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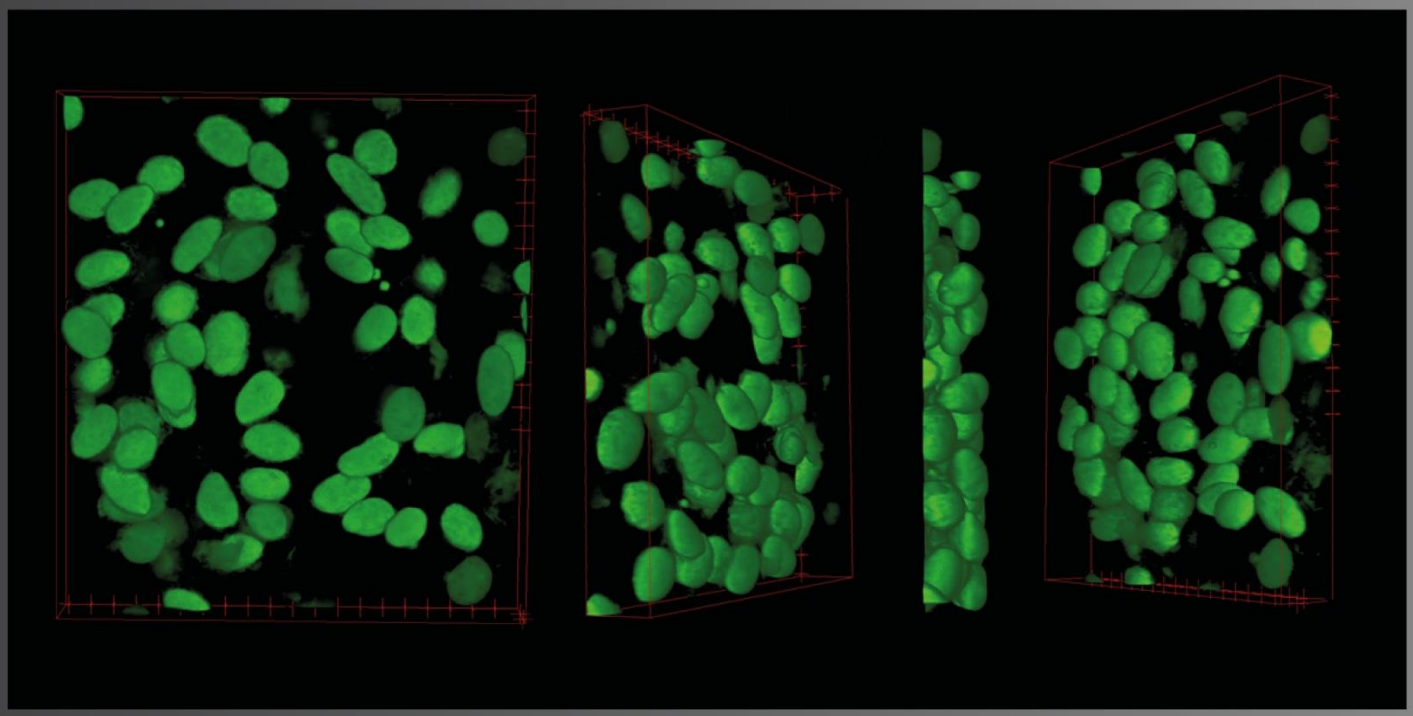

Confocal laser scanning microscopy of a fragment of the construct stained with SYTOX® Green.
Three-dimensional reconstruction was performed using ImageJ (Rasband, 1997–2018)
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ДОНСКОЙ ГОСУДАРСТВЕННЫЙ ТЕХНИЧЕСКИЙ УНИВЕРСИТЕТ

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«Ветеринарная патология» — рецензируемый научно-практический журнал, в котором публикуются результаты оригинальных исследований и обзорные статьи в области ветеринарной медицины. Освещаются вопросы паразитологии, физиологии, фармакологии, экологии, уделяется внимание инфекционным болезням и другим аспектам ветеринарии домашних, сельскохозяйственных и диких животных.

