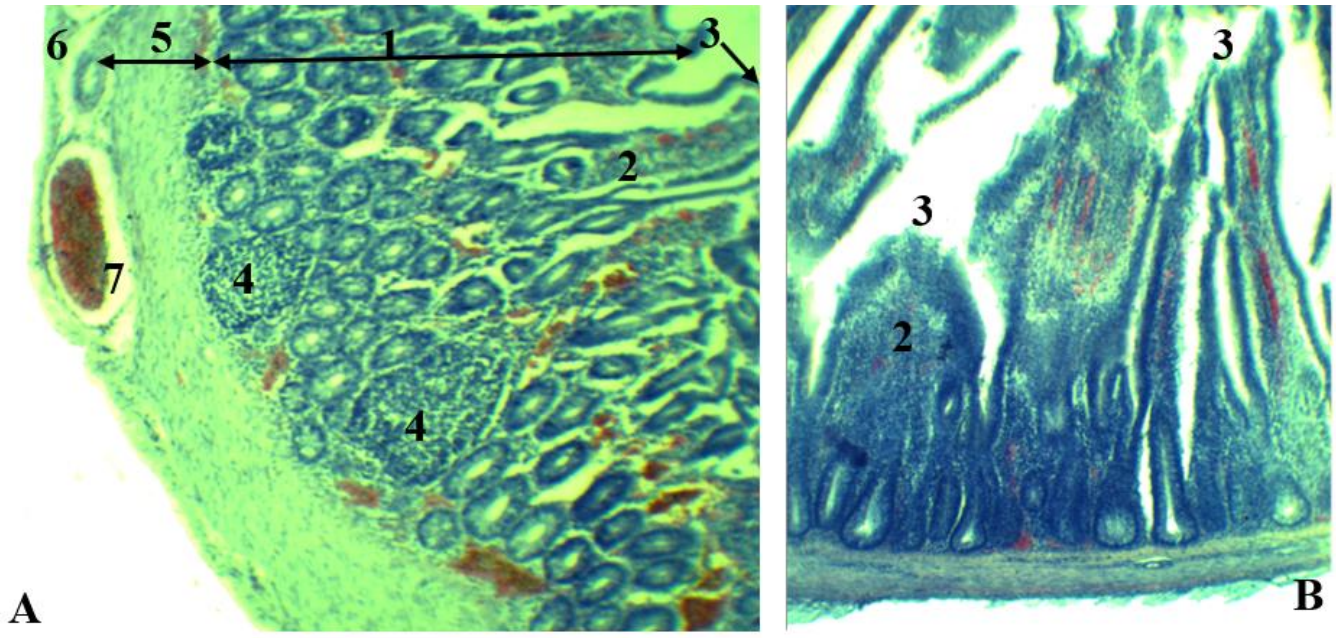

Figure 4. Fragment of a histologic specimen of the pigeon ileum: A, B: 1 — mucous membrane, 2 — intestinal crypts, 3 — apical surface of intestinal villi, 4 — accumulation of lymphoid cells, 5 — muscular membrane, 6 — serous membrane, 7 — blood vessels. Hematoxylin and eosin, × 100 (A), × 400 (B).

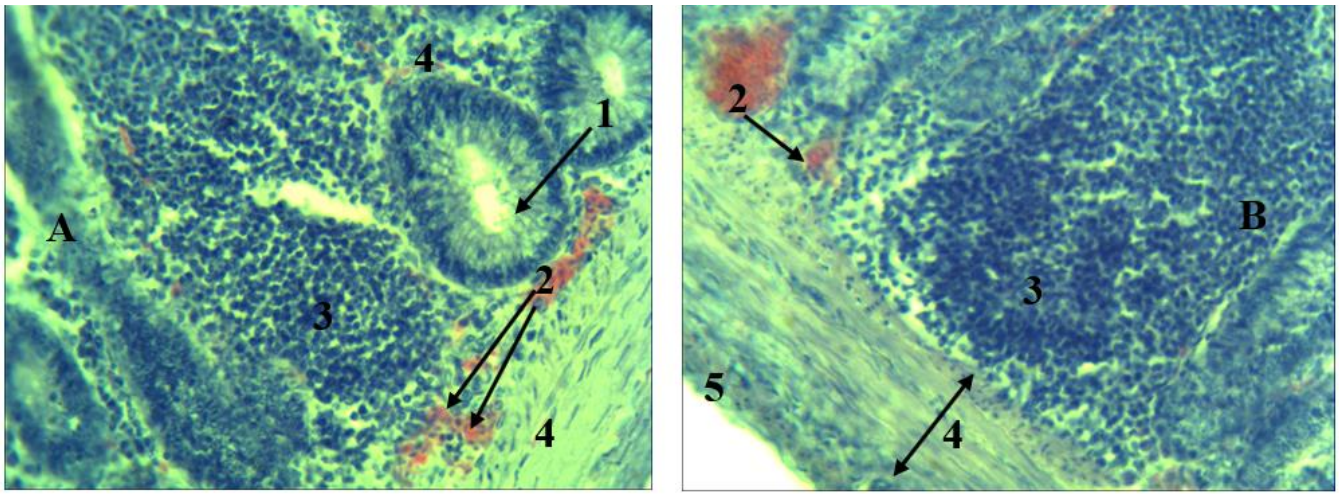

Figure 5. Fragment of a histologic specimen of the small intestine: A, B: 1 — intestinal crypts in cross-section, 2 — blood vessels of the mucosa and submucosa, 3 — accumulation of lymphoid cells, 4 — muscular membrane, 5 — serous membrane. Hematoxylin and eosin, × 400.

Figure 6. Section of the large intestine with paired blind appendages.

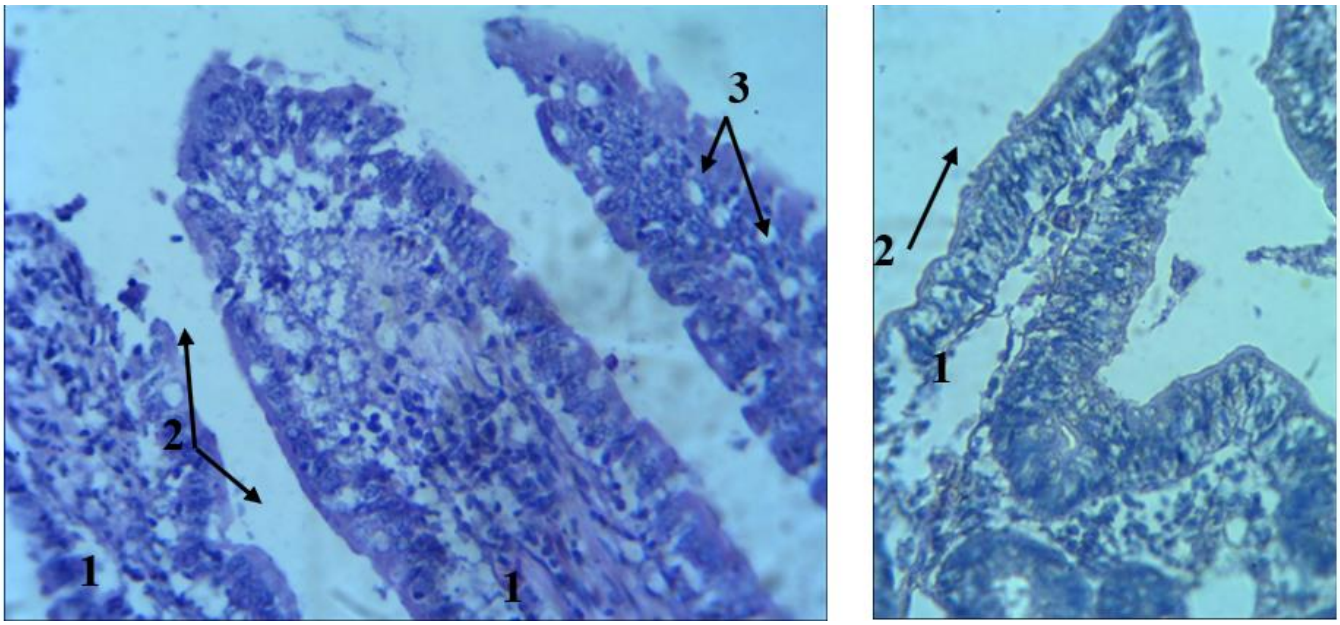

Figure 7. Fragment of a histologic specimen of the large intestine's proximal part: 1 — villi of the mucous membrane, 2 — single-layer prismatic epithelium, 3 — goblet cells. Hematoxylin and eosin, × 400.

The muscle layer is the most developed. Myocytes form longitudinal folds and are located in the circular and longitudinal direction the main function is to provide elastic movements. From the outside, the large intestine is covered with a serous membrane, which is a thin layer of simple flat epithelium with flattened nuclei.

The rectum is similar in structure to the small intestine. It was found that the muscular layer of the rectum was much more developed, in contrast to the small intestine, which has a more pronounced mucous membrane.

The cloaca has a complexly differentiated structure, where the rectum opens into the cranial part — the coprodeum, and the urogenital ducts (urodeum) open into the medial part. The caudal part (proctodeum) ensures the removal of waste products through a sphincter that opens to the outside.

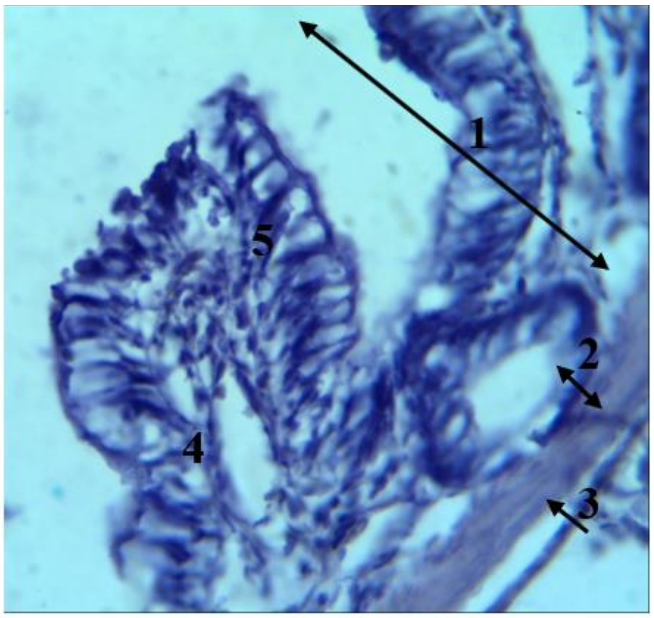

Figure 8. Fragment of a histologic specimen of a large intestine: 1 — mucous membrane, 2 — muscular membrane, 3 — serous membrane, 4 — leaf-like villi of the large intestine, 5 — single-layer prismatic epithelium, 6 — goblet cells. Hematoxylin and eosin, × 400.

Discussion. Most of the evolutionary processes that transform the anatomical structures of the birds' digestive tract are associated with the ability to fly and the process of egg-laying (Klasing, 1999).

In pigeons, the digestive tract is shorter compared to the digestive tube of mammals, which is indicated by the studies of most scientists.

Because the main way of moving birds is flight, which requires significant energy costs, the search for compensatory mechanisms that ensure sufficient energy accumulation for birds is underway (Lavin et al., 2008; Oakley et al., 2014).

According to Lavin et al. (2008), birds have an increased surface area of the intestinal mucosa, which is provided by a larger area of intestinal villi. Similar conclusions were reached by Oakley et al. (2014), who proved that flying birds and bats are under selective pressure to reduce the size and weight of the intestines they carry.

Our morphological studies indicate the presence of numerous protrusions in the small intestine, which increase the area of absorption due to the developed mucous membrane.

In the gastrointestinal tract of pigeons, proteins are split using a mixture of proteases (pepsin, trypsin, and chymotrypsin) to obtain a protein hydrolysate, which is subsequently absorbed in the small and partially large intestine (Price et al., 2015). According to other data, due to the large number of nerve fibers located in the digestive tract, enhanced functional regulation of the pigeon's intestine is carried out, which is a key role in the compensatory mechanisms of the reduced digestive tract (Hamdi et al., 2013; Ratnayani et al., 2019; Scanes and Pierzchala-Koziec, 2014).

In the alimentary canal of pigeons, lymphatic tissue is organized in the submucosa of its proper membrane in the form of diffusely located lymphatic tissue and lymphoid follicles. The epithelium in the intestines has lymphocytic infiltration with the formation of lymphoepithelium. Lymph nodes are located not only in the submucosa itself but also in the muscular and serous membranes of the intestines.

The small intestine contains numerous protrusions that can be traced macroscopically, which increase the area of absorption.

The duodenum and ileum are characterized by a developed mucous membrane containing numerous villi (crypts), which are processes of the mucous membrane. Development of the mucous membrane depending on age. Thus, Ratnayani et al. (2019), Klasing (1999), and Price et al. (2015) proved that the layers of the mucous membrane were most developed in mature pigeons, compared to young ones (Lee, Kil and Sul, 2017; Nasrin et al., 2012).

Conclusions. The digestive tract of the feral pigeon (*Columba livia f. urbana*) begins with the oropharynx, which lacks the palatal fold, lips, teeth, and gums. The rostral part of the skull is represented by a beak. The mucous membrane of the oropharynx contains numerous salivary glands that ensure the permeation of the fodder lump. In the wild, fodder is accumulated and processed with digestive enzymes, and both sexes produce a secret — 'bird's milk', which is fed to the young through regurgitation.

The mucous membrane of the esophagus forms longitudinal folds, the apical part of which is covered with a multilayered flat keratinized epithelium. Blood, lymphatic vessels, and nerve fibers are located in the submucosa. The muscle membrane is represented by several layers of myocytes, located in a circular and longitudinal orientation.

The proventriculus contains a large number of digestive glands, and the cavity of the muscular stomach contains gastroliths, which act as teeth. The small intestine consists of the duodenum and ileum, which are joined by the mesentery to form three loops. The mucous membrane is developed and contains numerous crypts, the central part of which is formed by connective tissue. The outer crypts are covered with a single-layered prismatic epithelium with numerous inclusions of exocrine cells.

The liver consists of the right and left parts, whereas the right part is much larger. The left part is not divided, and the gall bladder is absent. Bile moves along the hepatic-intestinal ducts and opens into the duodenum.

The large intestine is represented by the paired rectal cecum and the rectum. In the mucous membrane of the

large intestine, circular folds are replaced by leaf-like villi. The apical surface is covered with a single-layer prismatic epithelium alternating with glandular goblet cells. The muscle layer is the most developed, myocytes form longitudinal folds located in circular and longitudinal directions.

References

Adil, S. and Magray, S. N. (2012) 'Impact and manipulation of gut microflora in poultry: A review', *Journal of Animal and Veterinary Advances*, 11(6), pp. 873–877. doi: 10.3923/javaa.2012.873.877.

Bailey, T. A., Mensah-Brown, E. P., Samour, J. H., Naldo, J., Lawrence, P. and Garner, A. (1997) 'Comparative morphology of the alimentary tract and its glandular derivatives of captive bustards', *Journal of Anatomy*, 191(3), pp. 387–398. doi: 10.1046/j.1469-7580.1997.19130387.x.

Bindari, Y. R. and Gerber, P. F. (2022) 'Centennial review: Factors affecting the chicken gastrointestinal microbial composition and their association with gut health and productive performance', *Poultry Science*, 101(1), p. 101612. doi: 10.1016/j.psj.2021.101612.

CE (The Council of Europe). (1986) *European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes*. (European Treaty Series, No. 123). Strasbourg: The Council of Europe. Available at: https://conventions.coe.int/treaty/en/treaties/html/123.htm.

CEC (The Council of the European Communities) (2010) 'Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes', *The Official Journal of the European Communities*, L 276, pp. 33–79. Available at: http://data.europa.eu/eli/dir/2010/63/oj.

Gugołek, A., Jastrzębska, A. and Strychalski, J. (2016) 'Use of pigeons and other bird species in human recreation' [Wykorzystanie gołębi i innych gatunków ptaków w rekreacji człowieka], *Wiadomości Zootechniczne*, 54(2), pp. 90–95. URL: https://wz.izoo.krakow.pl/files/WZ_2016_2_art13.pdf. [in Polish].

Hamdi, H., El-Ghareeb, A.-W., Zaher, M. and AbuAmod, F. (2013) 'Anatomical, histological and histochemical adaptations of the avian alimentary canal to their food habits: II — *Elanus caeruleus*', *International Journal of Scientific & Engineering Research*, 4(10), pp. 1355–1364. URL: https://www.ijser.org/researchpaper/anatomical-histological-and-histochemical-adaptations-of-the-avian-alimentary.pdf.

Hamoda, H. and Farag, A. (2018) 'Histological characterizations of the gut associated lymphatic tissue in pigeon', *Alexandria Journal of Veterinary Sciences*, 59(2), p. 157–164. doi: 10.5455/ajvs.16178.

Klasing, K. C. (1999) 'Avian gastrointestinal anatomy and physiology', *Seminars in Avian and Exotic Pet Medicine*, 8(2), pp. 42–50. doi: 10.1016/S1055-937X(99)80036-X.

Kolomak, I. O. and Kruchynenko, O. V. (2017) 'Bird lice (Mallophaga, Philopteridae, Menoponidae) of domestic pigeons on specialized pigeon breeding farms in Poltava', *Vestnik Zoologii*, 51(6), pp. 487–492. URL: http://mail.izan.kiev.ua/vz-pdf/2017/6/Kolomak.pdf.

Lavin, S. R., Karasov, W. H., Ives, A. R., Middleton, K. M. and Garland Jr., T. (2008) 'Morphometrics of the avian small intestine compared with that of nonflying mammals: A phylogenetic approach', *Physiological and Biochemical Zoology*, 81(5), pp. 526–550. doi: 10.1086/590395.

Lee, K.-C., Kil, D. Y. and Sul, W. J. (2017) 'Cecal microbiome divergence of broiler chickens by sex and body weight', *Journal of Microbiology*, 55(12), pp. 939–945. doi: 10.1007/s12275-017-7202-0.

McWhorter, T. J., Caviedes-Vidal, E. and Karasov, W. H. (2009) 'The integration of digestion and osmoregulation in the avian gut', *Biological Reviews*, 84(4), pp. 533–565. doi: 10.1111/j.1469-185X.2009.00086.x.

Nasrin, M. Siddiqi, M. N. H. Masum M. A. and Wares M. A. (2012) 'Gross and histological studies of digestive tract of broilers during postnatal growth and development', *Journal of Bangladesh Agricultural University*, 10(1), pp. 69–77. URL: https://baures.bau.edu.bd/wp-content/uploads/2018/06/12-JBAU-480-11.pdf.

Oakley, B. B., Lillehoj, H. S., Kogut, M. H., Kim, W. K., Maurer, J. J., Pedroso, A., Lee, M. D., Collett, S. R., Johnson, T. J. and Cox, N. A. (2014) 'The chicken gastrointestinal microbiome', *FEMS Microbiology Letters*, 360(2), pp. 100–112. doi: 10.1111/1574-6968.12608.

Price, E. R., Brun, A., Caviedes-Vidal, E. and Karasov, W. H. (2015) 'Digestive adaptations of aerial lifestyles', *Physiology*, 30(1), pp. 69–78. doi: 10.1152/physiol.00020.2014.

Ratnayani, K., Suter, I. K., Antara, N. S. and Putra, I. N. K. (2019) 'Effect of *in vitro* gastrointestinal digestion on the Angiotensin Converting Enzyme (ACE) inhibitory activity of pigeon pea protein isolate', *International Food Research Journal*, 26, pp. 1397–1404. URL: http://www.ifrj.upm.edu.my/26%20(04)%202019/30%20-%20IFRJ19030.R1-Final.pdf.

Santos, H. M., Tsai, C.-Y., Catulin, G. E. M., Trangia, K. C. G., Tayo, L. L., Liu, H.-J. and Chuang, K. P. (2020) 'Common bacterial, viral, and parasitic diseases in pigeons (*Columba livia*): A review of diagnostic and treatment strategies', *Veterinary Microbiology*, 247, p. 108779. doi: 10.1016/j.vetmic.2020.108779.

Scanes, C. G. and Dridi, S. (eds.) (2021). *Sturkie's Avian Physiology*. 7th ed. San Diego: Academic Press. doi: 10.1016/C2019-0-00060-X.