Цель журнала заключается в повышении профессионального уровня знаний профильной аудитории и распространении по всему миру высокоэффективных, тщательно проверенных ветеринарных научных исследований, используя только онлайн-формат открытого доступа для максимальной доступности.

Журнал включен в перечень рецензируемых научных изданий, в котором должны быть опубликованы основные научные результаты диссертаций на соискание ученой степени кандидата наук, на соискание ученой степени доктора наук (Перечень ВАК) по следующим научным специальностям:

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Original Empirical Research

Trichinellosis in Badgers in the Rostov Region (Southern Russia)

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Abstract.

Introduction. Nematodes of the genus *Trichinella* are the causative agents of trichinellosis, which affects humans and animals and causes significant economic damage to agricultural and food industries, especially with regard to food safety. Currently, the genus *Trichinella* includes 10 species and 3 genotypes, which are divided into 2 clades: encapsulated and non-encapsulated. In the wildlife, a wide range of animals participate as carriers in circulation of Trichinella, and badgers are one of the main natural reservoirs for this disease. The article provides the information on detection of the first case of Trichinella larvae in a badger in the Rostov region.

Materials and Methods. The object of the study was a carcass of a badger hunted in the Sholokhov district of the Rostov region in August 2024. Detection with further study of larvae and capsules in the samples was carried out by the method of compressor trichinelloscopy. Afterwards, histological sections were made from the individual muscles, which were stained with hematoxylin and eosin according to the standard technique.

Results. Capsules of Trichinella were found in all the examined muscles of an animal. The largest number of them was found in the diaphragm and its crura. The larvae found belonged to the encapsulated ones and were located in capsules in groups or one at a time. Histological sections showed thick collagen shells of the capsules surrounded from the outside by thin membranes of connective tissue. The results of capsule morphometry showed that their size didn't differ depending on their location in the badger's body; the shape of the capsules in all muscles was almost round.

Discussion and Conclusion. The capsule shape indices calculated based on the morphometric results are closer to the corresponding indices of *T. nativa* than *T. spiralis*, however, this does not allow us to make fully accurate conclusions about the species membership of Trichinella, since the molecular genetic studies or the use of Western blot are required for precise diagnostics. Thus, the study of Trichinella species distribution and composition remains an extremely relevant objective that requires further research both in our region and worldwide.

Keywords: *Trichinella*, *Meles meles*, trichinellosis, morphometry, larvae, capsules

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Оригинальное эмпирическое исследование

Трихинеллез у барсука в Ростовской области (юг России)

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Аннотация

Введение. Нематоды рода *Trichinella* — возбудители трихинеллеза, поражающего людей и животных и наносящего значительный экономический ущерб сельскому хозяйству и пищевой промышленности, особенно в сфере безопасности продуктов питания. В настоящее время род *Trichinella* включает 10 видов и 3 генотипа, которые разделяются на 2 клады: капсулообразующие и бескапсульные. Циркуляция трихинелл в дикой природе происходит при участии обширного круга животных-трихинеллоносителей, и барсук является одним из основных природных резервуаров заболевания. В статье приводятся сведения о первом случае обнаружения личинок трихинелл у барсука в Ростовской области.

Материалы и методы. Объектом исследования послужила туша барсука, добытая охотниками в Шолоховском районе Ростовской области в августе 2024 г. Выявление и изучение личинок и капсул в образцах проводили методом компрессорной трихинеллоскопии. В дальнейшем из отдельных мышц были изготовлены гистологические срезы, которые окрашивались гематоксилином и эозином по стандартной методике.