Scanes, C. G. and Pierzchala-Koziec, K. (2014) 'Biology of the gastrointestinal tract in poultry', *Avian Biology Research*, 7(4), pp. 193–222. doi: 10.3184/175815514X14162292284822.

Simmonds, R. C. (2017) 'Chapter 4. Bioethics and animal use in programs of research, teaching, and testing', in Weichbrod, R. H., Thompson, G. A. and Norton, J. N. (eds.) *Management of Animal Care and Use Programs in Research, Education, and Testing*. 2nd ed. Boca Raton: CRC Press, pp. 35–62. doi: 10.1201/9781315152189-4.

VRU (Verkhovna Rada Ukrainy) (2006) 'Law of Ukraine No. 3447-IV of 21.02.2006 'About protection of animals from cruel treatment' [Zakon Ukrainy № 3447-IV vid 21.02.2006 'Pro zakhyst tvaryn vid zhorstokoho povodzhennia'], *News of the Verkhovna Rada of Ukraine [Vidomosti Verkhovnoi Rady Ukrainy]*, 27, art. 230. Available at: https://zakon.rada.gov.ua/laws/3447-15. [in Ukrainian].

Wang, F., Zhang, X., Zhong, Y., Tang, X., Hu, X., Yang, S., Zhong, S., Zhou, Z., Liu, J., Yuan, X. and Li, Y. (2020) 'Expression and localization of VIP in different segments of pigeon intestine', *Pakistan Journal of Zoology*, 52(6), pp. 2299–2304. doi: 10.17582/journal.pjz/20200208160224.

UDC 619:615.326:553.61 DOI 10.36016/JVMBBS-2024-10-1-3

PROSPECTS OF USING CLAY WITH MEDICINAL PROPERTIES IN VETERINARY MEDICINE AND AGRICULTURE

Mogilyovskyy V. M., Kibkalo D. V., Sobakar Y. V., Kravchenko N. O.

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

Summary. The article explores the potential applications of clays and clay materials in agriculture, emphasizing their role in developing effective remedies for common animal diseases and environmental cleanup. The study relies on electronic resources such as ScienceDirect, Scopus, PubMed, and ResearchGate, employing analysis and generalization as research methods. The focus is on scientific publications from 2000 to the present. Throughout history, mankind has recognized the healing properties of clay, utilized both internally through geophagy and externally in the form of medicinal poultices and rubs prepared by ancient healers. The analysis of publications highlights the focus on studying the physical and chemical properties of clay, establishing its therapeutic effects, and exploring its practical applications in medicine and veterinary medicine. The article outlines promising areas and proposes the use of clay, particularly of local origin, in domestic veterinary medicine. A notable application is in addressing gastrointestinal diseases in young animals and treating animal poisoning caused by pesticides and mycotoxins

Keywords: therapy, gastrointestinal diseases, sorbents, environmental protection

Introduction. The advancement of human and veterinary medicine in the modern era necessitates the exploration and development of effective medications suitable for both normal and extreme conditions. Criteria such as drug availability, cost, and versatility are crucial in this context. Clays, known for their potent therapeutic properties, are abundant in nature. A comprehensive investigation into clay properties enables the formulation of novel medicinal combinations that significantly enhance their efficacy.

Contemporary environmental challenges underscore the urgency of finding solutions. The escalation of environmental and food contamination by heavy metals, nitrates, pesticides, herbicides, organic compounds, and other toxins demands the creation and implementation of new, efficient, and cost-effective means for neutralizing and eliminating pollutants from diverse environmental entities. Various sorbents, including activated carbon, ion exchange resins, natural sorbents, zeolites, and vermiculites, are employed to extract and eliminate harmful substances. Clay, boasting exceptional healing and absorption properties, aligns perfectly with the requirements for its utilization in both animal health and environmental protection.

The aim of the study was analyze scientific publications from 2000 to the present about the potential applications of clays and clay materials in agriculture with focus on their role in developing effective remedies for common animal diseases and environmental cleanup.

Materials and methods. The research materials were the electronic resources Science Direct, Scopus, PubMed, and ResearchGate. Research methods – analysis and generalization. Scientific publications for the period from 2000 to the present were studied.

Results and discussion. Clay therapy, also known as bolucotherapia (from the Greek *bolos* — clay + *therapia* — treatment), or peloid therapy (from the Greek *pelos* — silt, clay + *therapia* — treatment), has been explored in this study (Konovalova, 2010).

Clay is a fine-grained sedimentary rock that is powdery when dry and malleable when wet. It is composed of one or more minerals from the kaolinite group, which is named after the Gaoling village in China, montmorillonite, or other aluminosilicates (clay minerals). It may also contain sand and carbonate particles. The main rock-forming mineral in clay is kaolinite ($Al_4Si_4O_{108}$), which contains 47% silicon oxide (SiO_2), 39% aluminium oxide (Al_2O_3), and 14% water (H_2O). Al_2O_3 and SiO_2 are significant components of clays of various colors.

The color of the clay, ranging from yellow, brown, blue, green, and purple to black, is caused by impurities of chromophore ions, including oxides of iron, titanium, magnesium, copper, nickel, and chromium (Grim, 1967). In official medicine, only white clay is used, while in folk medicine, white, blue, green, and other types are utilized. Each type of medicinal clay has a special chemical composition and healing properties. Blue clay, considered the most healing, contains elements like K, Ca, Mg, Si, Na, P, Cl, Fe, Al, I, Co, Cd, Mg, Li, Cu, Mo, Zn, Se, Ra, and others, exhibiting enveloping, bactericidal, anti-inflammatory, and other healing properties. It has a detrimental effect on pathogens such as tuberculosis, cholera, and many others, and is used to treat diseases of the throat, bronchi, and lungs. Red clay, mainly composed of iron oxide and copper, is used to treat allergies, anemia, and boost immunity (Samoilovich, 2009; Henry and Cring, 2013; Kravets and Riabiev, 2016; Young and Miller, 2019).

The most commonly used types of clay for therapeutic purposes are kaolinite (white or Chinese clay — Bolus alba, $Al_2O_3{\times}2SiO_2{\times}2H_2O$), hydrargillite

($Al_2O_3 \times 3H_2O$), diaspore ($Al_2O_3 \times H_2O$), and montmorillonite ($MgO \times Al_2O_3 \times 3SiO_2 \times 1.5H_2O$). The therapeutic effect of clay therapy is based on neurohumoral mechanisms, providing therapeutic, stimulating, and trophic effects on the pathological process. Methods of clay therapy include external use: lotions, applications, rubbing, wraps, compresses, masks, baths; and internal use: solution, powder.

History of the Use of Medicinal Clays. The healing properties of natural mineral clays have been known to man since ancient times, actively used not only in cosmetology but also in medicine. Ancient healers crafted various poultices and rubs from clay, and it was taken internally when its absorption effect was needed. Clay therapy aided in various poisonings, epidemics, and muscle pain.

The practice of eating earth, clay, and other minerals (geophagy, lithophagy) is known among both animals and humans, dating back to ancient times, and is still common in some cultures today. Clays have been used as therapeutic agents throughout human existence. Primitive tribes traditionally used different types of clay, such as bentonite, kaolinite, montmorillonite, smectite, and pascalite (Ca^{2+} montmorillonite from Wyoming, USA) (Droy-Lefaix and Tateo, 2006). Primitive tribes have used different types of clay to combat toxicity (Johns and Duquette, 1991). In Côte d'Ivoire, where clay is consumed primarily by pregnant women, the soil was found to be approximately 68% kaolinite, 27% smectite and vermiculite, and 5% other minerals (Abe et al., 2006). The use of clay is believed to address mineral deficiencies in the diet and alleviate many gastrointestinal diseases (Kikouama et al., 2002, 2009).

The earliest written sources that mention the health benefits of clay can be traced back to ancient Egyptian papyri. Even today, Egyptian and Syrian clays are recognized and popular as excellent cosmetic products. However, systematic data on using clay dates to a later historical period, specifically in Antiquity. Hippocrates (460–370 BC), often regarded as the father of medicine, was the first physician to detail the properties of clay in his works. His writings provided a comprehensive description of therapeutic and surgical methods, as well as pharmacology, emphasizing the diverse applications of clay (Hippocrates, 1936–1944).

During the medieval period, physicians like Avicenna and Amasiatsi referenced information about clay from earlier works, including Dioscorides (40–90 AD), Celsus (II century AD), and Galen (129–216 AD). However, the information from this period remains somewhat fragmentary. Avicenna, an outstanding Persian scientist, provided detailed descriptions of the properties and applications of more than twenty varieties of clay. In his Canon of Medical Science, Avicenna emphasized the importance of cleansing the body using printing clay (Tin Makhtum) as a hemostatic, wound, and ulcer healing agent, and a preventive measure against poisoning (Celsus, 1971; Avicenna, 1979–1982).

The end of the Middle Ages witnessed significant scientific and technological advancements, and medicine was no exception. In the early eighteenth century, Dr. John Quincy published *The New Dispensatorium* in London, a pharmacopeia that included descriptions of the types of clay and their applications (Lewis and Quincy, 1753). Clay was also utilized by healers known as 'hlyniuky' among the Zaporizhzhia Cossacks, and it played an active role in renowned resorts and clinics across Europe (Green Planet, 2022).

During the 20th century, clay's physical and chemical properties were extensively studied, substantiating its therapeutic effects and practical applications in medicine and animal husbandry. Both domestic and foreign scientists have contributed to the understanding of medicinal clays, and the term 'medical clays' was officially proposed by French scientists in 2006 (Tria, Zherom and Dyubuk, 2006).

For an extended historical period, spanning up to the early twentieth century, clay and clay products held a significant role in the traditional household culture, folk medicine, veterinary medicine, and magical healing practices of Ukrainians. In regions like Poltava and Slobozhanshchyna, the use of clay and its products for medicinal purposes, particularly in the preparation of homeopathic remedies, was widespread (Metka, 2010).

The current state of the problem reflects ongoing studies on the medicinal properties of clays by scientists from various countries. Essential properties of clay minerals, such as adsorption capacity (Tenorio Arvide et al., 2008) and the ability to release trace elements (Tateo and Summa, 2007; Gomes and Silva, 2007), have been established. These findings lay the foundation for the development of new drugs in both human and veterinary medicine.

Research has confirmed the effectiveness of clay minerals in binding and removing harmful compounds from the body (Xu, Han and Wang, 2004) and their antibacterial effects (Haydel, Remenih and Williams, 2008; Mpuchane et al., 2010). Clays are recognized as excellent adsorbents with physical and chemical interactions with microorganisms. They are further classified into bactericidal and non-bactericidal, with the bactericidal mechanism being chemical. Such clays demonstrate a detrimental effect on a wide range of microorganisms, including antibiotic-resistant strains (Korchak et al., 2014; Williams, 2019; Gomes C., Gomes J. and Da Silva, 2020).

Since 2006, animal health and growth have been significantly impacted by the banning of antibiotics as growth promoters in EU member states.

The use of clay minerals is considered a potential solution due to their adsorption capacity and lack of primary toxicity, making them effective preventive and

therapeutic agents and feed additives that can ensure animal health and productivity (Slamova et al., 2011).

The adverse effects of non-steroidal anti-inflammatory drugs (NSAIDs) are widely recognized. Clay minerals have been reported to protect against these side effects. Experiments involving pigs and rats showed that oral administration of aspirin and phenylbutazone caused severe ulcerative damage, which was significantly reduced after treatment with smectite (Droy-Lefaix et al., 1992; Peignot, Giral and Plique, 1997).

Clays and clay minerals have been found to have a stabilizing effect on the mucosal barrier, offering protection against various lesions of the gastrointestinal mucosa. Experiments on rats demonstrated that smectite can inhibit damage caused by pathological dysregulation, preventing hemorrhagic lesions, significant bleeding, and localized ulcerative lesions (Leonard, Droy-Lefaix and Allen, 1994).

Clays have demonstrated the ability to adsorb various toxic substances, including aflatoxin (Phillips, Lemke, and Grant, 2002; Marroquín-Cardona et al., 2011), as well as toxins produced by *Yersinia pseudotuberculosis* (Carnoy et al., 2000).

The exceptional adsorption capacity of bentonites, attributed to their fine particle size, fine particle shape, and high specific surface area (Dixon et al., 2008; Tenorio Arvide, 2008), allows them to absorb large organic molecules, polymers, and complex ions. This enables them to bind mycotoxins, heavy metals, bacteria, and viruses, particularly smectites like montmorillonites (Murray, 2000; Szajewska, Dziechciarz and Mrukowicz, 2006). Kaolin, on the other hand, has been found effective in binding enterotoxins that cause diarrhea (Dominy, Davoust and Minekus, 2004).

Clays, due to their high adsorption capacity, can protect the gastrointestinal tract of animals from damage. This makes the use of clays a promising avenue for the treatment of gastrointestinal diseases in young animals and in cases of pesticide poisoning. Studies have shown that the non-selective herbicide diquat can cause erosion of the intestinal mucosa. Treatment with smectite in diquat-treated rats led to the normalization of mucus rheological properties and intestinal permeability (Theodorou et al., 1994). Both montmorillonite and bentonite are effective adsorbents that are recommended for use in the treatment of pesticide poisoning (Meredith and Vale, 1987).

In the context of dairy calves, where the incidence of morbidity exceeds 34%, and the most common diseases are gastrointestinal and respiratory, there is a growing interest in complementary and alternative therapies. Medicinal clays, being effective supplements, are easy to incorporate into daily calf care practices. However, the diverse physical and chemical compositions of different clay types necessitate systematic research (Williams and Haydel, 2010).

Research on the natural zeolite clinoptilolite revealed its effect on the absorption of immunoglobulins from colostrum and the incidence of gastrointestinal diseases in newborn calves. Adding 1.0 g of clinoptilolite per kg of body weight per day to colostrum and milk reduced diarrhea, though its impact on passive immunity was insignificant. Higher doses hurt passive immunity and caused diarrhea (Sadeghi and Shawrang, 2008).

Another study on the administration of clinoptilolite to dairy calves found a statistically higher level of antibodies against *E. coli* in the blood serum of calves fed clinoptilolite. Clinoptilolite also significantly reduced the frequency of diarrhea. The addition of clinoptilolite to colostrum and milk during the first 10 days after calving proved effective in enhancing intestinal absorption of antibodies against enterotoxigenic *E. coli* strains and reducing the frequency and duration of diarrhea in calves (Pourliotis et al., 2012).

Studies on the therapeutic efficacy of sepiolite in neonatal diarrhea in calves demonstrated that adding 2% sepiolite to the feed had no side effects on the animals. Sepiolite, as a good absorbent, effectively provides hydration by retaining water, eliminating etiological factors, and preventing diarrhea. The inclusion of sepiolite in the feed positively influenced the increase in live weight and productivity of the animals. This positive effect was observed both in the treatment and prevention of calf diarrhea, and the use of sepiolite indirectly contributed to human health by preventing the emergence of antibiotic resistance (Elitok and Fatih Baser, 2016). Consequently, utilizing clays for infections lacking effective antimicrobial agents holds promise.

Another promising application of clays lies in creating slow-release formulations and water purification. Studies have explored the modification of montmorillonite and other clays by interacting with organic cations to produce slow-release herbicide preparations and effectively remove pollutants from water through filtration. The contaminants successfully removed include hydrophobic and anionic organic molecules (herbicides), dissolved organic matter, pharmaceuticals (antibiotics and non-steroidal drugs), inorganic anions (perchlorate), microorganisms, bacteria, including cyanobacteria, and their toxins. The modification of smectite clay minerals with natural organic cations, such as L-carnitine, dimethyl ester of L-cystine, and thiamine, enhances the clay's properties for removing the herbicide simazine from the environment (Cruz-Guzmán et al., 2004; Undabeytia et al., 2020).

The efficiency of removing tetracycline and sulfonamide antibiotics from water by benzyl dimethyl hexadecylammonium (BDMHDA) micelles pre-adsorbed on montmorillonite was investigated. Micellar clay complexes (1% w/w) were found to remove 96–99.9% of antibiotics from their aqueous solutions containing 5–50 mg/l of drugs. Micellar clay complexes

were found to be highly effective in removing 89–99% of tetracycline and sulfamethizole from initial solutions containing 10 mg/l of antibiotics and 8 mg/l of humic acid or 9 mg/l of fulvic acid in the presence of dissolved soil organic matter. These findings suggest that micellar clay complexes are a promising method for purifying water from tetracycline and sulfonamide antibiotics (Polubesova et al., 2006).

Simple clays, known for their significant absorption capacity for gases and organic matter, have been explored for their potential to absorb various compounds from the gaseous phase. Although this topic has not been fully explored, there are reports on the use of minerals such as kaolinite, halloysite, montmorillonite, bentonite, saponite, vermiculite, illite, sepiolite, and palygorskite to control emissions of a wide range of pollutants, including CO_2, CH_4, SO_2, H_2S, NH_3, etc. (Wal, Rutkowski and Stawiński, 2021).

Having one of the best absorption properties, the clay fully meets the requirements for its use in environmental cleaning. Since these properties of clays are still limited in livestock production, further research and implementation are promising areas. At the same time, the unsubstantiated use of clay, in addition to its benefits, may have adverse effects arising from parasites and/or harmful microorganisms and toxic substances present in the clay consumed. Current evidence indicates that geophagy poses potential risks such as heavy metal toxicity and diseases caused by clay consumption, which binds nutrients and beneficial pharmaceuticals in the gut. However, research also suggests that geophagy may have benefits in protecting against harmful pathogens and toxins through two different physiological pathways. Future research should investigate the causal relationship between geophagy and iron deficiency. Additionally, it should explore the biological and psychosocial factors associated with geophagy (Young and Miller, 2019).

In addition, the negative effects of clay may be due to the high adsorption capacity of clay. As a result, it can cause anemia by binding iron. Iron is an element that is often present in the soil, consumed by animals and humans, and thus can be a source of this mineral for the body. However, it can also be a contributing factor to anemia, according to the chelating capacity of soil clay (Kawai et al., 2009).

As a natural compound, clay is versatile: its anti-toxicity, rich mineral composition, and sorption properties, as discussed above, suggest its wider use.

Clay is richer in minerals than vegetables and fruits, and its absorption properties allow it to remove pathogens from the body. For the successful use of clay in the treatment of various diseases, it is necessary to comprehensively study their mineral composition. It is especially important to study and use local clay samples, as it is desirable to use clay not imported but taken from the place where the patient was born or lives.

In this regard, the absorption properties and ionic composition of samples of therapeutic clays located in the village of Luzhok, Derhachi District, Kharkiv Region (Ukraine) were studied. Methods for determining the trace element composition (Fe, Co, Zn, Al, Cu) in medicinal clays for medicine and veterinary medicine were developed and certified, allowing to control and regulate the concentration of the main components of medicines. The developed methods differ from the existing ones by their expressiveness, selectivity, and accuracy. A comparative analysis of the results obtained by nuclear and X-ray fluorescence spectrometry was carried out using the Q-test. It was found that the results belong to the same general population and are not burdened with systematic errors. The possibility of using clay and its combined preparations (ozone clay solutions and products for internal and external use with a regulated content of trace elements and active oxygen) in medicine and veterinary medicine for the treatment of various pathologies, disinfection of environmental objects, and the possibility of using clay for the prevention and treatment of animals with certain infectious diseases was investigated. It has been shown that combined clays do not adsorb carotene, vitamin B_{12}, and some enzymes (Melnik and Shevtsov, 2002; Melnik et al., 2002a, 2002b, 2003).

Conclusions. Given the above, we propose:

— investigate the effectiveness of clay application for gastrointestinal diseases in young animals and pesticide and mycotoxin poisoning in animals;

— develop a standard sample of therapeutic clay and study its absorption properties;

— utilize the absorption properties of clays for purifying industrial emissions from compounds (Cr, Pb, Co, Ni, Cu, Mg), treating pigsty wastewater, and improving the quality of air in livestock environments;

— create and employ combined preparations, such as ozone clay solutions, in the fields of medicine and veterinary medicine;

— explore the development of pharmaceuticals derived from local clay for both internal and external use, ensuring the regulated content of trace elements and active oxygen;

— develop regulatory documentation for the use of therapeutic clay in veterinary medicine and animal husbandry, including industry guidelines.

Acknowledgements. The author expresses sincere gratitude to Victor Vasilyevich Melnyk, an esteemed teacher, scientist-chemist, and associate professor of the Department of Chemical Metrology at V. N. Karazin Kharkiv National University.

References

Abe, S. S., Masunaga, T., Honna, T. and Wakatsuki, T. (2006) 'Comprehensive assessment of the clay mineralogical composition of lowland soils in West Africa', *Soil Science and Plant Nutrition*, 52(4), pp. 479–488. doi: 10.1111/j.1747-0765.2006.00060.x.

Avicenna (1979–1982) *The Canon of Medicine [Kanon vrachebnoy nauki]* (5 vols). 2nd ed. Tashkent: Fan. [in Russian].

Carnoy, C., Muller Alouf, H., Mullet, C., Droy-Lefaix, M. T. and Simonet, M. (2000) 'Oral infection of mice with superantigenic toxic producing *Yersinia pseudotuberculosis*. Effect of diosmectite', *International Journal of Medical Microbiology*, 290(30), p. 83.

Celsus, A. C. (1971) *De Medicina*. Cambridge: Harvard University Press. ISBN 9780434992928.

Cruz-Guzmán, M., Celis, R., Hermosín, M. C. and Cornejo, J. (2004) 'Adsorption of the herbicide simazine by montmorillonite modified with natural organic cations', *Environmental Science & Technology*, 38(1), pp. 180–186. doi: 10.1021/es030057w.

Dixon, J. B., Kannewischer, I., Tenorio Arvide, M. G. and Barrientos Velazquez, A. L. (2008) 'Aflatoxin sequestration in animal feeds by quality-labeled smectite clays: An introductory plan', *Applied Clay Science*, 40(1–4), pp. 201–208. doi: 10.1016/j.clay.2007.10.010.

Dominy, N. J., Davoust, E. and Minekus, M. (2004) 'Adaptive function of soil consumption: an *in vitro* study modeling the human stomach and small intestine', *Journal of Experimental Biology*, 207(2), pp. 319–324. doi: 10.1242/jeb.00758.

Droy-Lefaix, M. T. and Tateo, F. (2006) 'Chapter 11.6 Clays and clay minerals as drugs', in Bergaya, F., Theng, B. K. G. and Lagaly, G. (eds) *Handbook of Clay Science*. (Developments in Clay Science, Vol. 1). Amsterdam: Elsevier, pp. 743–752. doi: 10.1016/S1572-4352(05)01025-1.

Droy-Lefaix, M. T., Plique, O., Géraud, G. and Drouet, Y. (1992) 'Protective effect of diosmectite on the decrease of the adherent mucus gel thickness induced by phenylbutazone on rat stomach', *Hellenic Journal of Gastroenterology*, 5(70), p. 279.

Elitok, B. and Fatih Baser, D. (2016) 'Investigation of the therapeutic efficacy of sepiolite in Neonatal calf diarrhea', *International Biological and Biomedical Journal*, 2(3), pp. 110–119. Available at: https://ibbj.org/article-1-83-en.pdf.

Gomes, C. and Silva, J. (2007) 'Minerals and clay minerals in medical geology', *Applied Clay Science*, 36(1–3), pp. 4–21. doi: 10.1016/j.clay.2006.08.006.

Gomes, C. F., Gomes, J. H. and Da Silva, E. F. (2020) 'Bacteriostatic and bactericidal clays: An overview', *Environmental Geochemistry and Health*, 42(11), pp. 3507–3527. doi: 10.1007/s10653-020-00628-w.

Green Planet [Zelena Planeta] (2022) *Clay Adds Health [Hlyna dodaie zdorovia]*. Available at: https://www.zelena-planeta.ua/glina-sporidnena-z-lyudinoyu. [in Ukrainian].

Grim, R. E. (1967) *Applied Clay Mineralogy [Mineralogiya i prakticheskoe ispol'zovanie glin]*. Moscow: Mir. [in Russian].

Haydel, S. E., Remenih, C. M. and Williams, L. B. (2008) 'Broad-spectrum in vitro antibacterial activities of clay minerals against antibiotic-susceptible and antibiotic-resistant bacterial pathogens', *The Journal of Antimicrobial Chemotherapy*, 61(2), pp. 353–361. doi: 10.1093/jac/dkm468.

Henry, J. M. and Cring, F. D. (2013). 'Geophagy: An anthropological perspective', in Brevik, E. C. and Burgess, L. C. (eds) *Soils and Human Health*. Boca Raton: CRC Press, pp. 196–215. doi: 10.1201/b13683-13.

Hippocrates (1936–1944) *Works [Sochineniya]* (3 vols). Moscow: Medgiz. [in Russian].

Johns, T. and Duquette, M. (1991) 'Detoxification and mineral supplementation as functions of geophagy', *The American Journal of Clinical Nutrition*, 53(2), pp. 448–456. doi: 10.1093/ajcn/53.2.448.

Kawai, K., Saathoff, E., Antelman, G., Msamanga, G. and Fawzi, W.W. (2009) 'Geophagy (soil-eating) in relation to Anemia and Helminth infection among HIV-infected pregnant women in Tanzania', *The American Journal of Tropical Medicine and Hygiene*, 80(1), pp. 36–43. doi: 10.4269/ajtmh.2009.80.36.

Kikouama, O. J. R., Sei, J., Soro, S.N., Touré, A., Atindehou, E. and Bonnet, J. P. (2002) 'Clay consumption in Ivory Coast: Characterization and therapeutic use' [Argiles consommées en Côte d'Ivoire: Caractérisation et usages thérapeutiques], *Afrique Bio Médicale*, 7, pp. 34–40. [in French].

Kikouama, J., Konan, K., Katty, A., Bonnet, J., Balde, L. and Yagoubi, N. (2009) 'Physicochemical characterization of edible clays and release of trace elements', *Applied Clay Science*, 43(1), pp. 135–141. doi: 10.1016/j.clay.2008.07.031.

Konovalova, O. O. (2010) 'Glinolithic treatment' [Hlynolikuvannia], in *Pharmaceutical Encyclopedia [Farmatsevtychna entsyklopediia]*. Available at: https://www.pharmencyclopedia.com.ua/article/2983/glinolikuvannya. [in Ukrainian].

Korchak, G. I., Surmasheva, Ye. V., Romanenko, L. I., Marinin, A. I., Olishevsky, V. V. and Marchenko, A. B. (2014) 'Adsorptive activity of kaolin (microbiological study)' [Adsorbtsiina aktyvnist kaolinu (mikrobiolohichni doslidzhennia)], *Enviroment & Health [Dovkillia ta zdorovia]*, 4, pp. 37–41. Available at: http://nbuv.gov.ua/UJRN/dtz_2014_4_9. [in Ukrainian].

Kravets, O. M. and Riabiev, A. A. (2016) *Lecture Notes on the Discipline 'Resortology' [Konspekt lektsii z dystsypliny 'Kurortolohiia']*. Kharkiv: O. M. Beketov National University of Urban Economy in Kharkiv. Available at: http://eprints.kname.edu.ua/43523. [in Ukrainian].

Leonard, A., Droy-Lefaix, M. T. and Allen, A. (1994) 'Pepsin hydrolysis of the adherent mucus barrier and subsequent gastric mucosal damage in the rat: Effect of diosmectite and 16,16 dimethyl prostaglandin E2', *Gastroentérologie Clinique et Biologique*, 18(6–7), pp. 609-616.

Lewis, W. and Quincy, J. (1753) *The New Dispensatory ... : the Whole Interspersed with Practical Cautions and Observations: Intended as a Correction, and Improvement of Quincy*. London: Printed for J. Nourse, opposite Catharine Street in the Strand. doi: 10.5962/bhl.title.125385.

Marroquín-Cardona, A., Deng, Y., Garcia-Mazcorro, J. F., Johnson, N. M., Mitchell, N. J., Tang, L., Robinson, A., Taylor, J. F., Wang, J.-S. and Phillips, T. D. (2011) 'Characterization and safety of uniform particle size NovaSil clay as a potential aflatoxin enterosorbent', *Applied Clay Science*, 54(3–4), pp. 248–257. doi: 10.1016/j.clay.2011.09.009.

Melnik, V. V. and Shevtsov, N. I. (2002) 'Use of X-ray fluorescence spectrometry to determine the ionic composition of green clay in the treatment of animals in extreme conditions' [Ispol'zovanie rentgenofluorestsentnoy spektrometrii dlya opredeleniya ionnogo sostava zelenoy gliny pri lechenii zhivotnykh v ekstremal'nykh usloviyakh], *International Scientific-Practical Conference 'Information Technologies of Management of Emergencies, Environmental Protection, Resources' [Mezhdunarodnaya nauchno-prakticheskaya konferentsiya 'Informatsionnye tekhnologii upravleniya*

chrezvychaynymi situatsiyami, okhranoy okruzhayushchey sredy, resursami'], Crimea, 8–11 September: book of abstracts. Crimea, p. 99. [in Russian].

Melnik, V. V. et al. (2002a) 'Atomic absorption determination of ionic composition of green clay, used in human and veterinary medicine' [Atomno-absorbtsionnoe opredelenie ionnogo sostava zelenoy gliny, ispol'zuemoy v chelovecheskoy i veterinarnoy meditsine], *First International Symposium 'Methods of Chemical Analysis' [Pervyy mezhdunarodnyy simpozium 'Metody khimicheskogo analiza'], Sevastopol*, Crimea, 1–3 October: book of abstracts. Sevastopol, p. 64. [in Russian].

Melnik, V. V. et al. (2002b) 'The development and attestation of methodics of determination of microelements in propolis', *First International Symposium 'Methods of Chemical Analysis' [Pervyy mezhdunarodnyy simpozium 'Metody khimicheskogo analiza'], Sevastopol*, Crimea, 1–3 October: book of abstracts. Sevastopol, p. 72. [in Russian].

Melnik, V. V. et al. (2003) 'Evaluation of methods for determining the ozone in solutions, which are used in human and veterinary medicine' [Otsenka metodov opredeleniya ozona v rastvorakh, kotorye ispol'zuyutsya v chelovecheskoy i veterinarnoy meditsine], Actual Problems of Modern Analytical Chemistry [Aktual'nye problemy sovremennoy analiticheskoy khimii], Uzhgorod, 19–23 May: book of abstracts. Uzhgorod, p. 84–85. [in Russian].

Meredith, T. J. and Vale, J. A. (1987) 'Treatment of paraquat poisoning in man: Methods to prevent absorption', *Human Toxicology*, 6(1), pp. 49–55. doi: 10.1177/096032718700600108.

Metka, L. O. (2010) 'Clay and clay products in folk medicine, veterinary, medical practice magic Ukrainian of Northern Left-Bank Dipper area in the second half of XIX–early XX century' [Hlyna ta hlyniani vyroby u narodnii medytsyni, veterynarii, likuvalnii mahichnii praktytsi ukraintsiv Pivnichno-Dniprovskoho Livoberezhzhia u druhii polovyni XIX–pershii polovyni XX stolittia], *Nizhyn Antiquity [Nizhynska Starovyna]*, 10(13), pp. 58–64. Available at: http://dspace.nbuv.gov.ua/handle/123456789/24176. [in Ukrainian].

Mpuchane, S. F., Ekosse, G.-I. E., Gashe, B. A., Morobe, I. and Coetzee, S. H. (2010) 'Microbiological characterisation of southern African medicinal and cosmetic clays', *International Journal of Environmental Health Research*, 20(1), pp. 27–41. doi: 10.1080/09603120903254025.

Murray, H. H. (2000) 'Traditional and new applications for kaolin, smectite, and palygorskite: A general overview', *Applied Clay Science*, 17(5–6), pp. 207–221. doi: 10.1016/S0169-1317(00)00016-8.

Peignot, J. F., Giral, P. and Plique, O. (1997), 'A multicentric, double-blind, placebo controlled study of the efficacy of diosmectite in the treatment of secondary stomach pain due to administration of non steroid antiinflammatory agents', *Médecine et Chirurgie Digestive*, 26, pp. 233–241.

Phillips, T. D., Lemke, S. L. and Grant, P. G. (2002) 'Characterization of clay-based enterosorbents for the prevention of aflatoxicosis', in DeVries, J. W., Trucksess, M. W. and Jackson, L. S. (eds) *Mycotoxins and Food Safety*. (Advances in Experimental Medicine and Biology, Vol. 504). Boston, MA: Springer, pp. 157–171. doi: 10.1007/978-1-4615-0629-4_16.

Polubesova, T., Zadaka, D., Groisman, L. and Nir, S. (2006) 'Water remediation by micelle–clay system: Case study for tetracycline and sulfonamide antibiotics', *Water Research*, 40(12), pp. 2369–2374. doi: 10.1016/j.watres.2006.04.008.

Pourliotis, K., Karatzia, M. A., Florou-Paneri, P., Katsoulos, P. D. and Karatzias, H. (2012) 'Effects of dietary inclusion of clinoptilolite in colostrum and milk of dairy calves on absorption of antibodies against *Escherichia coli* and the incidence of Diarrhea', *Animal Feed Science and Technology*, 172(3–4), pp. 136–140. doi: 10.1016/j.anifeedsci.2011.12.001.

Sadeghi, A. A. and Shawrang, P. (2008) 'Effects of natural zeolite clinoptilolite on passive immunity and diarrhea in newborn Holstein calves', *Livestock Science*, 113(2–3), pp. 307–310. doi: 10.1016/j.livsci.2007.08.010.

Samoilovich, V. (2009) 'The use of clay in medical practice and at home' [Zastosuvannia hlyny v likuvalnii praktytsi ta v domashnikh umovakh], *Internal Medicine [Vnutrishnia medytsyna]*, 3, pp. 60–66. Available at: http://www.mif-ua.com/archive/article/10270. [in Ukrainian].

Slamova, R., Trckova, M., Vondruskova, H., Zraly, Z. and Pavlik, I. (2011) 'Clay minerals in animal nutrition', *Applied Clay Science*, 51(4), pp. 395–398. doi: 10.1016/j.clay.2011.01.005.

Szajewska, H., Dziechciarz, P. and Mrukowicz, J. (2006) 'Meta-analysis: Smectite in the treatment of Acute infectious diarrhoea in children', *Alimentary Pharmacology & Therapeutics*, 23(2), pp. 217–227. doi: 10.1111/j.1365-2036.2006.02760.x.

Tateo, F. and Summa, V. (2007) 'Element mobility in clays for healing use', *Applied Clay Science*, 36(1–3), pp. 64–76. doi: 10.1016/j.clay.2006.05.011.

Tenorio Arvide, M. G., Mulder, I., Barrientos Velazquez, A. L. and Dixon, J. B. (2008) 'Smectite clay adsorption of aflatoxin *vs.* octahedral composition as indicated by FTIR', *Clays and Clay Minerals*, 56(5), pp. 571–578. doi: 10.1346/CCMN.2008.0560510.

Theodorou, V., Fioramonti, J., Droy-Lefaix, M. T., Plique, O. and Buéno, L. (1994) 'Protective action of diosmectite treatment on digestive disturbances induced by intestinal anaphylaxis in the guinea-pig', *Alimentary Pharmacology & Therapeutics*, 8(3), pp. 295–299. doi: 10.1111/j.1365-2036.1994.tb00291.x.

Tria, Zh. M., Zherom, M. S. and Dyubuk, Zh. P. (2006) 'Therapeutic use of medicinal clays in gastroenterology' [Terapevticheskoe primenenie meditsinskikh glin v gastroenterologii]. *Russian Medical Journal [Russkiy meditsinskiy zhurnal]*, 8(1), pp. 35–38. Available at: https://www.rmj.ru/articles/bolezni_organov_pishchevareniya/Terapevticheskoe_primenenie_medicinskih_glin_v_gastroenterologii. [in Russian].

Undabeytia, T., Shuali, U., Nir, S. and Rubin, B. (2020) 'Applications of chemically modified clay minerals and clays to water purification and slow release formulations of herbicides', *Minerals*, 11(1), p. 9. doi: 10.3390/min11010009.

Wal, K., Rutkowski, P. and Stawiński, W. (2021) 'Application of clay minerals and their derivatives in adsorption from gaseous phase', *Applied Clay Science*, 215, p. 106323. doi: 10.1016/j.clay.2021.106323.

Williams, L. B. (2019) 'Natural antibacterial clays: Historical uses and modern advances', *Clays and Clay Minerals*, 67(1), pp. 7–24. doi: 10.1007/s42860-018-0002-8.

Williams, L. B. and Haydel, S. E. (2010) 'Evaluation of the medicinal use of clay minerals as antibacterial agents', *International Geology Review*, 52(7–8), pp. 745–770. doi: 10.1080/00206811003679737.

Xu, Z. R., Han, X. Y. and Wang, Y. Z. (2004) 'Effects on growth and cadmium residues from feeding cadmium-added diets with and without montmorillonite nanocomposite to growing pigs', *Veterinary and Human Toxicology*, 46(5), pp. 238–241. PMID: 15487642.

Young, S. L. and Miller, J. D. (2019) 'Medicine beneath your feet: A biocultural examination of the risks and benefits of geophagy', *Clays and Clay Minerals*, 67(1), pp. 81–90. doi: 10.1007/s42860-018-0004-6.

Part 2. Biotechnology

UDC 619:602.3:[579.864.1+579.873.13]:615.24.012 DOI 10.36016/JVMBBS-2024-10-1-4

DEVELOPMENT OF TECHNOLOGY FOR THE PRODUCTION OF SYMBIOTIC BIOLOGICALLY ACTIVE SUPPLEMENT FOR ANIMALS BASED ON *LACTOBACILLUS* AND *BIFIDOBACTERIUM*

Gujvinska S. O.

National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Kharkiv, Ukraine, e-mail: biotechvet2024@gmail.com

Summary. The study aimed to develop a technological procedure for the production of a symbiotic biologically active supplement for animals based on *Lactobacillus* and *Bifidobacterium*. Three pilot batches of the symbiotic preparation were manufactured. The technology for the production of a symbiotic biologically active supplement for animals has been developed. The scheme of the technological process of manufacturing a symbiotic is proposed: production of nutrient media and working solutions; cultivation of cultures of lacto- and bifidobacteria for the preparation of a symbiotic biological supplement for animals; freeze-drying of cultures of lacto- and bifidobacteria for a symbiotic biological supplement for animals; obtaining mass for a symbiotic biological supplement for animals; control of the finished product before release; packaging, labeling, transportation and storage of a symbiotic biological supplement for animals. The formulation of a symbiotic biologically active supplement is proposed: a mixture of freeze-dried cultures of *Lactobacillus plantarum* No. 7-317 and *Bifidobacterium adolescentis* No. 17-316 (55–65%), inulin (1.0–2.5%), lactose (1.0–2.5%), fructose (1.0–2.5%), starch (42–27.5%). The number of microbial cells per 1 cm^3 of symbiotic is lactobacilli ≥ 10^8, bifidobacteria ≥ 10^8

Keywords: *Lactobacillus plantarum* No. 7-317, *Bifidobacterium adolescentis* No. 17-316, freeze-drying

Introduction. Scientific literature indicates that an imbalance between members of the intestinal microbial community and the host organism leads to dysbiosis (Gujvinska and Paliy, 2018; Floch et al., 2011; Khyzhniak, 2017). It should be noted that the use of antibiotics, various pharmaceuticals, and chemotherapy leads to the development of diseases of the gastrointestinal tract and other organs and systems of the animal body (Gujvinska, 2015, 2019; Starovoitova et al., 2012). As a result of scientific research, veterinary medicine has been enriched with biological bacterial preparations that have proven to be effective in the prevention and treatment of gastrointestinal diseases (Roberfroid, 2000).

According to researchers, until recently, only probiotic drugs were available on the market. Today, the range of probiotic drugs is expanding (Vastano et al., 2013; Ohland and MacNaughton, 2010). Domestic medical science has not ignored probiotics and has contributed its priority materials to the world of medical science. Special merits in this area are Kovalenko N. K., Kigel N. F., Shenderov B. Ya., Pidhorskykh V. S., and others. Research institutes have published many works on probiotic drugs.

Recently, complex biological preparations based on probiotics have become increasingly popular. Many scientists claim that symbiotics are preparations containing a combination of probiotic cultures and prebiotics (Krupytska and Kaprelyants, 2016; Krupytska et al., 2018; Candela et al., 2010; Vitali et al., 2010). From the above, it can be concluded that the effect of symbiotics is the synergistic effect of living bacteria and non-living biologically active factors (Kianifar et al., 2014; Maydeo, 2010; Samuylenko et al., 2011). With the help of symbiotics, probiotic microorganisms colonize the epithelium of the gastrointestinal tract of animals, and their own microbiota is stimulated.