Результаты исследования. Капсулы трихинелл были обнаружены во всех исследованных мышцах животного. Наибольшее их количество найдено в диафрагме и ее ножках. Обнаруженные личинки относились к инкапсулирующим и располагались в капсулах группами или по одной. На гистологических срезах капсулы имели толстые коллагеновые стенки, окруженные снаружи тонкими соединительнотканными оболочками. Результаты морфометрии капсул показали, что они не отличаются по размерам в зависимости от локализации в теле барсука, форма капсул во всех мышцах была почти круглой.

Обсуждение и заключение. Индексы формы капсул, рассчитанные по результатам морфометрии, более близки к соответствующим показателям *T. nativa*, чем *T. spiralis*, однако это не позволяет делать точные выводы относительно видовой принадлежности трихинелл, поскольку для точной диагностики необходимы молекулярно-генетические исследования или использование вестерн-блота. Таким образом, изучение распространения и видового состава трихинелл по-прежнему остается крайне актуальной задачей, требующей дальнейших исследований как в нашем регионе, так и по всему миру.

Ключевые слова: *Trichinella*, *Meles meles*, трихинеллез, морфометрия, личинки, капсулы

Благодарности. Авторы статьи выражают благодарность охотникам, предоставившим для исследования тушу барсука. Также авторы признательны М.С. Капле (ДГТУ) за изготовление гистологических срезов.

Для цитирования. Евсюков А.П., Потапенко И.О., Зеленков А.П., Зеленкова Г.А. Трихинеллез у барсука в Ростовской области (юг России). *Ветеринарная патология*. 2025;24(3):7–13. https://doi.org/10.23947/2949-4826-2025-24-3-7-13

Introduction. Nematodes of the genus *Trichinella* of the family Trichinellidae, are the causative agents of trichinellosis, the intestinal and tissue type of helminthiasis deemed to be one of the most serious and widespread zoonotic infections threatening human health [1]. In humans, this disease has been reported in 55 countries worldwide [2]. Apart from its negative impact on public health, trichinellosis causes significant economic damage to agricultural and food industries, particularly with regard to food safety.

Trichinella have a simple yet unique life cycle. The enteric phase begins with the consumption of meat containing tissue parasite capsules. Larvae are released from the capsules due to gastric acid activity and, within a few hours, migrate to the small intestine, where they penetrate into the

columnar epithelium. There, they undergo four molts to mature and begin mating 30 hours after infestation. On the fourth day, newborn larvae migrate through the blood-vascular system to the striated muscles and penetrate into muscle cells. Here, encapsulated species form collagen capsules and develop into an invasive form after 15–20 days [3]. Larvae of non-encapsulated species are likely to migrate between muscle layers [4]. Thus, a characteristic feature of the Trichinella life cycle can be identified: any animal they parasitize in becomes for them both a definitive and intermediate host.

At present, the genus *Trichinella* includes 10 species and 3 genotypes having no taxonomic status. All known species and genotypes are clearly divided into 2 clades. One clade includes Trichinella that encapsulate in the muscle tissue of a host and are capable of infecting mammals. It includes *T. spiralis, T. nativa, T. britovi, T. murrelli, T. nelsoni, T. patagoniensis, T. chanchalensis* and genotypes designated as T6, T8 and T9. The second clade includes three species of Trichinella that do not encapsulate: among them, one species infests mammals and birds (*T. pseudospiralis*); and two species parasitize in mammals and reptiles (*T. papuae and T. zimbabwensis*) [3, 5]. All of the above-mentioned species and genotypes of Trichinella pose danger to both animals and humans. Primarily one species was previously reported in the Rostov Region: *T. spiralis* [e.g., 6]. In the Krasnodar region, cases of finding the non-encapsulated species *T. pseudospiralis* in pigs have been recorded [7].