The work aimed to develop a technology for the production of a symbiotic biologically active supplement for animals based on *Lactobacillus* and *Bifidobacterium*.

Materials and methods. The development of technological regulations for the production of a symbiotic biologically active supplement for animals based on *Lactobacillus* and *Bifidobacterium* was carried out in the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine'. Three pilot batches of the symbiotic drug were produced. To create the symbiotic, we considered the following cultures: *Bifidobacterium bifidum* No. 3, *Bifidobacterium longum* No. 5, *Lactobacillus plantarum* No. 7-317, *Bifidobacterium adolectentis* No. 17-316, *Lactobacillus casei* No. 12. The bacteria were cultured on nutrient media in various combinations.

Comparing the level of the main biological parameters (number of microbial cells, acid formation activity, milk coagulation rate, etc.), *Bifidobacterium*

adolectentis No. 17-316 and *Lactobacillus plantarum* No. 7-317 strains were selected for the production of the symbiotic. Additional prebiotic substances are inulin, lactose, fructose, and starch.

Based on the research, a scheme for the production of a symbiotic biologically active supplement for animals was developed. Scheme of the technological process of symbiotic production: production of nutrient media and working solutions; cultivation of lacto- and bifidobacteria cultures for the preparation of a symbiotic biological additive for animals; freeze-drying of lacto- and bifidobacteria cultures for a symbiotic biological additive for animals; obtaining mass for the animal symbiotic; control of the finished product before release; labeling, packaging, transportation, storage of a symbiotic biological supplement for animals.

Lactobacilli and bifidobacteria were cultured on De Man–Rogosa–Sharpe agar (MRS agar), Blaurock medium, and skim milk for 24–48 h at a temperature 37 °C.

Microbiological control of the symbiotic was carried out at all stages of cultivation of probiotic cultures from the stage of bacterial recovery to the stage of control of the finished product. Control was performed by Gram staining of smears. Bifidobacteria and lactobacilli are colored purple.

Freeze-drying of bifidobacteria and lactobacilli was carried out on the LZ-4527 unit. The drying was performed according to the following technological regime: the temperature was increased from –72 °C to +26 °C, and the duration of drying of bacteria was from 26 h to 28 h.

The following composition of the symbiotic is proposed: dry biomass of *Lactobacillus plantarum* No. 7-317 and *Bifidobacterium adolectentis* No. 17-316, inulin, starch, lactulose, fructose. The content of lyophilized bacteria in the symbiotic supplement is not less than 10^8 CFU.

Symbiotic control was performed according to the following criteria: determination of appearance; microbiological purity (bacterioscopic control and absence of foreign microflora); harmlessness; specific activity (number of live bacteria in one dose of the drug).

Appearance and color were determined visually.

Microbiological purity was determined in accordance with DSTU 4483:2005 'Veterinary Immunobiological Preparations. Methods for Determination of Bacterial and Fungous Contamination' (DSSU, 2005). The symbiotic should not be contaminated with bacterial and fungal microflora. The symbiotic should not contain any microflora other than lacto- and bifidobacteria.

To determine the number of live microbial cells in 1 dose of the symbiotic drug, the contents of the sachet were dissolved in 0.9% sodium chloride solution at a rate of 1.0 ml per 1 dose of the drug. The number was then determined by serial dilutions of the resulting suspension in saline followed by inoculation of 0.1 cm^3 of bacteria from 10^6 dilutions onto MRS agar and Blaurock medium.

The biochemical activity was determined by a conventional method. The symbiotic was inoculated into test tubes with skim milk (inoculation dose — 0.2 cm^3 per 5 cm^3 of milk). The inoculated symbiotic should curdle the milk within 48–72 hours with the formation of a characteristic solid clot without gas puffs. A symbiotic that does not meet the requirements is rejected.

The symbiotic drug should be harmless to white mice weighing 20 ± 1 g when administered orally in an amount corresponding to one dose of freeze-dried drug. The symbiotic was dissolved in 0.9% sodium chloride solution at the rate of 0.5 ml per dose. The resulting solution was orally administered to 12 mice weighing 20 ± 1 g into the stomach (using a special nozzle on a 1 ml syringe) — 0.5 ml each. The mice were observed for 21 days.

Experiments on animals were conducted following the recommendations of the 'European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes' (CE, 1986) and Council Directive 2010/63/EU (CEC, 2010), and 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). The research program was reviewed and approved by the Bioethics Committee of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' under the current procedure.

All experiments were performed in triplicate. The results were processed by methods of variation statistics using Microsoft Excel for Windows 2010. To compare mean values Student's *t*-test was used (Van Emden, 2019).

Results and discussion. To create a symbiotic dietary supplement for animals, according to the results of preliminary studies, we have selected *Lactobacillus plantarum* No. 7-317 and *Bifidobacterium adolectentis* No. 17-316 as the most promising probiotic cultures. Additionally, the product contains prebiotics — inulin, starch, and lactulose — which accelerate, stabilize, and enhance the vital activity of lactic acid and bifidobacteria in the gastrointestinal tract.

A formulation of a symbiotic dietary supplement was developed: a mixture of freeze-dried cultures of *Lactobacillus plantarum* No. 7-317 and *Bifidobacterium adolescentis* No. 17-316 (55–65%), inulin (1.0–2.5%), lactose (1.0–2.5%), fructose (1.0–2.5%), starch (the rest). The content of lyophilized probiotic bacteria in the finished product is not less than 10^8 CFU. Three pilot batches of the symbiotic dietary supplement for animals were prepared for the experiments (Table 1).

Based on literature data and our research, the most promising sample of symbiotic No. 2 was obtained (Table 2).

Table 1 — Formulation of a symbiotic dietary supplement

Experimental series	Formulation of a symbiotic dietary supplement, %				
	Bacterial biomass	Inulin	Lactulose	Fructose	Starch
1	40.0±2.5	1.0±0.02	1.0±0.02	1.0±0.02	57.0±3.06
2	50.0±3.0	1.5±0.03	1.5±0.03	1.5±0.03	45.0±2.17
3	60.0±3.2	2.5±0.05	2.5±0.05	2.5±0.05	32.5±2.01

Table 2 — Biological characteristics of the bacteria included in the symbiotic preparation

Indicators	Bacteria that are part of a symbiotic dietary supplement	
	Lactobacillus plantarum No. 7-317	*Bifidobacterium adolescentis* No. 17-316
Number of live bacteria, CFU/cm^3	$3.7 \pm 0.12 \times 10^8$	$4.1 \pm 0.17 \times 10^9$
Acid formation activity, °T	250 ± 5	230 ± 7
Milk fermentation rate, h	18–24	18–24

Table 2 shows that in the experimental series, the number of live bacteria ranged from $3.7 \pm 0.12 \times 10^8$ CFU/cm^3 to $4.1 \pm 0.17 \times 10^9$ CFU/cm^3, and the acid formation activity ranged from 230 ± 7 °T to 250 ± 5 °T. It should be noted that the rate of milk fermentation for lactobacilli and bifidobacteria was within 18–24 h.

The next stage of our work was to develop a technology for the production of a symbiotic biologically active additive for animals. The scheme of the manufacturing technology:

Preparation of nutrient media for the cultivation of bacteria *Lactobacillus plantarum* No. 7-317 and *Bifidobacterium adolescentis* No. 17-316

↓

Sterilization of culture media 110 ± 1.0 °C for 20 min

↓

Cooling of culture media up to 30 °C

↓

Introduction of 5% daily cultures *Lactobacillus plantarum* No. 7-317 and *Bifidobacterium adolescentis* No. 17-316 in a ratio of 1:1

↓

Cultivation of bacterial mass at a temperature of 37.0 ± 1.0 °C for 48 h

↓

Freeze-drying of cultures of lacto- and bifidobacteria

↓

Obtaining mass for symbiotic biologically active supplement for animals

↓

Control of the finished product before its release

↓

Labeling, packaging, transportation, storage of symbiotic biologically active supplement for animals

During the production of a symbiotic, one of the most important conditions is the control of the technological process. A symbiotic dietary supplement for oral use is controlled by the following indicators: appearance, microbiological purity (bacterioscopic control and absence of foreign microflora); harmlessness; specific activity (number of live bacteria in one dose of the symbiotic), and acid formation activity of cultures (Table 3).

Table 3 — Standard quality indicators of symbiotic dietary supplements for animals

Indicators	Obtained results	Control methods
Description	Powder of white-cream color	Visually
Microbiological purity of a symbiotic dietary supplement	The product was not contaminated with bacterial and fungal microflora and contained only lacto- and bifidobacteria	In accordance with DSTU 4483:2005 (DSSU, 2005)
Harmlessness	Symbiotic was harmless when tested on white mice	In accordance with the TUU
Specific activity	The number of lactobacilli was ≥ 10^8, the number of bifidobacteria was ≥ 10^8.	
Biochemical activity of the drug	The diluted preparation coagulates skim milk in test tubes within 18–24 h. Incubation at a temperature of 37 ± 0.5 °C	

The test results showed that it was a white-cream-colored powder. The symbiotic dietary supplement was not contaminated with bacterial and fungal microflora, containing only lacto- and bifidobacteria. Based on the experiments, it is clear that twelve white mice weighing 20.0 ± 1.0 g remained alive and healthy for 21 days after oral administration of the diluted symbiotic dietary supplement at a dose of 0.5 cm^3. During the experiments, it was found that the inoculated symbiotic on skim milk curdled it within 18–24 h with the formation of a characteristic solid clot without gas puffs. The number of

microbial cells in 1 cm^3 of the symbiotic was: lactobacilli $\geq 10^8$, bifidobacteria $\geq 10^8$. The symbiotic dietary supplement was packed in plastic sachets, labeled, and then placed in boxes of 10. The symbiotic was stored in a dry place, protected from direct light, at a temperature between +4 °C and +8 °C.

Conclusion. The formulation of a symbiotic dietary supplement was developed: a mixture of freeze-dried cultures of *Lactobacillus plantarum* No. 7-317 and *Bifidobacterium adolescentis* No. 17-316 (55–65%), inulin (1.0–2.5%), lactose (1.0–2.5%), fructose (1.0–2.5%), starch (42–27.5%).

References

Candela, M., Maccaferri, S., Turroni, S., Carnevali, P. and Brigidi, P. (2010) 'Functional intestinal microbiome, new frontiers in prebiotic design', *International Journal of Food Microbiology*, 140(2–3), pp. 93–101. doi: 10.1016/j.ijfoodmicro.2010.04.017.

CE (The Council of Europe). (1986) *European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes*. (European Treaty Series, No. 123). Strasbourg: The Council of Europe. Available at: https://conventions.coe.int/treaty/en/treaties/html/123.htm.

CEC (The Council of the European Communities) (2010) 'Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes', *The Official Journal of the European Communities*, L 276, pp. 33–79. Available at: http://data.europa.eu/eli/dir/2010/63/oj.

DSSU [State Committee for Technical Regulation and Consumer Policy] (2005) DSTU 4483:2005. *Veterinary Immunobiological Preparations. Methods for Determination of Bacterial and Fungous Contamination [Preparaty veterynarni imunobiolohichni. Metody vyznachannia bakterialnoi i hrybkovoi kontaminatsii]*. Kyiv: Derzhspozhyvstandart Ukrainy. [in Ukrainian].

Floch, M. H., Walker, W. A., Madsen, K., Sanders, M. E., Macfarlane, G. T., Flint, H. J., Dieleman, L. A., Ringel, Y., Guandalini, S., Kelly, C. P. and Brandt, L. J. (2011) 'Recommendations for probiotic use—2011 Update', *Journal of Clinical Gastroenterology*, 45, pp. 168–171. doi: 10.1097/MCG.0b013e318230928b.

Gujvinska, S. O. (2015) 'Determination of storage stability of the probiotics' [*Vyznachennia stabilnosti probiotyka u protsesi zberihannia*], *Veterinary Medicine [Veterynarna medytsyna]*, 101, pp. 208–211. Available at: https://jvm.kharkov.ua/sbornik/101/10_61.pdf. [in Ukrainian].

Gujvinska, S. (2019) 'Study of the biological properties of lactobacilli and bifidobacteria', *4th Annual BTRP Ukraine Regional One Health Research Symposium*, Kyiv, Ukraine, 20–24 May 2019: abstr. Kyiv, p. 328.

Gujvinska, S. O. and Paliy, A. P. (2018) 'Determination of antagonistic and adhesive properties of *Lactobacterium* and *Bifidobacterium*' [Vyznachennia antahonistychnykh ta adhezyvnykh vlastyvostei laktobakterii ta bifidobakterii], *Microbiological Journal [Mikrobiolohichnyi Zhurnal]*, 80(1), pp. 36–44. doi: 10.15407/microbiolj80.01.036. [in Ukrainian].

Khyzhniak, O. S. (2017) *Development of the Composition and Biotechnology of the Multipurpose Probiotic Preparation [Rozrobka skladu ta biotekhnolohii otrymannia kompleksnoho probiotychnoho preparatu].* The dissertation thesis for the scientific degree of the candidate of pharmaceutical sciences. Kharkiv: National Pharmaceutical University. Available at: https://nrat.ukrintei.ua/searchdoc/0417U002705. [in Ukrainian].

Kianifar, H., Ahanchian, H., Grover, Z., Jafari, S., Noorbakhsh, Z., Khakshour, A., Sedaghat, M. and Kiani, M. (2014) 'Synbiotic in the management of infantile colic: A randomised controlled trial', *Journal of Paediatrics and Child Health*, 50(10), pp. 801–805. doi: 10.1111/jpc.12640.

Krupytska, L. O. and Kaprelyants, L. V. (2016) 'Fatty acid composition of dietary supplements with the inclusion of propionic acid bacteria' [Zhyrnokyslotnyi sklad biolohichno aktyvnykh dobavok z vkliuchenniam propionovokyslykh bakterii], *Visnyk of Lviv University. Biological Series [Visnyk Lvivskoho universytetu. Seriia biolohichna]*, 73, p. 442. Available at: http://nbuv.gov.ua/UJRN/VLNU_biol_2016_73_123. [in Ukrainian].

Krupytska, L. O., Kaprelyants, L. V., Kylymenchuk, O. O. and Velichko, T. O. (2018) 'Non-waste biotechnology of obtaining a symbiotic and a metabolite on the basis of *Bifidobacterium longum* – Ya 3 and *Propionibacterium shermanii* – 4' [Bezvidkhodna biotekhnolohiia otrymannia symbiotyka i metabiotyka na osnovi *Bifidobacterium longum* – Ya 3 ta *Propionibacterium shermanii* – 4], *Scientific Messenger of Lviv National University of Veterinary Medicine and Biotechnologies named after S. Z. Gzhytskyj. Series: Food Technologies [Naukovyi visnyk Lvivskoho natsionalnoho universytetu veterynarnoi medytsyny ta biotekhnolohii imeni S. Z. Gzhytskoho. Seriia: Kharchovi tekhnolohii]*, 20(85), pp. 148–154. doi: 10.15421/nvlvet8527. [in Ukrainian].

Maydeo, A. (2010) 'Lactitol or lactulose in the treatment of chronic constipation: Result of a systematic', *Journal of the Indian Medical Association*, 108(11), pp. 789–792. PMID: 21510584.

Ohland, C. L. and MacNaughton, W. K. (2010) 'Probiotic bacteria and intestinal epithelial barrier function', *American Journal of Physiology. Gastrointestinal and Liver Physiology*, 298(6), pp. 807–819. doi: 10.1152/ajpgi.00243.2009.

Roberfroid, M. B. (2000) 'Prebiotics and probiotics: Are they functional foods?', *The American Journal of Clinical Nutrition*, 71(6), pp. 1682-1687. doi: 10.1093/ajcn/71.6.1682S.

Samuylenko, A. Ya., Shkolnikov, Ye. E., Pavlenko, I. V., Rayewsky, A. A., Eremets, V. I., Skichko, N. D., Anisimova, L. V. and Andrianova, E. N. (2011) 'Development of symbiotic lysine-synthesizing preparation for growing broiler chickens' [Razrabotka simbioticheskogo preparata na osnove *E. coli* VL-613 i primenenie ego v broylernom ptitsevodstve], *Veterinary Medicine [Veterynarna medytsyna]*, 95, pp. 183–185. Available at: http://nbuv.gov.ua/UJRN/vetmed_2011_95_80. [in Russian].

Simmonds, R. C. (2017) 'Chapter 4. Bioethics and animal use in programs of research, teaching, and testing', in Weichbrod, R. H., Thompson, G. A. and Norton, J. N. (eds.) *Management of Animal Care and Use Programs in Research, Education, and Testing*. 2nd ed. Boca Raton: CRC Press, pp. 35–62. doi: 10.1201/9781315152189-4.

Starovoitova, S. O., Skrotska, O. I., Penchuk, Yu. M. and Pyroh, T. P. (2012) *The Technology of Probiotics [Tekhnolohiia probiotykiv]*. Kyiv: National University of Food Technologies. ISBN 9789666121274. Available at: https://dspace.nuft.edu.ua/handle/123456789/10384. [in Ukrainian].

Van Emden, H. F. (2019) *Statistics for Terrified Biologists*. 2nd ed. Hoboken, NJ: John Wiley & Sons. ISBN 9781119563679.

Vastano, V., Capri, U., Candela, M., Siciliano, R. A., Russo, L., Renda, M. and Sacco, M. (2013) 'Identification of binding sites of Lactobacillus plantarum enolase involved in the interaction with human plasminogen', *Microbiological Research*, 168(2), pp. 65–72. doi: 10.1016/j.micres.2012.10.001.

Vitali, B., Ndagijimana, M., Cruciani, F., Carnevali, P., Candela, M., Guerzoni, M. E. and Brigidi, P. (2010) 'Impact of a synbiotic food on the gut microbial ecology and metabolic profiles', *BMC Microbiology*, 10(1), p. 4. doi: 10.1186/1471-2180-10-4.

VRU (Verkhovna Rada Ukrainy) (2006) 'Law of Ukraine No. 3447-IV of 21.02.2006 'About protection of animals from cruel treatment' [Zakon Ukrainy № 3447-IV vid 21.02.2006 'Pro zakhyst tvaryn vid zhorstokoho povodzhennia'], *News of the Verkhovna Rada of Ukraine [Vidomosti Verkhovnoi Rady Ukrainy]*, 27, art. 230. Available at: https://zakon.rada.gov.ua/laws/3447-15. [in Ukrainian].

UDC 602.3:578.842.1:595.771 DOI 10.36016/JVMBBS-2024-10-1-5

SOME BIOLOGICAL PROPERTIES OF CHLORIRIDOVIRUS FROM *CULISETA* MOSQUITOES (DIPTERA: CULICIDAE)

Buchatsky L. P., Filenko O. M., Sutugina L. P.

Taras Shevchenko National University of Kyiv, Kyiv, Ukraine, e-mail: iridolpb@gmail.com

Summary. Two strains of mosquito iridescent viruses (*Chloriridovirus*) were isolated in Ukraine from seek larvae of bloodsucking mosquitoes *Culiseta annulata* (Schrank, 1776) and *Culiseta morsitans* (Theobald, 1901). Electron microscopic study of tissues from these larvae revealed icosahedral virus particles ranging from 180 nm to 5 nm in diameter, containing dense, pleomorphic nucleotides. Viruses were assembled in the cytoplasm within spherical virosomes. Both viruses contained DNA and 11 polypeptides with molecular weights varying from 16 kD to 98 kD. A DNA restriction analysis of both strains of mosquito chloriridovirus of the genus *Culiseta* and cloning of fragments of their genomes by genetic engineering methods was performed. Isolated strains were sensitive to ultraviolet insolation and heating and were stable to organic solvents such as ether and chloroform. Both isolated strains grew well in mosquito (*Aedes aegypti*, *Aedes pseudoscutellaris*, *Aedes albopictus*) and in Lepidoptera (*Euxoa scandens*, *Antheraea pernyi*) cell lines. A close antigenic relationship has been found between isolated strains and *Chloriridovirus* from *Aedes cantans.* Some antigenic relationship was also demonstrated between isolated strains and still unclassified iridovirus from carp (*Cyprinus carpio*). These findings imply that both strains share some similarity in structural and biochemical characteristics and belong to the *Chloriridovirus* genus of the Iridoviridae family

Keywords: electron microscopy, carp, Iridoviridae

Introduction. The family Iridoviridae consists of six genera: *Lymphocystivirus*, *Iridovirus*, *Megalocytivirus*, *Decapodiridovirus*, *Ranavirus*, and *Chloriridovirus* (Chinchar et al., 2017). The main members of the genus *Chloriridovirus* are isolated from mosquitoes, midges, and some moths (Wong et al., 2011; Piegu et al. 2013; Huang et al., 2015). The type species for the genus *Chloriridovirus* is IIV-3, which was isolated from *Aedes taeniorhinchus* larvae (Chinchar et al., 2017). Mosquito chloryridoviruses are among the most important pathogens of mosquitoes worldwide (Muttis, Micieli and García, 2023). They were found in *Aedes* mosquito larvae in Czechoslovakia (Weiser, 1965), USA (Clark, Kellen and Lum, 1965), Great Britain (Tinsley et al., 1971), Kazakhstan (Torybaev and Dubitskiy, 1971), Ukraine (Buchatsky and Sheremet. 1974), Argentina (Muttis et al., 2012), and other countries (Williams, 2008).

In Ukraine, up-to-date mosquito chloryridovirus was found among 13 species of *Aedes* mosquito larvae (Buchatsky, 1975; Buchatsky and Sherban, 1976; Buchatsky, Victorov-Nabokov and Sheremet, 1976). In addition to the genus *Aedes*, chloriridovirus was also isolated from blood-sucking mosquitoes of genera *Culex* (Buchatsky, Victorov-Nabokov and Sheremet, 1976; Muttis et al, 2012), *Psorophora* (Chapman et al., 1966), *Anopheles* (Huang et al., 2015), and from non-blood-sucking mosquito *Mochlonyx culiciformis* (Buchatsky, Victorov-Nabokov and Sheremet, 1976). The first record of chloriridovirus isolated from the genus *Culiseta* was in Ukraine (Buchatsky, Victorov-Nabokov and Sheremet, 1976; Buchatsky, 1977).

Therefore, our study aimed to compare the antigenic, ultrastructural, and other characteristics of the two strains of chloriridovirus the genus *Culiseta* and made a comparison between them.

Materials and methods. *Cell and viruses.* Mosquito tissue cultures were grown at 27 °C in medium KC-13, and lepidopteran cells — in medium JC-7, containing 5% and 10% fetal calf serum, respectively.

An isolate of mosquito chloriridovirus, obtained from *Culiseta annulata* (CaChIV) was passaged 27 times in honeycomb moth *Galleria mellonella* larvae as was described Buchatsky, Kaniuka and Lebedinets (1976). Following infection and incubation for 48 h cells and debris were removed by centrifugation and the released virus in the culture medium was stored at a temperature 4 °C.

Both strains were found in different geographical settings of the country. The *Culiseta morsitans* chloriridovirus (CmChIV) was isolated from mosquito larvae in natural small ponds in Sumy Region; CaChIV — in the vicinity of Kyiv.

Infection trials. The susceptibility of honeycomb moth larvae *Galleria mellonella* to mosquito chloriridovirus following intrahaemoceal injection (0.02 ml 10-fold serial dilutions of homogenates from infected larvae) provides a good model for infection trials. The honeycomb moth larvae were examined for the presence of the virus on 14th day of post-infection. The presence of the virus was confirmed by the method of 'colored pellets' (Buchatsky, Kaniuka and Lebedinets, 1976). Infectious titers of chloriridoviruses were counted as described in Reed and Mench (1939).

Electron microscopy. For negative contrast electron microscopy triturated tissues of both infected *Culiseta* species were adsorbed to cooper grids and negatively

stained with 2% phosphotungstic acid (PTA) adjusted to pH 6.8 with 1 M KOH.

For thin layer electron microscopy infected larvae were fixed in phosphate-buffered 2.5% glutaraldehyde, post-fixed in 1% osmium tetroxide, dehydrated in graded alcohols, and embedded in 812 Epon resin.

Ultra-thin sections were double stained in uranyl and lead citrate. Particles exhibiting a hexagonal outline were measured vertex to vertex. Examinations were performed with a JEMB-100 B electron microscope.

Purification of viruses. Both strains of mosquito chlorirodovirus were purified as described previously (Buchatsky, Kuznetsova and Prima, 1983).

Constants of sedimentation. The viruses were resuspended in 0.05 tris-HCl (pH 7.2) in different concentrations (from 0.5 mg/ml to 1 mg/ml) and centrifuged in 'MOM 3175' under 5,000 rpm at a temperature 20 °C.

Constants of sedimentation were measured as described in Bowen (1976).

Histology. Infected larvae were placed into Bouin's fixative and neutral formalin and allowed to fix for 24 h at room temperature. They were then stored at a temperature 4 °C for 1 week before being dehydrated and processed for paraffin embedding.

Tissue sections were stained with hematoxylin and eosin.

Sensitivity to the organic solvents, UV, and heat. Susceptibilities of isolated strains to organic solvents were tested after they were mixed (1:1) and incubated at a temperature 4 °C for 12 h. After this ether or chloroform were moved off and viruses were titrated on *Galleria mellonella* larvae. Susceptibility of chloriridovirus to ultraviolet was tested on viruses with a concentration of 1 mg/ml contained in Petri dishes at a distance of 100 cm from ultraviolet source (OMB-30) power 1800 WA. Infectious trials were performed as above.

Antigenic relationships. The antiserum to chloriridoviruses were prepared as described previously (Filenko, Lebedinetz and Buchatsky, 1995). Antigenic relationships were tested by using the Ouchterlony method (Bailey, 1996).

SDS-PAGE analysis of virion polypeptides. Viruses were boiled for 2 min and then subjected to SDS-PAGE on a 12% polyacrylamide (Buchatsky, Kuznetsova and Sherban, 1982). The gel was run for 5 h at 11 mA. The polypeptide bands were stained with 1% Coomassie Blue. The gel was treated twice with dimethyl sulfoxide for 30 min and then washed with water.

DNA cloning. Mosquito chloroiridovirus DNA was isolated and purified by the phenol-detergent method (Buchatsky, Kuznetsova and Prima, 1983) and by Wesley and Tuthill (1982). For the treatment of DNA preparations with EcoR1 and Msp1 restriction enzymes, an incubation mixture of 20 μl was used, containing 1 μg of chloroiridovirus DNA, a buffer solution, and 5 units of EcoR1 or Msp1 restriction enzymes. The reaction mixture was incubated at a temperature 37 °C for 2 h. To obtain fragments with a known molecular weight (markers), phage λ DNA was treated with EcoR1 and EcoRV restriction enzymes. Electrophoretic separation of DNA fragments was carried out in a 0.7% horizontal agarose gel (130×84×4 mm) at a constant current of 10 mA and a voltage of 30 V for 17–18 h until the band of bromophenol blue reaches the end of the plate. The molecular mass of DNA fragments was determined using the program Scheffer and Sederoff (1981). Cloning of *Culiseta annulata* chloroiridovirus DNA was carried out at the Pst1 site in *E. coli* bacteria. The shuttle plasmid PMK 419, which can replicate in *E. coli* and *Bacillus subtilis* (Sullivan, Yasbin and Young, 1984), was used as a vector.

Results. *Clinical signs.* Infected mosquito larvae displayed classical clinical signs associated with the iridoviral infection. In sunlight, only the thorax of infected larvae was iridescent (from bluish to violet) because of the dark natural color of larvae of these species. The larvae became violet at an advanced stage of the infection and were hardly visible against the black leaf litter at the bottom of the pond. Before death, infected larvae became sluggish and inactive. Their body was neither swollen nor had any cysts, which are common in protozoan infections found in infected tissues. Neither bacteriological nor parasitological examination yielded any significant findings.

Electron microscopy. A preliminary electron microscopical examination of crude hemolymph extracts from infected mosquitoes revealed the presence of icosahedral virions (Fig. 1).

Similar virions were also detected in various tissues from mosquitoes infected with both viral strains (Fig. 2).

When calibrated relatively tomato mosaic virus, the size of hexagonal virions was estimated as 185 ± 5 nm (corner to corner) and 165 ± 5 nm (facet to facet) in the lest compressed direction. The size and cytoplasmic position of the particles together with icosahedral capsid presumptively identified them as members of the family Iridoviridae.

In thin sections of infected larvae, no major differences were noted for each virus strain in different mosquito hosts. Within the infected cells, virions were observed in different stages of assembly (namely capsid fragments, and incomplete and complete capsids).

Histological findings. The most severely affected tissues of mosquitoes were fat bodies and hypoderma. All larvae with overt infection died before pupation. The fat bodies contain reduced supplying substances. A great number of cytoplasmic organelles were destroyed. Infected cells contain also big inclusions with oval or irregular forms.

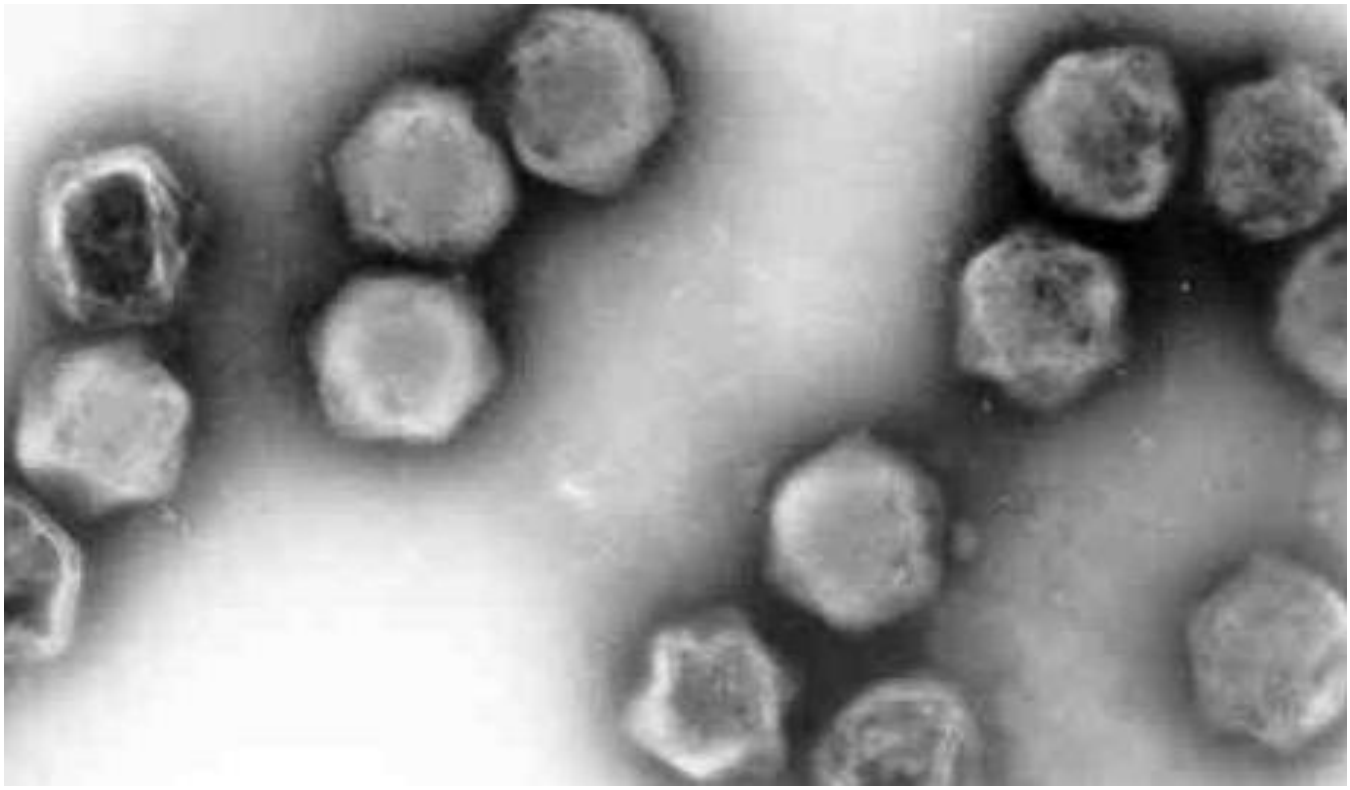

Figure 1. Virus particles of *Culiseta morsitans* chloriridovirus. Negative contrast with uranyl acetate. Virus particles range from 180 nm to 5 nm in diameter.

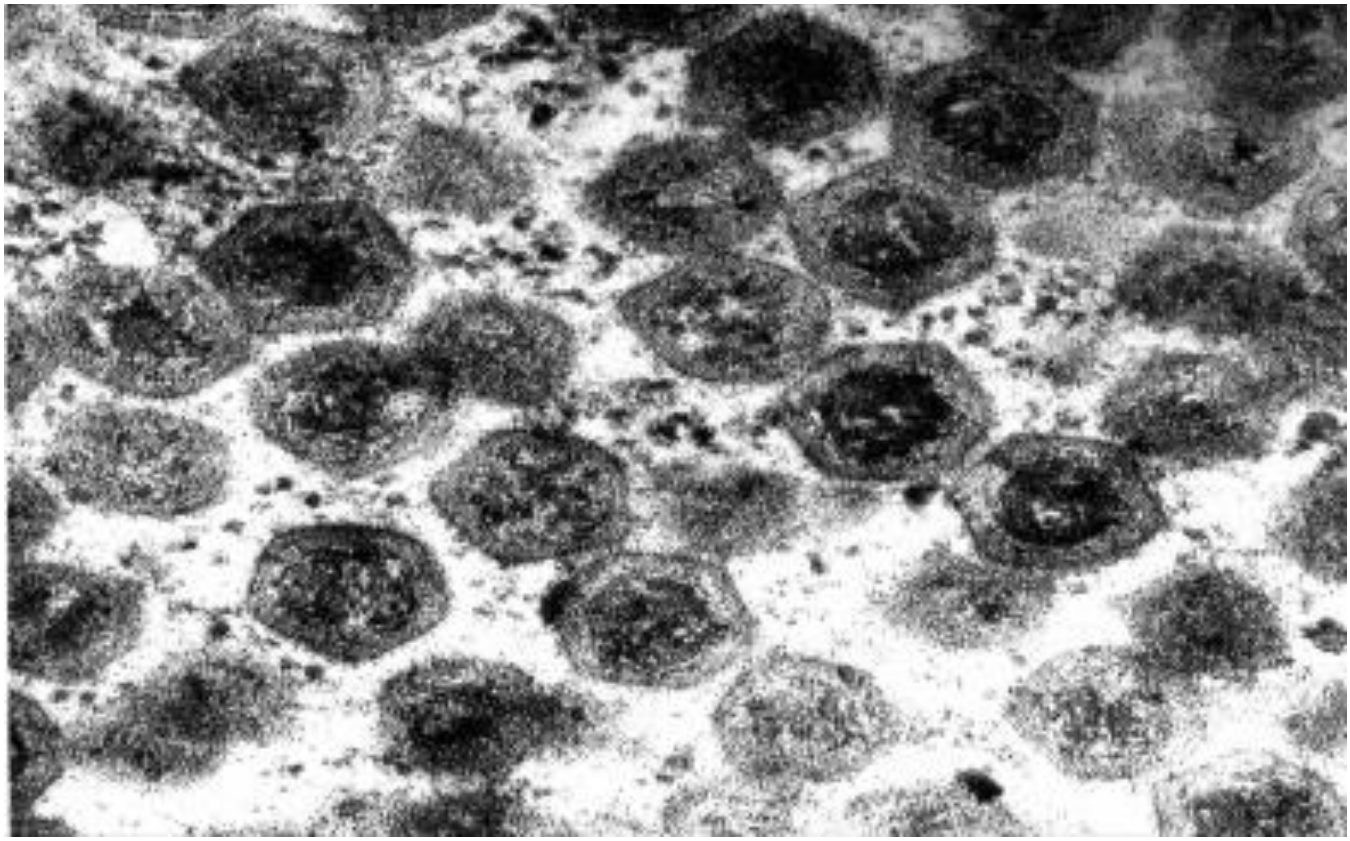

Figure 2. Chloriridovirus particles in the cytoplasm of infected fat body tissue of *Culiseta annulata*. Virus particles range from 180 nm to 5 nm in diameter.

Viral growth in cell culture. Cytopathic effect was observed in cultured *Aedes* mosquito cells 7 days after inoculation with diluted viral suspension. Small dense grayish patches were first seen in the cell's monolayer, which developed into plaques with a border formed by a compact layer of spherical cells, containing refractile cytoplasmic inclusions.

At high inoculum concentrations (> 100 ID_{50}/ml) cytopathic effect was seen in all types of cells in 2–4 h after inoculation. Well-defined syncytia were formed in the infected cells (Fig. 3).

Growth media withdrawn from the cultured cells infected with low concentrations of the virus were titrated on the honeycomb moth larvae.

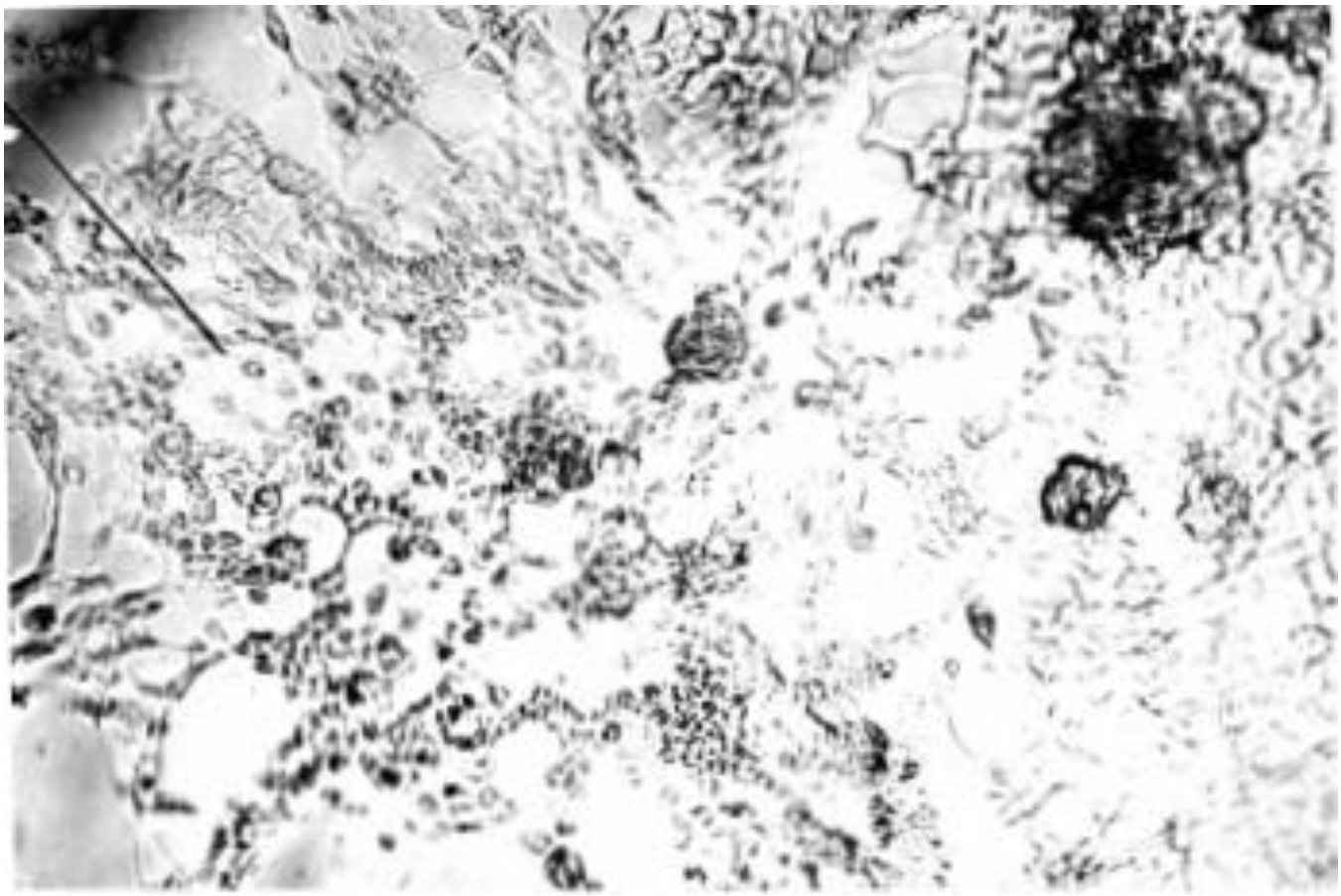

Figure 3. Cytopathic effect of *Culiseta annulata* chloriridovirus (100 ID_{50}/ml) in *Aedes aegypti* cells 4 h after inoculation.