In the wildlife, a wide range of animals participate as carriers in circulation of Trichinella, which includes approximately 100 diverse species of carnivorous and omnivorous animals [8]. A badger (Meles meles) is one of the main natural reservoirs of trichinellosis [9]. In the Rostov Region, as well as in the whole country, badgers are often the main prey for hunters. According to data published on the website of the Ministry of Natural Resources and Environment in July 2024[1], the population of badgers in the Rostov Region equals to 2 556 specimens, and the authorised quota for hunting is 3.91% of the population. Although this is quite a high level, poaching is still taking place. As a result, the uninspected meat can be consumed by humans or animals, which can lead to their infectation with trichinellosis.

In the article, we report the first case of detecting Trichinella larvae in a badger in the Rostov Region.

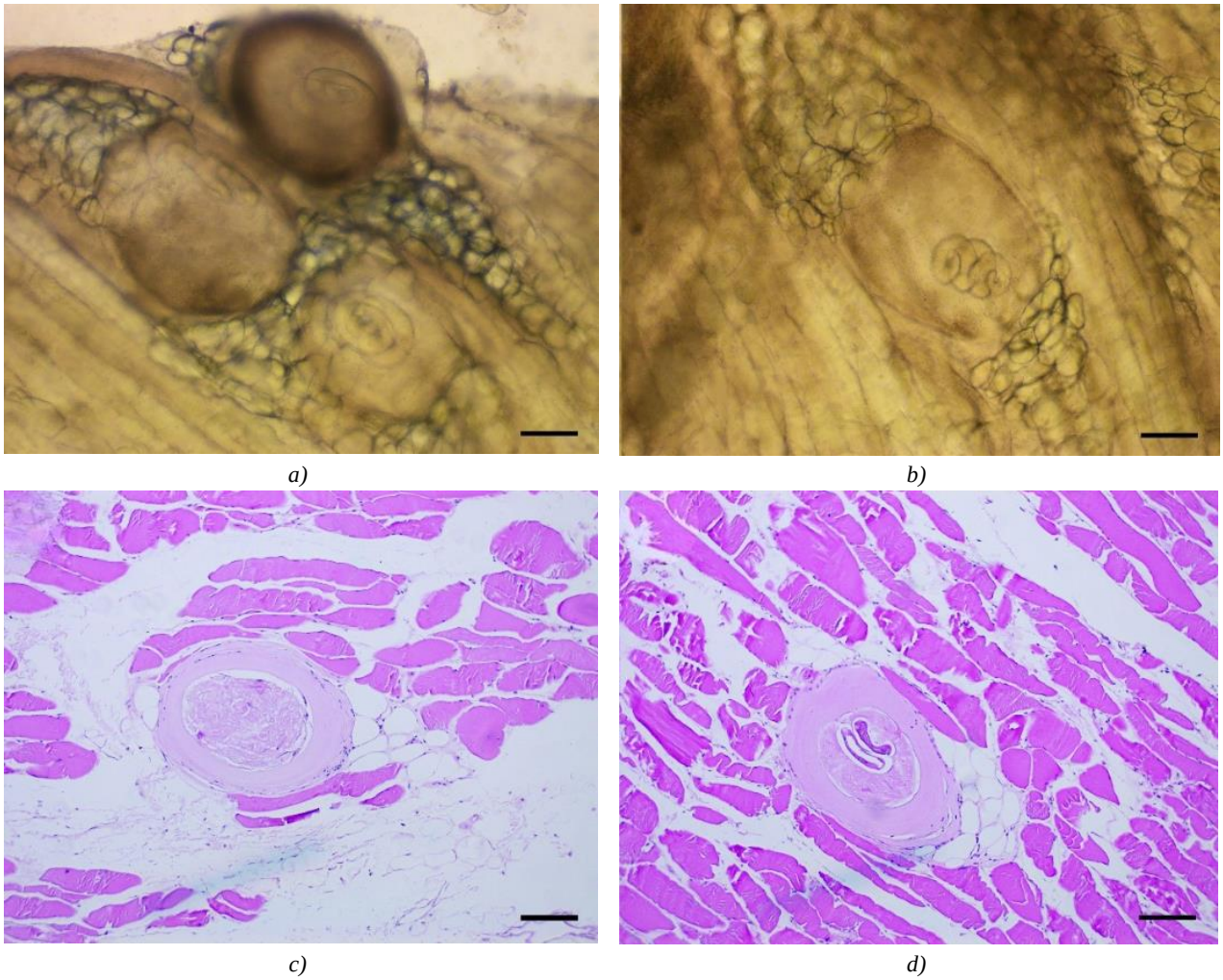
Materials and Methods. The object of the study was a carcass of a badger hunted on the territory of the Veshensky Production and Experimental Hunting Area (Sholokhov District, northern part of Rostov Region) in August 2024. Various muscle groups were sampled, including the crura of the diaphragm, diaphragm, intercostal muscles, neck muscles, and thigh muscles. Larvae and capsules in these samples were isolated and examined using compressor trichinoscopy. Histological sections were then prepared from seperate muscles and stained with hematoxylin and eosin according to the standard techniques. Capsules in native samples were measured and photographed using a Levenhuk MED D45 microscope (Levenhuk Inc., China) and LevenhukLite software. The capsule shape index (V) was calculated as the ratio of the capsule length (L) to its diameter (D). This index is used to evaluate the shape of round objects, including Trichinella capsules [10, 11**].**