Titration experiments revealed the reproduction of both strains of mosquito chloriridovirus in all cell lines (Table 1). Although the levels of virus reproduction were similar in all cell lines, the cytopathic effect was observed only in *Aedes aegypti* cell line.

Properties of viruses. Both strains of mosquito chloriridovirus purified from infected larvae were dark blue colored. They had the same (2,800 S) constant of sedimentation. Molecular weights were calculated by the following formula:

$$S_{20}, w = 1.114 \times 1O \times M^{0.175},$$

where S — constant of sedimentation at a temperature 20 °C,

M — molecular weight.

Nucleic acids isolated from both strains reacted positively with diphenylamine and negatively with orcin suggesting that viruses contain DNA. Isolated DNA of both strains had a constant of sedimentation 70 S, their points of melting were 86.3 °C and their buoyant density in CzCl solution was 1.7043 g/cm^3.

Table 1 — Titration of the cultural media from insect cells infected with chloriridoviruses on *Galleria mellonella* larvae (lg ID_{50}/ml)

No.	Cell lines	CmChIV	CaChIV	CPD	P
1	*Aedes aegypti*	4.7	5.0	+	> 0.05
2	*Aedes pseudoscutellaris*	5.0	4.7	–	> 0.05
3	*Aedes albopictus*	4.0	4.3	–	> 0.05
4	*Euxoa scandens*	4.3	4.2	–	> 0.05
5	*Antheraea pernyi*	4.3	4.1	–	> 0.05

Table 2 — Molecular weights (kDa) of polypeptides isolated from chloriridovirus of *Culiseta annulata*, *Culiseta morsitans* and *Aedes cantans*

CaChIV	CmChIV	AcChIV
98	98	95
89	89	87
74	74	77
50	50	62
40	40	54
36	36	36
28	28	28
25	25	22
20	20	16.5
17	17	
16	16	

Antigenic relationships. In gel immunodiffusion tests only one line of precipitation was detected in homologous as well as heterologous systems (Fig. 4). These results suggest a close antigenic relationship between *Culiseta* and *Aedes* chloridoviruses as well as between both strains of *Culiseta* viruses and chloridovirus from *Cyprinus carpio*.

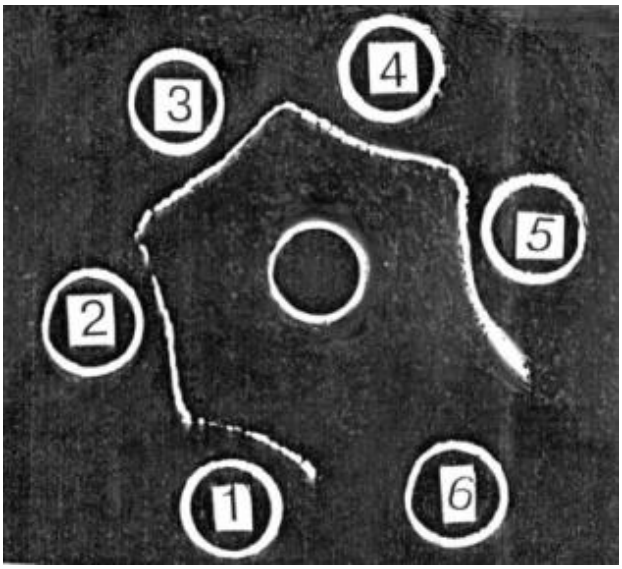

Figure 4. Antigenic relationships of two strains of *Culiseta* chloriridovirus with chloriridovirus from *Aedes* genera. Cloriridoviruses from: 1 — *Culiseta annulata*, 2 — *Culiseta morsitans*, 3 — *Aedes cantans*, 4 — *Aedes flavescens*, 5 — iridovirus from *Cyprinus carpio*, 6 — tissue homogenate from *Galleria mellonella* larvae. Central hole — antiserum to *Aedes cantans* chloriridovirus.

The results of passive agglutination also indicated that carp (*Cyprinus carpio*) iridovirus gave a positive reaction with antisera to chloriridovirus from *Aedes cantans* in titers $^1/_{16}$–$^1/_{64}$, but in homologous systems were higher titers — $^1/_{512}$–$^1/_{1,024}$.

Resistance of mosquito chloriridovirus to UV-light, heat, and organic solvents. Purified chloriridovirus from both strains lost their infectivity after 15 min exposure under UV light (Fig. 5). Non-purified chloriridovirus (supernatant of crude homogenates from infected larvae) appeared to be more resistant and lost the infectivity only after 30 min exposition.

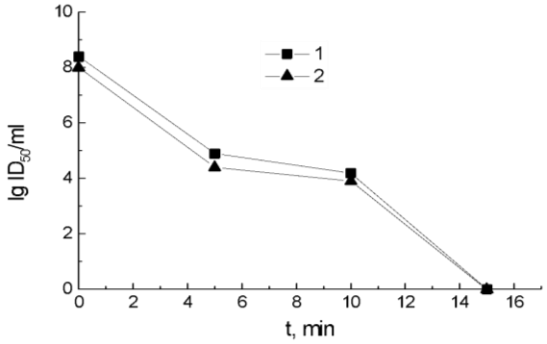

Figure 5. Influence of UV irradiation on two strains of *Culiseta* chloriridoviruses: 1 — CaChIV, 2 — CmChIV.

Both strains of *Culiseta* chloridovirus were not sensitive to ether and chloroform. Titers of their infectivity remained stable after the treatments with these organic solvents (Table 3).

Table 3 — Effect of ether and chloroform on the infectivity of chloriridoviruses from *Culiseta annulata* and *Culiseta morsitans*

Organic solvents	Infectious titers, lg ID_{50}/ml		P
	CaChIV	CmChIV	
Ether	8.9	8.8	> 0.05
Chloroform	8.8	8.9	> 0.05
Control	8.9	9.0	

One of the important characteristics of viruses is the level of their resistance to heating. We determined that both strains of *Culiseta* chloriridovirus were sensitive to high temperatures (60 °C). Incubation of viral suspension at a temperature 60 °C for 30 min resulted in a significant decrease of the infectivity, prolonged incubation (60 min) led to complete inactivation of viruses (Fig. 6).

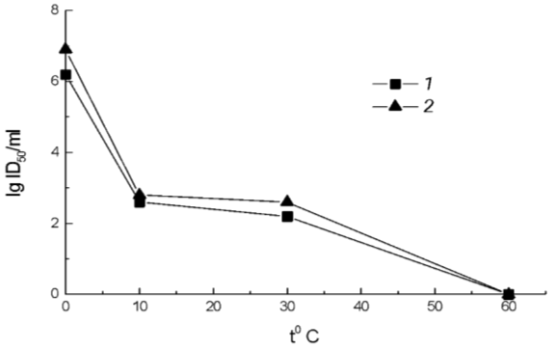

Figure 6. Infectivity of *Culiseta* chloriridoviruses at a temperature 60 °C: 1 — CaChIV, 2 — CmChIV.

The study of electropherograms of DNA fragments of chloroiridovirus from mosquitoes *Culiseta annulata* and *Culiseta morsitans* showed the identity of hydrolysis and mobility of DNA restrictions for these strains of iridoviruses. Upon hydrolysis of DNA from both strains of chloroiridovirus with EcoR1 restriction enzyme, 26 DNA fragments ranging in size from 24.7 kb to 1.7 kb were detected. After DNA treatment with Msp1 restriction enzyme, 31 fragments with sizes from 13.5 kb to 1.7 kb were detected. The sizes of the DNA of the two strains of chloroiridovirus obtained as a result of restriction analysis range from 148 kb to 154 kb, corresponding to approximately $110–115\times10^6$ Da. Thus, the results of DNA restriction analysis of chloriridovirus of *Culiseta annulata* and *Culiseta morsitans* mosquitoes allow us to conclude about a significant degree of homology of these strains of chloriridoviruses of blood-sucking mosquitoes. To clone the genome of the chloroiridovirus of *Culiseta annulata* mosquitoes, DNA was treated with Pst1 restriction enzyme. Plasmid RMK 419, which was used as a vector, had one restriction site for Pst1. Pst1 ligation of the chloroiridoviral DNA restriction and plasmid resulted in the formation of a recombinant DNA molecule that was used to transform competent *E. coli* cells. When the transformants were transferred to the medium containing IPTG and XGal, the colonies with the chloriridoviral DNA insert were colored white, and the colonies with the original plasmid were colored blue.

Treatment of the original plasmid with Pst1+Hind 111 restriction enzymes leads to the formation of two DNA bands during electrophoresis, and for the recombinant plasmid — to cleavage of the *Culiseta annulata* chloroiridovirus DNA fragment inserted at the Pst1 site and two fragments characteristic of the original plasmid. As a result of the work carried out, an insert of iridovirus DNA with 1.5 kb was isolated, which can be used in the construction of diagnostic probes.

Discussion. We report here about the biological properties of two strains of viruses isolated from the mosquito larvae of the genus *Culiseta*. The size and morphology of these viruses, the presence of lipid envelope, CPE in cultured cells from different insects, and the presence of DNA, are consistent with *Chloriridovirus* genus (Devauchelle et al., 1985). As shown (Stoltz, 1971, Wagner et al., 1973), chloriridoviruses have bigger sizes and contain fewer polypeptides in comparison with other iridoviruses of insects. Iridovirus from *Tipula paludosa* contains 28 polypeptides with molecular weight varying from 17.5 kDa to 30 kDa (Krell and Lee, 1974). Iridovirus from *Sericestis pruinosa* contains 22 polypeptides (Elliot, Lescott and Kelly, 1977), iridoviruses from *Simulium* and *Chilo* — 25 polypeptides (Devauchelle et al., 1985). In contrast, the mosquito chloriridoniruses isolated from *Aedes taeniorhinchus* or *Aedes cantans* contain only 11 (Wagner et al., 1973) and 9 polypeptides (Buchatsky, Kuznetsova and Sherban, 1982), respectively.

Our results described above show that *Culiseta* chloriridoviruses contain 11 polypeptides with molecular weights varying from 16 kDa to 98 kDa. Some of these polypeptides may possess toxic activity for cells as was shown for other iridoviruses (Cerruti and Devauchelle, 1979). We observed a toxic effect in all types of cell lines infected with large doses of *Culiseta* chloriridoviruses (> 100 ID_{50}/ml). No effect was detected for small doses of both strains of chloriridoviruses, except for *Aedes aegypti* culture.

Results of our experiments on the resistance of two strains of *Culiseta* chloriridovirus to heating are well consistent with data obtained for other iridoviruses. An increase in incubation temperature led to the loss of iridovirus infectivity (Carter, 1975).

Iridoviruses are sensitive to exposure to light (Klump, Beaumais and Devauchelle, 1983). However, some protectors may increase viral resistance to the damaging effect of UV light. Higher resistance to the UV light observed for *Culiseta* chloriridovirus maintained in crude homogenates from infected larvae in comparison with purified viral preparations suggests that host tissues (cells) may contain protective factors.

Our experiments show that both strains of *Culiseta* chloriridovirus are resistant to ether and chloroform. As hypothesized (Balange-Orange and Devauchelle, 1982) such resistance of iridoviruses to organic solvents could be a result of high content of phosphatidylinositol (acid phospholipids) found in viral capsid. Alternatively (Kelly and Vance, 1973; Kelly et al., 1979) it is speculated that the lipid bilayer is located within the virion's capsid and, therefore, is not accessible to solvents.

In our experiments, close antigenic relationships between both strains of *Culiseta* chloriridovirus, *Aedes cantans* chloriridovirus and iridovirus from carp were shown. Antigenic relationships among different genera of iridoviruses are poorly investigated. It was shown (Cunningham and Tinsley, 1968) that mosquito chloriridovirus is not antigenically related to small iridoviruses of insects from the genus *Iridovirus*. No antigenic difference was found between the two strains (R and T) of chloriridovirus from *Aedes taeniorhynchus* (Hall and Love, 1972). Antigenic relationships among small iridoviruses vary from none to close. Mosquitoes are also susceptible to invertebrate iridescent virus 6 (IIV-6) which was originally isolated from *Chilo suppressalis* (Lepidoptera: Pyralidae) (Marina et al., 1999, 2003) which is the type species for the genus *Iridovirus* (Chinchar et al., 2017).

At present no similarity between mosquito chloriridoviruses and fish iridovirus has been reported. Our previous results (Yaremenko and Buchatsky, 2003)

demonstrate that still unclassified iridovirus from carp shares many similar biological properties with chloriridoviruses and like chloriridovirus, grows well in the honeycomb moth larvae. In addition, using PCR it was shown that the chloriridovirus of mosquitoes can multiply in the body of carp (Rud and Buchatskyi, 2009). We suggest that one of the possible candidates for the role of the etiological agent of gill necrosis of carp may be mosquito chloriridovirus. We base our suggestion on the similarity in biological, structural, and antigenic properties found between two strains of mosquito *Culiseta* chloriridovirus and carp iridovirus.

Conclusions. A close antigenic relationship has been found among two isolated virus strains from *Culiseta* and *Chloriridovirus* from *Aedes cantans*. Some antigenic relationship was also demonstrated between isolated strains and still unclassified iridovirus from carp (*Cyprinus carpio*). These findings imply that both chloriridovirus strains from *Culiseta* mosquitoes share some similarity in structural and biochemical characteristics and may belong to the genus *Chloriridovirus* from the family Iridoviridae.

References

Bailey, G. S. (1996) 'Ouchterlony double immunodiffusion', in Walker, J. M. (ed.) *The Protein Protocols Handbook*. Totowa, NJ: Humana Press, pp. 749–752. doi: 10.1007/978-1-60327-259-9_135.

Balange-Orange, N. and Devauchelle, G. (1982) 'Lipid composition of an iridescent virus type 6 (CIV)', *Archives of Virology*, 73(3–4), pp. 363–367. doi: 10.1007/BF01318091.

Bowen, R. M. (1976) 'Part 1 — Theory of mixtures', in Eringen, A. C. (ed.) *Continuum Physics, Volume III: Mixtures and EM Field Theories*. New York: Academic Press, pp. 1–127. doi: 10.1016/B978-0-12-240803-8.50017-7.

Buchatsky, L. P. (1975) 'Viruses pathogenic for mosquitoes (Diptera, Culicidae)' [Virusy, patogennye dlya komarov (Diptera, Culicidae)], *Problems of Virology [Voprosy virusologii]*, 3, pp. 268–273. PMID: 240230. [in Russian].

Buchatsky, L. P. (1977) 'An iridovirus from larvae of *Culiseta annulata* and *Culex territans*', *Acta Virologica*, 21(1), pp. 85–86. PMID: 15443.

Buchatsky, L. P. and Sherban, S. D. (1976) 'Various biological properties of *Aedes cantans* irides iridescent virus' [Nekotorye biologicheskyie svoistva iridovirusa komarov iz *Aedes cantans*], *Problems of Virology [Voprosy virusologii]*, 4, pp. 432–435. PMID: 1007219. [in Russian].

Buchatsky, L. P. and Sheremet, V. P. (1974) 'Detection of iridoviruses in mosquitoes in the Ukraine' [Vyyavlenie iridovirusa komarov v Ukraine], *Problems of Virology [Voprosy virusologii]*, 2, pp. 226–228. PMID: 4446562. [in Russian].

Buchatsky, L. P., Kaniuka, V. I. and Lebedinets, N. N. (1976) 'Sensitivity of the honeycomb moth to mosquito iridescent virus' [Chuvstvitel'nost' bol'shoy voshchinnoi moli k iridovirusu komarov], *Microbiology Journal [Mikrobiolohichnyi Zhurnal]*, 38(5), pp. 605–607. PMID: 1012084. [in Russian].

Buchatsky, L. P., Kuznetsova, M. A. and Prima, V. I. (1983) 'Isolation and study of the physicochemical properties of the DNA of the iridescent virus from the mosquito *Aedes cantans*' [Izolatsiya i izuchenie DNK iz iridovirusa komarov *Aedes cantans*], *Problems of Virology [Voprosy virusologii]*, 3, pp. 355–358. PMID: 6613094. [in Russian].

Buchatsky, L. P., Kuznetsova, M. A. and Sherban, S. D. (1982) 'Investigation of mosquito iridescent virus proteins' [Izuchenie belkov iridovirusa komarov], *Microbiology Journal [Mikrobiolohichnyi Zhurnal]*, 44(6), pp. 77–79. [in Russian].

Buchatsky, L. P., Victorov-Nabokov, O. V. and Sheremet, V. P. (1976) 'New hosts of the mosquito iridescent virus in the Ukraine and in Karelia' [Novye khozyaeva iridovirusa komarov na Ukraine i v Karelii], *Microbiology Journal [Mikrobiolohichnyi Zhurnal]*, 38(4), pp. 502–505. PMID: 11396. [in Russian].

Carter, J. B. (1975) 'The effect of temperature upon *Tipula* iridescent virus infection in *Tipula oleracea*', *Journal of Invertebrate Pathology*, 25(1), pp. 115–124. doi: 10.1016/0022-2011(75)90291-8.

Cerutti, M. and Devauchelle, G. (1979) 'Cell fusion induced by an invertebrate virus', *Archives of Virology*, 61(1–2), pp. 149–155. doi: 10.1007/BF01320599.

Chapman, H. C., Clark, T. B., Woodard, D. B. and Kellen, W. R. (1966) 'Additional mosquito hosts of the Mosquito Iridescent Virus', *Journal of Invertebrate Pathology*, 8(4), pp. 545–546. doi: 10.1016/0022-2011(66)90089-9.

Chinchar, V. G., Hick, P., Ince, I. A., Jancovich, J. K., Marschang, R., Qin, Q., Subramaniam, K., Waltzek, T. B., Whittington, R., Williams, T., Zhang, Q.-Y., and ICTV Report Consortium (2017) 'ICTV Virus Taxonomy Profile: Iridoviridae', *Journal of General Virology*, 98(5), pp. 890–891. doi: 10.1099/jgv.0.000818.

Clark, T. B., Kellen, W. R. and Lum, P. T. M. (1965) 'A mosquito iridescent virus (MIV) from *Aedes taeniorhynchus* (Wiedemann)', *Journal of Invertebrate Pathology*, 7(4), pp. 519–521. doi: 10.1016/0022-2011(65)90133-3.

Cunningham, J. C. and Tinsley, T. W. (1968) 'A serological comparison of some iridescent non-occluded insect viruses', *Journal of General Virology*, 3(1), pp. 1–8. doi: 10.1099/0022-1317-3-1-1.

Devauchelle, G., Attias, J., Monnier, C., Barray, S., Cerutti, M., Guerillon, J. and Orange-Balange, N. (1985) '*Chilo* iridescent virus', in Willis, D. B. (ed.) *Iridoviridae*. (Current Topics in Microbiology and Immunology, Vol. 116). Berlin, Heidelberg: Springer, pp. 37–48. doi: 10.1007/978-3-642-70280-8_3.

Elliott, R. M., Lescott, T. and Kelly, D. C. (1977) 'Serological relationships of an iridescent virus (type 25) recently isolated from *Tipula* sp. with two other iridescent viruses (types 2 and 22)', *Virology*, 81(2), pp. 309–316. doi: 10.1016/0042-6822(77)90147-7.

Filenko, O. M., Lebedinetz, N. N. and Buchatsky, L. P. (1995) 'Antigenic affinity of mosquito iridoviruses' [Antigennoe rodstvo iridovirusov komarov], *Bulletin of Taras Shevchenko National University of Kiev. Series: Biology [Visnyk Kyivskoho natsionalnoho universytetu imeni Tarasa Shevchenka: Biolohiia]*, 26, pp. 139–146.

Hall, D. W. and Lowe, R. E. (1972) 'Physical and serological comparisons of "R" and "T" strains of mosquito iridescent virus

from *Aedes taeniorhynchu*', *Journal of Invertebrate Pathology*, 19(3), pp. 317–324. doi: 10.1016/0022-2011(72)90228-5.

Huang, Y., Li, S., Zhao, Q., Pei, G., An, X., Guo, X., Zhou, H., Zhang, Z., Zhang, J. and Tong, Y. (2015) 'Isolation and characterization of a novel invertebrate iridovirus from adult *Anopheles minimus* (AMIV) in China', *Journal of Invertebrate Pathology*, 127, pp. 1–5. doi: 10.1016/j.jip.2015.01.007.