Results. Trichinella capsules were found in all the examined muscles of an animal. The largest number was found in the diaphragm and its crura. It has been acknowledged in the previous studies that the most heavily infested muscles in badgers are the head muscles, particularly the mylohyoid muscle [12]. However, these muscles were not within the scope of the present research.

The larvae discovered belong to the encapsulated ones. They were located in capsules in groups or one at a time (Fig. 1 *a–b*). Histological sections revealed that the capsules had thick shells covered by thin connective tissue membranes formed by the organism of a host (Fig. 1 *c–d*).

Based on the capsule morphometric data obtained (Table 1), it is possible to state that capsule location in the badger's body does not affect its size to any significant extent. The shape of capsules in all the muscles was nearly round (V≈0.79).

[1] Directive of the Governor of the Rostov Region of July 17, 2024 No. 175 "On approval of quota for obtaining hunting resources in the Rostov Region for the period from August 1, 2024 to August 1, 2025". URL: https://минприродыро.рф/upload/uf/5b8/n4ub6dzirqjhmt4tivn963j39r4zpdks/limity.pdf (accessed: 05.06.2025 г.).



a) *b)* *c)* *d)*

Fig. 1. Trichinella capsules with larvae in muscles of a badger: *a–b* — native preparation; *c–d* — histological sections stained with hematoxylin and eosin. Scale: 0.1 mm

Table 1

Morphometric parameters of Trichinella capsules in various muscles of a badger

Muscles examined	Number of capsules	Capsule diameter (D), mm	Capsule length (L), mm	Shape index (V)
Crura of the diaphragm	20	0,441±0,010	0,349±0,008	0,797±0,020
Diaphragm	20	0,438±0,007	0,342±0,020	0,791±0,010
Intercostal muscles	20	0,440±0,009	0,348±0,010	0,790±0,008
Neck muscles	20	0,438±0,011	0,349±0,015	0,798±0,012
Thigh muscles	10	0,437±0,020	0,350±0,011	0,801±0,020

Discussion and Conclusion. The capsule shape indices calculated based on the morphometric results correspond more to the respective indices of *T. nativa* ratether than *T. spiralis* [11]. The former species used to be registered in the Central Russia, but has not been previously observed in the south of the country or in the Caucasus [13]. The –4 °C isotherm in January is likely to be a southern boundary for this species [4], which makes the possibility of finding this species in the Rostov Region very low. However, it is impossible to draw precise conclusions regarding the species membership of Trichinella detected by us in the badger, since the molecular genetic studies [14] or the use of Western blot technique [15] are required for a reliable diagnosis. Moreover, it is worth noting that most Trichinella species have a sympatric distribution [3]. Thus, in the Rostov region there could potentially be detected two encapsulated species (*T. spiralis* — reported here before and distributed worldwide, and *T. britovi* —disseminated from the Iberian Peninsula to Kazakhstan, Turkiye and Iran [4]), and one non-encapsulated species — *T. pseudospiralis* registered in North America, Eurasia and Tasmania [16]. No evident dependence of different Trichinella species to host animals has been revealed [17], however, different invasion rate by different Trichinella species have been recorded during experimental infestation [18]. All the above makes species identification of Trichinella extremely difficult.

Thus, studying the distribution and species composition of Trichinella remains a highly relevant objective, requiring further research both in south of Russia and worldwide. Badger meat must undergo mandatory trichinoscopy to prevent potential infestations in humans. Unused carcasses and their remains must be disposed of to exclude infestation of domestic and wild animals.

References

1. Dubinsky P, Antolova D, Reiterova K. Human *Trichinella* Infection Outbreaks in Slovakia, 1980–2008. *Acta Parasitologica*. 2016;61:205–211. https://doi.org/10.1515/ap-2016-0029

2. Pozio E. World Distribution of *Trichinella* Spp. Infections in Animals and Humans. *Veterinary Parasitology*. 2007;149(1–2):3–21. https://doi.org/10.1016/j.vetpar.2007.07.002

3. Zarlenga D, Thompsona P, Pozio E. *Trichinella* Species and Genotypes. *Research in Veterinary Science*. 2020;133:289–296. https://doi.org/10.1016/j.rvsc.2020.08.012

4. Pozio E, Zarlenga DS. Recent advances on the taxonomy, systematics and epidemiology of *Trichinella*. *International Journal for Parasitology*. 2005; 35(11–12):1191–1204. https://doi.org/10.1016/j.ijpara.2005.07.012

5. Pozio E. *Trichinella* and Trichinellosis in Europe. *Veterinarski Glasnik*. 2019;73(2):65–84. https://doi.org/10.2298/VETGL190411017P

6. Tverdokhlebova TI, Dumbadze OS, Ermakova LA, Kovalev EV, Nenadskaya SA, Guzeeva TM, et al. Epidemiological Aspects of Trichinellosis in the South of Russia. In: *Proceedings of the Scientific and Practical Conference "Topical Issues in the Study of Especially Dangerous and Natural Focal Diseases"*. Novosibirsk: "Prodvizhenie" Publ.; 2019. P. 125–130. (In Russ.)

7. Sapunov AYa. *Improving Measures of Combating Trichinellosis in the North-West Region of the Caucasus.* Extended Abstract of Dr.Sci. (Veterinary Sciences) Dissertation. Moscow; 2000. 48 p. (In Russ.).

8. Tverdokhlebova TI, Khutoryanina IV, Chernikova MP. Epidemiological Aspects of Trichinellosis. *Natsional'nye Prioritety Rossii (National priorities of Russia).* 2021;(3(42)):279–282. (In Russ.)

9. Gorodovich NM, Gorodovich SN. *Fighting against Trichinellosis in the Wildlife: Methodological Guidelines.* Blagoveshchensk: Far Eastern Zonal Research Veterinary Institute; 2009. 7 p. (In Russ.)

10. Romashov BV, Vasilenko VV, Rogov MV. *Trichinellosis in the Central Black Earth Region (Voronezh Region): Ecology and Biology of Trichinella, Epizootology, Prevention and Monitoring of Trichinellosis.* Voronezh: Voronezh State University; 2006. 181 p. (In Russ.)

11. Andreyanov ON. Comparative Morphology of Capsules of Larvae Trichinella from Different Types of Hosts. *Actual Questions of Veterinary Biology*. 2014;(2(22)):27–29. (In Russ.)