Kelly, D. C. and Vance, D. E. (1973) 'The lipid content of two iridescent viruses', *Journal of General Virology*, 21(2), pp. 417–423. doi: 10.1099/0022-1317-21-2-417.

Kelly, D. C., Ayres, M. D., Lescott, T., Robertson, J. S. and Happ, G. M. (1979) 'A small iridescent virus (type 29) isolated from *Tenebrio molitor*: A comparison of its proteins and antigens with six other iridescent viruses', *Journal of General Virology*, 42(1), pp. 95–105. doi: 10.1099/0022-1317-42-1-95.

Klump, H., Beaumais, J. and Devauchelle, G. (1983) 'Structural and thermodynamic investigation of the *Chilo* iridescent virus (Iridovirus type 6)', *Archives of Virology*, 75(4), pp. 269–276. doi: 10.1007/BF01314892.

Krell, P. and Lee, P. E. (1974) 'Polypeptides in *Tipula* iridescent virus (TIV) and in TIV-infected hemocytes of *Galleria mellonella* (L.) larvae', *Virology*, 60(2), pp. 315–326. doi: 10.1016/0042-6822(74)90327-4.

Marina, C. F., Arredondo-Jiménez, J. I., Castillo, A. and Williams, T. (1999) 'Sublethal effects of iridovirus disease in a mosquito', *Oecologia*, 119(3), pp. 383–388. doi: 10.1007/s004420050799.

Marina, C. F., Ibarra, J. E., Arredondo-Jiménez, J. I., Fernández-Salas, I., Valle, J. and Williams, T. (2003) 'Sublethal iridovirus disease of the mosquito *Aedes aegypti* is due to viral replication not cytotoxicity', *Medical and Veterinary Entomology*, 17(2), pp. 187–194. doi: 10.1046/j.1365-2915.2003.00422.x.

Muttis, E., Micieli, M. V. and García, J. J. (2023) 'Pathology of an iridescent virus in immature *Culex pipiens* L. (Diptera, Culicidae)', *Anais da Academia Brasileira de Ciências*, 95(4), p. e20200558. doi: 10.1590/0001-3765202320200558.

Muttis, E., Miele, S. A. B., Belaich, M. N., Micieli, M. V., Becnel, J. J., Ghiringhelli, P. D. and García, J. J. (2012) 'First record of a mosquito iridescent virus in *Culex pipiens* L. (Diptera: Culicidae)', *Archives of Virology*, 157(8), pp. 1569–1571. doi: 10.1007/s00705-012-1302-2.

Piégu, B., Guizard, S., Spears, T., Cruaud, C., Couloux, A., Bideshi, D. K., Federici, B. A. and Bigot, Y. (2013) 'Complete genome sequence of invertebrate iridescent virus 22 isolated from a blackfly larva', *Journal of General Virology*, 94(9), pp. 2112–2116. doi: 10.1099/vir.0.054213-0.

Reed, L. J. and Mench, H. A. (1939) 'A simple method for estimating fifty percent end point', *American Journal of Hygiene,* 27, pp. 493–497.

Rud, Yu. P. and Buchatsky, L. P. (2009) 'Experimental infection of the carp by mosquito iridovirus' [Eksperymentalne infikuvannia koropa irydovirusom komara], *Fisheries Science of Ukraine [Rybohospodarska nauka Ukrainy]*, 1, pp. 102–106. Available at: https://fsu.ua/index.php/en/2009/1-2009-7/2009-01-102-106. [in Ukrainian].

Schaffer, H. E. and Sederoff, R. R. (1981) 'Improved estimation of DNA fragment lengths from agarose gels', *Analytical Biochemistry*, 115(1), pp. 113–122. doi: 10.1016/0003-2697(81)90533-9.

Stoltz, D. B. (1971) 'The structure of icosahedral cytoplasmic deoxyriboviruses', *Journal of Ultrastructure Research*, 37(1–2), pp. 219–239. doi: 10.1016/S0022-5320(71)80052-7.

Sullivan, M. A., Yasbin, R. E. and Young, F. E. (1984) 'New shuttle vectors for *Bacillus subtilis* and *Escherichia coli* which allow rapid detection of inserted fragments', *Gene*, 29(1–2), pp. 21–26. doi: 10.1016/0378-1119(84)90161-6.

Tinsley, T. W., Robertson, J. S., Rivers, C. F. and Service, M. W. (1971) 'An iridescent virus of *Aedes cantans* in Great Britain', *Journal of Invertebrate Pathology*, 18(3), pp. 427–428. doi: 10.1016/0022-2011(71)90051-6.

Torybaev, Kh. K. and Dubitskiy, A. M. (1971) '*Iridovirus* in blood-sucking mosquitoes in southeast Kazakhstan' [Raduzhnyy virus u krovososushchikh komarov na yugo-vostoke Kazakhstana], *Bulletin of Agricultural Science of Kazakhstan [Vestnik sel'skokhozyaystvennoy nauki Kazakhstana]*, 12, pp. 101–102. [in Russian].

Wagner, G. W., Paschke, J. D., Campbell, W. R. and Webb, S. R. (1973) 'Biochemical and biophysical properties of two strains of mosquito iridescent virus', *Virology*, 52(1), pp. 72–80. doi: 10.1016/0042-6822(73)90399-1.

Weiser, J. (1965) 'A new virus infection of mosquito larvae', *Bulletin of the World Health Organization*, 33(4), pp. 586–588. PMID: 5295003.

Wesley, R. D. and Tuthill, A. E. (1984) 'Genome relatedness among African swine fever virus field isolates by restriction endonuclease analysis', *Preventive Veterinary Medicine*, 2(1–4), pp. 53–62. doi: 10.1016/0167-5877(84)90048-5.

Williams, T. (2008) 'Natural invertebrate hosts of iridoviruses (Iridoviridae)', *Neotropical Entomology*, 37(6), pp. 615–632. doi: 10.1590/S1519-566X2008000600001.

Wong, C. K., Young, V. L., Kleffmann, T. and Ward, V. K. (2011) 'Genomic and proteomic analysis of invertebrate iridovirus type 9', *Journal of Virology*, 85(15), pp. 7900–7911. doi: 10.1128/JVI.00645-11.

Yaremenko, D. M. and Buchatskyi, L. P. (2003) 'Study of reproduction of iridovirus of gill necrosis of carp' [Izuchenie reproduktsii virusa zhabernogo nekroza karpa], *Veterinary Medicine of Ukraine* [*Veterynarna medytsyna Ukrainy*], 3, pp. 29–30. [in Russian].

Part 3. Biosafety

UDC 619:616.995.42/.7:615.285.036:636.7/.8.083.8 DOI 10.36016/JVMBBS-2024-10-1-6

EFFECTIVENESS OF MODERN ANTIPARASITIC ANIMAL COLLARS

Paliy A. P. [1], Sumakova N. V. [1], Bohach O. M. [1], Borovkov S. B. [1], Pavlichenko O. V. [1, 2], Ihnatieva T. M. [2], Dubin R. A. [3]

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

Summary. Despite the successes achieved in the prevention and control of parasitic diseases in companion animals, the issue of developing and implementing innovative, highly effective antiparasitic agents in veterinary practice is still relevant today. This study aimed to evaluate the efficacy of modern antiparasitic collars for dogs and cats in the prevention and treatment of ectoparasitic infections. The antiparasitic agents used in the experiments were 'Flea and tick collar Comfort for cats', 'Antiparasitic collar TM Healthy Pet, Oberig', 'Flea and tick collar Comfort for dogs', and 'Antiparasitic collar TM Compliment, Oberig'. The active ingredient in the collars tested is diazinon. The experimental studies were conducted under current methodological recommendations and practical guidelines. The study of shelter pets and stray animals revealed their infestation with lice, fleas, and parasitic ticks of varying intensity. Experimentally, a high insecticidal effect of the products 'Flea and tick collar Comfort for cats' and 'Antiparasitic collar TM Healthy Pet, Oberig' was established, 'Flea and tick collar Comfort for dogs', 'Antiparasitic collar TM Compliment, Oberig' against fleas (*Ctenocephalides felis*, *Ctenocephalides canis*), chewing lice (*Felicola subrostratus*), sucking lice (*Linognathus setosus*), ticks (*Ixodes ricinus*, *Dermacentor* spp., *Rhipicephalus* spp.) The effectiveness of the drugs is 100%. It has been experimentally proven that the studied collars with the active ingredient diazinon can be used for preventive and therapeutic purposes for companion animals in case of infestation with fleas, chewing lice, sucking lice, and ticks

Keywords: diazinon, dogs, cats, fleas, lice, ticks

Introduction. One of the most common diseases in pets is ectoparasitosis, which is caused by the parasitism of fleas, sucking lice, chewing lice, and ticks on the body of animals. Due to climate change, there are changes in the life of animal parasites (Bogach et al., 2020). In this regard, it is necessary to constantly monitor the distribution of parasitic insects, their species composition, etc.

To date, there are many reports on how to address this problem in different countries (Gizaw et al., 2021). A study of 200 dogs and 137 cats in Ethiopia reported that 97% of dogs and 90.5% of cats were carriers of at least one species of ectoparasite. The dominant species in both cats and dogs is *Ctenocephalides felis* (Kumsa, Abiy and Abunna, 2019). In Albania, out of 181 dogs and 26 cats examined, most were found to be carriers of ectoparasites in both mono- and mixed infections. The arthropod ectoparasite fauna of dogs included two species of ticks (*Rhipicephalus sanguineus* and *Ixodes ricinus*), three species of mites (*Sarcoptes scabiei* var. *canis, Otodectes cynotis*, and *Demodex canis*), three species of fleas (*Ctenocephalides canis, Ctenocephalides felis*, and *Pulex irritans*), and one species of chewing lice (*Trichodectes canis*) (Xhaxhiu et al., 2009). Of 120 dogs examined in the Shimoga Region of Karnataka, 59 (49.1%) had ectoparasites (Krishna Murthy, Ananda and Adeppa, 2017). The prevalence of infection in domestic animals in Iran ranges from 68.5% to 100% in some regions. The most common ectoparasites in dogs are fleas, followed by sucking lice, ticks, and flies (Ebrahimzade, Fattahi and Ahoo, 2016). In Nigeria, 92.5% of 1,041 dogs tested had one or more ectoparasites (Jajere et al., 2023).

As dogs and cats live in shared environments with humans, they are likely to be key reservoirs of pathogens that infect humans in the same environment (Colella et al., 2020). Some ectoparasites transmit serious human diseases, so regular monitoring of them is a major challenge to control arthropods and the diseases they transmit (Abdullah et al., 2019; Liodaki et al., 2022).

It has been confirmed that ectoparasites were the main vectors of plague during the second pandemic (Dean et al., 2018). They are the cause of bartonellosis (Frye et al., 2015). Some ectoparasites are vectors of disease-causing bacteria and viruses that are treated with antibiotic and antiviral drugs, ultimately contributing to antimicrobial overuse (Carvalho da Silva et al., 2023).

The urgency of the problem of ectoparasite control leads to the development and widespread introduction of various antiparasitic drugs of different forms into practical veterinary medicine (Lavan et al., 2022; Paliy et al., 2023).

Future research should explore both distinctions between, and overlap across, ectoparasite defense systems and pathogen avoidance systems, as doing so will not only illuminate proximate motivational systems, including disgust but may also reveal important clinical and social consequences (Kupfer and Fessler, 2018). The value of rotation of acaricides should be investigated for a range of compounds under field conditions (Rodriguez-Vivas, Jonsson and Bhushan, 2018).

The objective of this study was to evaluate the use of anti-parasitic collars for pets with diseases caused by ectoparasites.

Materials and methods. Studies to determine the effectiveness of antiparasitic drugs on dogs and cats were conducted at the Laboratory of Veterinary Sanitation and Parasitology of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine'. Some studies were conducted at the animal shelter in Balakliya (Kharkiv Region).

Modern domestic antiparasitic products were used in the experiments:

— 'Flea and tick collar Comfort for cats'. Polymeric tape with a fixative with a specific odor of the components (1 g of the collar contains the active ingredient diazinon — 60 mg; excipients: polyvinyl chloride, dye).

— 'Antiparasitic collar TM Healthy Pet, Oberig'. Polymeric tape (1.0 g of the collar tape contains the active ingredient diazinon — 60 mg).

— 'Flea and tick collar Comfort for dogs'. Polymeric tape with a fixative with a specific odor of the components (1 g of the collar contains the active ingredient diazinon — 60 mg; excipients: polyvinyl chloride, dye).

— 'Antiparasitic collar TM Compliment, Oberig'. Polymeric tape (1.0 g of the collar tape contains the active ingredient diazinon — 60 mg).

Clinical trials of animal collars to study the therapeutic effect were conducted in the following areas:

— clinical examination of pets, preliminary diagnosis, sampling of ectoparasites from the skin of animals for laboratory testing, constant clinical monitoring of the physiological state of experimental animals;

— microscopic examination of samples to determine pathogens of ectoparasitic diseases in biological material, their identification, and to determine the prevalence of infections in animals;

— formation of experimental groups of animals;

— application of collars, individually, according to leaflets, keeping animals in the shelter, taking samples for laboratory testing on 5th, 10th, 30th, and 45th days after the application of collars, determining the effectiveness of their action;

— daily clinical examination of the health of experimental animals throughout the experiment.

A total of 40 cats and 36 dogs of varying body weights were examined for ectoparasites. Of these, 22 cats and 16 dogs were found to have ectoparasites. The experimental animals were housed in standardized aviaries and fed an approved diet. The studies were conducted using visual and microscopic methods following established methodologies (Kumsa, Abiy and Abunna, 2019; Colella et al., 2020).

At the preliminary stage, *in vivo* diagnostics of ectoparasitoses were performed, and the number of ectoparasites was determined. Identification of ectoparasitic pathogens was performed through microscopic examination. The mean intensity was determined by counting ectoparasites per 10 cm^2 of animal skin area.

The sick animals were divided into groups that were separately administered the following drugs: 'Flea and tick collar Comfort for cats' (n = 11), 'Antiparasitic collar TM Healthy Pet, Oberig' (n = 11), 'Flea and tick collar Comfort for dogs' (n = 8), 'Antiparasitic collar TM Compliment, Oberig' (n = 8). The animals were clinically examined before, during, and after the application of the collars. The collars were applied individually to each animal, following the instructions provided.

The study recorded results after 5, 10, 20, 30, and 45 days of collar application, based on examination of the treated animals, counting live ectoparasites on them, and determining the prevalence of infection after treatment and the effectiveness of the collar.

To collect ectoparasites from animal skin, the animals were fixed in a lying position. The examination of the skin began with the head, followed by the neck, back, sides, abdomen, and limbs. During the examination, the hair was parted and combed. Any detected ectoparasites were removed from the animal's skin using tweezers. The ectoparasites that were removed were preserved in either Barbagallo's fluid (a 3% aqueous formalin solution in saline) or 70% ethanol. Some of the ectoparasites were transported to the laboratory alive in tubes or containers with damp filter paper inside. The tubes and jars were covered with a cloth and labeled.

Prevalence (P) was defined as the ratio of the number of infected animals to the number of examined animals, expressed as a percentage:

$$P = \frac{X}{Y} \times 100$$

where: X — number of animals with detected ectoparasites;

Y — total number of animals.

Mean intensity (MI) of infection was determined by the number of ectoparasites per 10 cm^2 of animal skin area.

Effectiveness of the collar was calculated by the number of treated animals in percentage that were completely free of parasites.

Experiments on animals were conducted following the recommendations of the 'European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes' (CE, 1986) and Council Directive 2010/63/EU (CEC, 2010), and 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). The research program was reviewed and approved by the Bioethics Committee of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' under the current procedure.

Results and discussions. As a result of clinical examination of animals (n = 22) housed in the shelter, 14 cats affected by ectoparasites were found, the prevalence of the infection was 63.6%. Clinical examination of the affected cats revealed redness, inflammation of the skin, a clearly visible itch reflex, papules, scales, seborrhea, and noticeable bald spots. A stable infection of cats with fleas (85.7%) was found, mixed infection with fleas and chewing lice was detected in two cats, and mixed infection with sucking lice and chewing lice in two cats (Table 1).

Table 1 — Prevalence and mean intensity of infection in cats housed in a shelter (n = 14)

Ectoparasite species	P, %	MI, insects/10 cm^2
Ctenocephalides felis	85.7	5.5 ± 2.0
Felicola subrostratus	14.3	1.5 ± 0.5
Linognathus setosus	28.6	2.0 ± 1.5

The data presented in Table 1 show that the mean intensity of infection in cats with fleas was 5.5 ± 2.0 insects per 10 cm^2 of animal skin area, sucking lice — 2.0 ± 1.5 insects per 10 cm^2 of animal skin area, and chewing lice — 1.5 ± 0.5 insects per 10 cm^2 of animal skin area.

In addition, during the clinical examination of animals (n = 18) housed in the shelter, 10 dogs with manifestations of skin lesions by ectoparasites were found, with a prevalence rate of 55.6%. Clinical examination of the affected dogs revealed redness, inflammation of the skin, a well-defined itch reflex, papules, scales, and seborrhea. Bald spots are noticeable. The skin is scratched. The study revealed the infection of dogs with fleas (60%). Mixed infection was detected in 40% of the infected animals (two dogs were infected with chewing lice, two with chewing lice and sucking lice) (Table 2).

Table 2 — Prevalence and mean intensity in dogs housed in the shelter (n = 10)

Ectoparasite species	P, %	MI, insects/10 cm^2
Ctenocephalides canis	30.0	3.5 ± 2.0
Pulex irritans	30.0	3.5 ± 2.0
Trichodectes canis	40.0	1.5 ± 0.5
Linognathus setosus	20.0	2.0 ± 0.5

The results presented in Table 2 show that dogs were infected with two species of fleas (*Ctenocephalides canis* and *Pulex irritans*) (the mean intensity of infection in dogs with fleas was 3.5 ± 2.0 insects per 10 cm^2 of animal skin area, sucking lice — 2.0 ± 0.5 insects per 10 cm^2 of animal skin area, chewing lice — 1.5 ± 0.5 insects per 10 cm^2 of animal skin area.

After diagnosis, the infected cats were divided into two groups of seven animals each. Collars were applied to the animals individually as described in the leaflets. The animals were monitored for 45 days (Table 3).

Table 3 — Collars' effectiveness against fleas and lice on cats housed in a shelter (n = 14)

Animal group	Before applying collars		After applying collars									
			5th day		10th day		20th day		30th day		45th day	
	P, %	MI, average	P, %	MI, insects/10 cm^2	P, %	MI, insects/10 cm^2	P, %	MI, insects/10 cm^2	P, %	MI, insects/10 cm^2	P, %	MI, insects/10 cm^2
'Flea and tick collar Comfort for cats' (n = 7)	100	4.5	14.3	1.5	0	0	0	0	0	0	0	0
'Antiparasitic collar TM Healthy Pet, Oberig' (n = 7)	100	4.75	14.3	2.0	0	0	0	0	0	0	0	0

After applying the collars, two cats with characteristic features of the Siamese breed in appearance in the experimental and control groups showed short-term salivation for 10 minutes. From the second day and during clinical observation of the experimental and control animals, no complications or changes in clinical condition were observed after the application of the collars.

From the second to the fifth day after the application of the collars, dead adult fleas and lice were found on the treated animals. From the 10th day, no adult fleas and lice were found on the animal bodies, and up to the 10th day, live eggs of lice were found on the animal fur. From the 10th to the 45th day, no adult fleas and lice, and live eggs of ectoparasites were found on the bodies of animals.

At the same time, we divided the dogs into two groups of five animals each. The animals of the experimental and control groups were individually fitted with collars as described in the leaflets. The animals were monitored for 45 days (Table 4).

Two dogs showed short-term salivation after wearing the collars. From the second day and during clinical observation of experimental and control animals, no complications or changes in their clinical state were observed after the application of the collars.

From the second to the fifth day after the application of the collars, dead flea and lice adults were found on the treated animals. On the 10th day, no adults of fleas and lice were found on the bodies of animals. Up to the 10th day, live eggs of lice were found on the fur of animals. From the 30th to the 45th day, no adult fleas and lice, and live eggs of ectoparasites were found on the body of animals.

Table 4 — Collars' effectiveness against fleas and lice on dogs housed in a shelter (n = 10)

Animal group	Before applying collars		After applying collars									
			5th day		10th day		20th day		30th day		45th day	
	P, %	MI, average	P, %	MI, insects/10 cm²	P, %	MI, insects/10 cm²	P, %	MI, insects/10 cm²	P, %	MI, insects/10 cm²	P, %	MI, insects/10 cm²
'Flea and tick collar Comfort for dogs' (n = 5)	100	3.5	20.0	1.5	0	0	0	0	0	0	0	0
'Antiparasitic collar TM Compliment, Oberig' (n = 5)	100	3.75	20.0	1.5	0	0	0	0	0	0	0	0

As a result of clinical examination of cats (n = 18) that moved freely in the city (animals were sterilized, chipped, and moderately nourished), a noticeable itching reflex was detected in 8 animals, with areas covered with scales on the skin. The prevalence of infection in stray cats was 44.4% (Table 5).

The study revealed a flea infection prevalence of 100% among the stray cats. Ticks were found on four cats. Mixed infection in animals amounted to 50%. The mean intensity of flea infection in stray cats was 1.5 ± 0.5 individuals per 10 cm² of animal skin area, which is lower than in animals kept indoors.

Table 5 — Prevalence and mean intensity of infection in stray cats (n = 8)

Ectoparasite species	P, %	MI, individuals/10 cm²
Ctenocephalides felis	100.0	1.5 ± 0.5
Ixodes ricinus	37.5	1.5 ± 0.5
Rhipicephalus spp.	25.0	0.7 ± 0.3
Dermacentor spp.	12.5	0.5 ± 0.3

The experimental and control groups of animals were individually applied with collars as described in the leaflets. The animals were monitored for 45 days during feeding (Table 6).

Table 6 — Collars' effectiveness against fleas and ticks on stray cats (n = 8)

Animal group	Before applying collars		After applying collars									
			5th day		10th day		20th day		30th day		45th day	
	P, %	MI, average	P, %	MI, individuals/10 cm²	P, %	MI, individuals/10 cm²	P, %	MI, individuals/10 cm²	P, %	MI, individuals/10 cm²	P, %	MI, individuals/10 cm²
'Flea and tick collar Comfort for cats' (n = 4)	100	4.5	25	1.5	0	0	0	0	0	0	0	0
'Antiparasitic collar TM Healthy Pet, Oberig' (n = 4)	100	4.75	25	2.0	0	0	0	0	0	0	0	0

From the second to the fifth day after applying the collars, dead fleas were found on the treated animals. From the fifth to 45th day, no fleas were found on the bodies of the animals. Ticks were not found on the animals for 45 days.

As a result of the clinical examination of stray dogs (n = 18) that moved freely in the city (animals were sterilized, chipped, moderately nourished), 6 animals had a clearly visible itch reflex, scales were present on the skin. The prevalence of infection in stray dogs was 33.3% (Table 7).

The study found the infection of stray dogs with fleas and ticks to be 100%. At the same time, the mean intensity of flea infection in stray dogs was 1.25 ± 0.5 insects per 10 cm^2 of animal skin area, which is lower than in animals kept indoors.

Table 7 — Prevalence and mean intensity of infection in stray dogs (n = 6)

Ectoparasite species	P, %	MI, individuals/10 cm^2
Ctenocephalides canis	76.7	1.5 ± 0.5
Pulex irritans	33.3	1.0 ± 0.5
Ixodes ricinus	100.0	2.5 ± 0.5
Rhipicephalus spp.	33.3	0.5 ± 0.3
Dermacentor spp.	16.7	0.5±0.3

The infected animals were divided into two groups of three animals each. The experimental and control groups of animals were individually collared as described in the leaflets. The animals were monitored for 45 days during feeding (Table 8).

Table 8 — Collars' effectiveness against fleas and ticks on stray dogs (n = 6)

Animal group	Before applying collars		After applying collars									
			5th day		10th day		20th day		30th day		45th day	
	P, %	MI, average	P, %	MI, individuals/10 cm^2	P, %	MI, individuals/10 cm^2	P, %	MI, individuals/10 cm^2	P, %	MI, individuals/10 cm^2	P, %	MI, individuals/10 cm^2
'Flea and tick collar Comfort for dogs' (n = 3)	100	2.5	33.3	1.5	0	0	0	0	0	0	0	0
'Antiparasitic collar TM Compliment, Oberig' (n = 3)	100	2.75	33.3	1.5	0	0	0	0	0	0	0	0

From the second to the fifth day after the application of the collars, dead fleas were found on the treated animals. After the fifth day and up to the 45th day, no fleas were found on the bodies of the animals. Ticks were not found on the animals for 45 days.

Thus, it was proved that the effectiveness of 'Flea and tick collar Comfort for cats' and 'Antiparasitic collar TM Healthy Pet, Oberig' in production conditions for cats affected by fleas, lice, and ticks was 100%. It was found that the effectiveness of the 'Flea and tick collar Comfort for dogs' and 'Antiparasitic collar TM Compliment, Oberig' in production conditions for the infection of dogs with fleas, lice, and ticks was 100%.

During our monitoring studies, we found a stable flea infection rate of 85.7% to 100% in cats. At the same time, mixed infection with fleas and chewing lice, as well as sucking lice and chewing lice, was detected in animals. The prevalence of infection in dogs ranged from 33.3% to 55.6%. The infection of the examined animals with fleas was 100%.

Other researchers have found that dogs are most often parasitized by fleas *Ctenocephalides felis* (95%), *Pulex irritans* (20.5%), *Echidnophaga gallinacea* (9%), *Xenopsylla cheopis* (0.5%), as well as ticks *Haemaphysalis leachi* (17.5%), *Amblyomma variegatum* (8.5%). A smaller percentage is represented by *Rhipicephalus sanguineus* (8%), *Rhipicephalus pulchellus* (5.5%) and *Rhipicephalus* (*Boophilus*) *decoloratus* (2.5%), as well as lice *Heterodoxus spiniger* (5%), *Linognathus setosus* (1.5%) and *Trichodectes canis* (0.5%). Along with this, fleas *Ctenocephalides felis* (61.7%), *Echidnophaga gallinacea* (24.1%), *Pulex irritans* (1.5%), *Xenopsylla cheopis* (0.7%), as well as ticks *Haemaphysalis leachi* (10.9%), *Amblyomma variegatum* (1.5%), and *Rhipicephalus sanguineus* (0.7%) (Kumsa, Abiy and Abunna, 2019).

In Albania, in dogs, the infection rate was 23.8% for *Rhipicephalus sanguineus*, 0.6% for *Ixodes ricinus*, 4.4% for *Sarcoptes scabiei* var. *canis*, 6.7% for *Otodectes cynotis*, 0.6% for *Demodex canis*, 75.7% for *Ctenocephalides canis*, 5.0% for *Ctenocephalides felis*, 8.3% for *Pulex irritans* and 6.6% for *Trichodectes canis*. Mixed infection with two or three species of ectoparasites was recorded in 38.1% of dogs. Fleas infected 75.7% of dogs, and ticks parasitized 24.3% of dogs. However, during the examination of cats, infection with only one species of ectoparasites *Ctenocephalides felis* was found in cats (Xhaxhiu et al., 2009).

Of the 59 infected domestic dogs, 22 (37.28%) were positive for fleas, 18 (30.5%) for ticks, 9 (15.2%) for lice, 7 (11.8%) for scaroptosis, and 3 (5.0%) for demodicosis. Two flea species were identified as *Ctenocephalides canis* (59%) and *Ctenocephalides felis* (41%). Tick and louse species were identified as *Riphicephalus sanguineus* and *Trichodectus canis*, respectively. The prevalence of ectoparasites was higher among stray and adult dogs compared to domestic dogs and puppies, respectively (Krishna Murthy, Ananda and Adeppa, 2017).

In Iran, arthropods isolated from domestic animals included fleas (77.5%), lice (50%), ticks (8.6%), and flies (6.8%). Among the ectoparasites of dogs, four species of fleas were found: *Ctenocephalides canis* (29.8%), *C. felis* (19.9%), *Pulex iritans* (2.9%), and *Xenopsiella cheopis* (0.7%). One species of lice, *Trichodectes canis* (41.3%), one species of tick, *Rhipicephalus sanguinus* (0.7%), and one species of fly, *Hippobosca* sp. (1.1%), were also identified (Ebrahimzade, Fattahi and Ahoo, 2016).

Both our studies and other reports have found a high species diversity and high frequency of ectoparasites on dogs and cats (Kumsa, Abiy and Abunna, 2019).

There is measurable resistance ectoparasites to most of the compounds that are commercially available, and this can be expected to increase. There is a need to develop and validate the efficacy of strategies for ectoparasite control that will delay the emergence of resistance (Rodriguez-Vivas, Jonsson and Bhushan, 2018).

Organophosphorus compounds are promising for the control of animal ectoparasites (El-Maghraby et al., 2022). An organophosphate pesticide that is widely used in agriculture for insect control and in veterinary medicine for ectoparasite control is diazinon (Jafari et al., 2012; Rahimi Anbarkeh et al., 2019; Legesse et al., 2022). The mechanism of action of diazinon is based on the inhibition of acetylcholinesterase, an enzyme necessary for the functioning of the insect nervous system. The insecticide has a half-life of 2 to 6 weeks. Diazinon promotes lipid accumulation and activates the adipogenic signaling pathway in an *in vitro* model (Smith, Yu, X. and Yin, 2018).

Our studies have proven the prospects of using antiparasitic drugs with the active ingredient diazinon when applied to pets. Our other studies have shown the high insecticidal efficacy of collars with the active ingredient fipronil (Paliy et al., 2021).

Thus, our results expand the range of existing antiparasitic collars for companion animals.

Conclusions. Based on experimental studies, it was found that 'Flea and tick collar Comfort for cats', 'Antiparasitic collar TM Healthy Pet, Oberig', 'Flea and tick collar Comfort for dogs', and 'Antiparasitic collar TM Compliment, Oberig' are well tolerated by animals and do not cause side effects or changes in the clinical state of animals.

The high insecticidal effect of the experimental collars against fleas (*Ctenocephalides felis, Ctenocephalides canis*), chewing lice (*Felicola subrostratus*), sucking lice (*Linognathus setosus*), ticks (*Ixodes ricinus*, *Dermacentor* spp., *Rhipicephalus* spp.) was established.

The effectiveness of the 'Flea and tick collar Comfort for cats', 'Antiparasitic collar TM Healthy Pet, Oberig', 'Flea and tick collar Comfort for dogs', and 'Antiparasitic collar TM Compliment, Oberig' in production conditions for the infection of pets with fleas, lice and ticks is 100%.

References

Abdullah, S., Helps, C., Tasker, S., Newbury, H. and Wall, R. (2019) 'Pathogens in fleas collected from cats and dogs: Distribution and prevalence in the UK', *Parasites & Vectors*, 12(1), p. 71. doi: 10.1186/s13071-019-3326-x.

Bogach, M. V., Paliy, A. P., Perots'ka, L. V., Pyvovarova, I. V., Stoyanova, V. Y. and Palii, A. P. (2020) 'The influence of hydro-meteorological conditions on the spread of Chicken cestodiasis', *Regulatory Mechanisms in Biosystems*, 11(3), pp. 414–418. doi: 10.15421/022063.

Carvalho Da Silva, R., Meisel, L., Farinha, N., Póvoa, O. and De Mello-Sampayo, C. (2023) 'Parasiticides: Weapons for controlling microbial vector-borne diseases in veterinary medicine; The potential of ethnobotanic/phytoparasiticides: An asset to One Health', *Antibiotics*, 12(2), p. 341. doi: 10.3390/antibiotics12020341.

CE (The Council of Europe). (1986) *European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes*. (European Treaty Series, No. 123). Strasbourg: The Council of Europe. Available at: https://conventions.coe.int/treaty/en/treaties/html/123.htm.

CEC (The Council of the European Communities) (2010) 'Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes', *The Official Journal of the European Communities*, L 276, pp. 33–79. Available at: http://data.europa.eu/eli/dir/2010/63/oj.

Colella, V., Nguyen, V. L., Tan, D. Y., Lu, N., Fang, F., Zhijuan, Y., Wang, J., Liu, X., Chen, X., Dong, J., Nurcahyo, W., Hadi, U. K., Venturina, V., Tong, K. B. Y., Tsai, Y.-L., Taweethavonsawat, P., Tiwananthagorn, S., Le, T. Q., Bui, K. L., Watanabe, M., Rani, P. A. M. A., Annoscia, G., Beugnet, F., Otranto, D. and Halos, L. (2020) 'Zoonotic vectorborne pathogens and ectoparasites of dogs and cats in eastern and southeast Asia', *Emerging Infectious Diseases*, 26(6), pp. 1221–1233. doi: 10.3201/eid2606.191832.

Dean, K. R., Krauer, F., Walløe, L., Lingjærde, O. C., Bramanti, B., Stenseth, N. Chr. and Schmid, B. V. (2018) 'Human ectoparasites and the spread of Plague in Europe during the Second Pandemic', *Proceedings of the National Academy of Sciences*, 115(6), pp. 1304–1309. doi: 10.1073/pnas.1715640115.

Ebrahimzade, E., Fattahi, R. and Ahoo, M. B. (2016) 'Ectoparasites of stray dogs in Mazandaran, Gilan and Qazvin Provinces, North and Center of Iran', *Journal of Arthropod-Borne Diseases*, 10(3), pp. 364–369. PMID: 27308294.

El-Maghraby, M., Mahmoud, A., Abdelkhalek, D., Sallam, N., Aly, A., Elaziz, A. and Soliman, E. (2022) 'Insecticidal efficacy and safety of Phoxim and influence on hematological, biochemical, and antioxidant profiles in German Shepherd dogs', *Open Veterinary Journal*, 12(6), p. 888. doi: 10.5455/OVJ.2022.v12.i6.15.

Frye, M. J., Firth, C., Bhat, M., Firth, M. A., Che, X., Lee, D., Williams, S. H. and Lipkin, W. I. (2015) 'Preliminary survey of ectoparasites and associated pathogens from Norway rats in New York City', *Journal of Medical Entomology*, 52(2), pp. 253–259. doi: 10.1093/jme/tjv014.

Gizaw, Z., Engdaw, G. T., Nigusie, A., Gebrehiwot, M. and Destaw, B. (2021) 'Human ectoparasites are highly prevalent in the rural communities of northwest Ethiopia: A community-based cross-sectional study', *Environmental Health Insights*, 15, p. 11786302211034463. doi: 10.1177/11786302211034463.

Jafari, M., Salehi, M., Ahmadi, S., Asgari, A., Abasnezhad, M. and Hajigholamali, M. (2012) 'The role of oxidative stress in diazinon-induced tissues toxicity in Wistar and Norway rats', *Toxicology Mechanisms and Methods*, 22(8), pp. 638–647. doi: 10.3109/15376516.2012.716090.

Jajere, S. M., Lawal, J. R., Shittu, A., Waziri, I., Goni, M. D. and Fasina, F. O. (2023) 'Ectoparasites of dogs (*Canis familiaris*) from northeastern Nigeria: An epidemiological study', *Parasitology Research*, 122(2), pp. 675–684. doi: 10.1007/s00436-022-07748-5.

Krishna Murthy, C. M., Ananda, K. J. and Adeppa, J. (2017) 'Prevalence of ectoparasites in dogs of Shimoga, Karnataka', *Journal of Parasitic Diseases*, 41(1), pp. 167–170. doi: 10.1007/s12639-016-0770-9.

Kumsa, B., Abiy, Y. and Abunna, F. (2019) 'Ectoparasites infesting dogs and cats in Bishoftu, Central Oromia, Ethiopia', *Veterinary Parasitology: Regional Studies and Reports*, 15, p. 100263. doi: 10.1016/j.vprsr.2019.100263.

Kupfer, T. R. and Fessler, D. M. T. (2018) 'Ectoparasite defence in humans: Relationships to pathogen avoidance and clinical implications', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 373(1751), p. 20170207. doi: 10.1098/rstb.2017.0207.

Lavan, R., Normile, D., Husain, I., Singh, A., Armstrong, R. and Heaney, K. (2022) 'An assessment of canine ectoparasiticide administration compliance in the USA', *Parasites & Vectors*, 15(1), p. 32. doi: 10.1186/s13071-021-05134-1.

Legesse, S., Hailemelekot, M., Tamrat, H. and Alemu, Y. F. (2022) 'Epidemiological and therapeutic studies on sheep lice in Sayint District, South Wollo Zone, Northeast Ethiopia', *Frontiers in Veterinary Science*, 9, p. 1008455. doi: 10.3389/fvets.2022.1008455.

Liodaki, M., Spanakos, G., Samarkos, M., Daikos, G. L., Christopoulou, V. and Piperaki, E.-T. (2022) 'Molecular screening of cat and dog ectoparasites for the presence of *Bartonella* spp. in Attica, Greece', *Acta Veterinaria Hungarica*, 70(1), p. 9–14. doi: 10.1556/004.2022.00004.

Paliy, A. P., Sumakova, N. V., Rodionova, K. O., Mashkey, A. M., Alekseeva, N. V., Losieva, Ye. A., Zaiarko, A. I., Kostyuk, V. K., Dudus, T. V., Morozov, B. S., Hurtovyi, O. O. and Palii, A. P. (2021) 'Efficacy of flea and tick collars against the ectoparasites of domestic animals', *Ukrainian Journal of Ecology*, 11(2), pp. 197–203. Available at: https://www.ujecology.com/abstract/efficacy-of-flea-and-tick-collars-against-the-ectoparasites-of-domestic-animals-70487.html.

Paliy, A. P., Sumakova, N. V., Bohach, O. M., Bogach, M. V., Pavlichenko, O. V., Ihnatieva, T. M. and Dubin, R. A. (2023) 'Assessing the efficacy of antiparasitic sprays', *Journal for Veterinary Medicine, Biotechnology and Biosafety*, 9(3), pp. 6–10. doi: 10.36016/JVMBBS-2023-9-3-2.

Rahimi Anbarkeh, F., Nikravesh, M. R., Jalali, M., Sadeghnia, H. R. and Sargazi, Z. (2019) 'The effect of diazinon on cell proliferation and apoptosis in testicular tissue of rats and the protective effect of vitamin E', *International Journal of Fertility and Sterility*, 13(2), pp. 154–160. doi: 10.22074/ijfs.2019.5612.

Rodriguez-Vivas, R. I., Jonsson, N. N. and Bhushan, C. (2018) 'Strategies for the control of *Rhipicephalus microplus* ticks in a world of conventional acaricide and macrocyclic lactone resistance', *Parasitology Research*, 117(1), pp. 3–29. doi: 10.1007/s00436-017-5677-6.

Simmonds, R. C. (2017) 'Chapter 4. Bioethics and animal use in programs of research, teaching, and testing', in Weichbrod, R. H., Thompson, G. A. and Norton, J. N. (eds.) *Management of Animal Care and Use Programs in Research, Education, and Testing*. 2nd ed. Boca Raton: CRC Press, pp. 35–62. doi: 10.1201/9781315152189-4.

Smith, A., Yu, X. and Yin, L. (2018) 'Diazinon exposure activated transcriptional factors CCAAT-enhancer-binding proteins α (C/EBPα) and peroxisome proliferator-activated receptor γ (PPARγ) and induced adipogenesis in 3T3-L1 preadipocytes', *Pesticide Biochemistry and Physiology*, 150, pp. 48–58. doi: 10.1016/j.pestbp.2018.07.003.

VRU (Verkhovna Rada Ukrainy) (2006) 'Law of Ukraine No. 3447-IV of 21.02.2006 'About protection of animals from cruel treatment' [Zakon Ukrainy № 3447-IV vid 21.02.2006 'Pro zakhyst tvaryn vid zhorstokoho povodzhennia'], *News of the Verkhovna Rada of Ukraine [Vidomosti Verkhovnoi Rady Ukrainy]*, 27, art. 230. Available at: https://zakon.rada.gov.ua/laws/3447-15. [in Ukrainian].

Xhaxhiu, D., Kusi, I., Rapti, D., Visser, M., Knaus, M., Lindner, T. and Rehbein, S. (2009) 'Ectoparasites of dogs and cats in Albania', *Parasitology Research*, 105(6), pp. 1577–1587. doi: 10.1007/s00436-009-1591-x.

Contents

Part 1. Veterinary medicine

Part 2. Biotechnology

Part 3. Biosafety