12. Trukhina TI, Solovieva IA, Bondarenko GA, Ivanov DA. Peculiarities of Trichinella Larvae Distribution in Badgers' Muscles that Inhabit in Amur Region. *Bulletin of NSAU (Novosibirsk State Agrarian University).* 2019;1(50):171–176. (In Russ.) https://doi.org/10.31677/2072-6724-2019-50-1-171-176

13. Andreyanov ON, Konyaev SV. Causative Agents of Trichinosis in the Central Region of Russia. In: *Proceedings of the All-Russian Scientific Conference with International Participation "Modern Problems of Parasitology and Ecology. Readings Dedicated to the Memory of S.S. Shulman"*. Tolyatti: "Anna" Publ.; 2018. P. 28–31. (In Russ.)

14. Konyaev SV, Krivopalov AV, Yanagida T, Nakao M, Sako Ya, Ito A, et al. The Molecular Genetic Investigation of Trichinella Spp. in Russia: First Results. In: *Proceedings of the International Scientific Conference "Modern Problems of General Parasitology".* Moscow: A.N. Severtsov Institute of Ecology and Evolution of RAS Publ.; 2012. P. 171–174. (In Russ.)

15. Gómez-Morales MA, Ludovisi A, Amati M, Cherchi S, Tonanzi D, Pozoi E. Differentiation of *Trichinella* Species (*Trichinella Spiralis*/*Trichinella Britovi* versus *Trichinella Pseudospiralis*) Using Western Blot. *Parasites & Vectors*. 2018;11:631. https://doi.org/10.1186/s13071-018-3244-3

16. Pozio E, Zarlenga DS. New Pieces of the *Trichinella* Puzzle. *International Journal for Parasitology*. 2013;43(12–13):983–997. https://doi.org/10.1016/j.ijpara.2013.05.010

17. Pozio E. Searching for *Trichinella*: Not All Pigs are Created Equal. *Trends in Parasitology*. 2014;30(1):4–11. https://doi.org/10.1016/j.pt.2013.11.001.

18. Garkavi BL. Trichinellosis Caused by Trichinella Pseudospiralis (Morphology and Bio-Logy, Epizootology and Epidemiology, Diagnostics, Control Measures and Preventive Maintenance). *Russian Journal of Parasitology.* 2007;(2):35–116. (In Russ.)

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Original Empirical Research

Anatomical and Morphological Characteristics of Burmese Python (*Python Molurus Bivittatus*) Raised in Captivity and Pathological Changes in It

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Abstract

Introduction. The Burmese python (*Python Molurus Bivittatus*) is the third largest snake species in the world. In the wildlife, this snake breed is most often found in the countries of South and Southeast Asia, however, in recent years, these beautiful and docile reptiles have become popular around the world as pets. Burmese pythons are kept and bred by specialists in zoos and at zoo exhibitions as well as by amateurs. For keeping at home, it is recommended to purchase a specimen born and raised in captivity and create for it conditions that imitate its natural habitat. Pythons originate from the tropical and subtropical zones, therefore, when kept at home, high air humidity should be maintained, and a water reservoir should be provided for the semi-aquatic reptiles to bathe, which is especially important for correct snake molting. Unfortunately, more and more often these exotic animals become patients of veterinary clinics or die due to various reasons. The aim of the study is to establish the anatomical and morphological characteristics of Burmese python specimens raised in captivity, as well as pathological changes in them upon death.

Materials and Methods. In 2024–2025, two corpses of Burmese python (male and female) from a private collection were delivered to “Khimera” veterinary clinic (Ussuriysk, Primorsky Territory). Cytological, bacteriological and histological studies were carried out to establish the cause of death. The studies were carried out in an artificially lighted dissection room using the method of complete autopsy during which the metric parameters of organs, as well as pathological changes in them were established.

Results. It was determined that the morphometric parameters of the body and internal organs in male and female Burmese pythons differed in size related to the age, whereas some organs were almost of the same length regardless of sex and age. It was established that death of the male python occurred due to acute pneumonia, which could become a background reason for probable drowning. The female python died from acute pneumonia caused by the bacterium *Ochrobactrum anthropi*.

Discussion and Conclusion. The data obtained during the study of the anatomical and morphological characteristics of the internal organs of male and female specimens of Burmese python, and pathological changes in the organs of reptiles upon death, can be used in the practical work of veterinarians and herpetologists.

Keywords: Burmese python, anatomical and morphological characteristics, pathological changes, pneumonia

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Оригинальное эмпирическое исследование

Анатомо-морфологические характеристики и патологические изменения у бирманского питона (*Python molurus bivittatus*), выращенного в условиях неволи

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Аннотация

Введение. Бирманский питон (*Python molurus bivittatus*) является третьим по величине видом змеи в мире. В дикой природе данная порода змей чаще всего встречается в странах Южной и Юго-Восточной Азии, но в последние годы эти красивые и покорные рептилии стали популярны во всем мире как домашние животные. Содержанием и разведением бирманских питонов занимаются как специалисты в зоопарках и на зоовыставках, так и непрофессиональные любители. Для содержания питона в домашних условиях рекомендуется приобретать особь, рожденную и выращенную в неволе, и создавать ей условия, имитирующие естественную среду обитания. Питоны — выходцы из тропической и субтропической зоны, поэтому в домашних условиях уровень влажности воздуха нужно поддерживать на высокой отметке, а рептилиям, ведущим полуводный образ жизни, обеспечить емкость с водой, в которой они будут иметь возможность купаться, что особенно важно для правильной линьки змей. К сожалению, все чаще эти экзотические животные становятся пациентами ветеринарных клиник или гибнут по разным причинам. Цель исследования — установить анатомо-морфологические характеристики у особей бирманского питона, выращенных в неволе, а также патологические изменения у них при гибели.

Материалы и методы. В 2024–2025 гг. в ветеринарную клинику «Химера» (г. Уссурийск, Приморский край) поступили два трупа бирманских питонов (самец и самка) из частной коллекции. Для установления причины гибели проводили цитологическое, бактериологическое и гистологическое исследование. Исследование осуществлялось в условиях секционного зала при искусственном освещении по методу полного патологоанатомического вскрытия с установлением метрических характеристик органов, а также патологических изменений в них.

Результаты исследования. Определено, что морфометрические показатели тела и внутренних органов у самца и самки бирманских питонов отличаются по размерам, что связано с возрастом, при этом некоторые органы имеют почти одинаковую длину независимо от пола и возраста. Установлено, что гибель самца произошла по причине острой пневмонии и вероятного утопления на ее фоне. Причиной гибели самки стала острая пневмония, вызванная бактерией *Ochrobactrum anthropi*.

Обсуждение и заключение. Полученные в ходе исследования данные, касающиеся анатомо-морфологических характеристик внутренних органов самца и самки бирманского питона, а также патологических изменений в органах при гибели рептилий, могут быть использованы в работе ветеринарных специалистов и герпетологов.

Ключевые слова: бирманский питон, анатомо-морфологическая характеристика, патологические изменения, пневмония

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Introduction. The Burmese python (*Python Molurus Bivittatus Kuhl, 1820*) is a large and robust snake with dark scales and light brown or yellowish markings along its body. These reptiles were long considered a subspecies of the Indian python *P. molurus*, but in 2009 they were recognised as a full value species due to distinctive morphological features, their co-occurrence within the respective habitats, and apparent lack of natural interbreeding [1]. The debates concerning the Burmese python taxonomy have been going on for 200 years and are still unfinished. According to the current scientific classification, the Burmese python belongs to the phylum *Chordata*, class *Reptilia*, order *Squamata*, family *Pythonidae*, genus *Python*, and species *P. bivittatus* [2].

In the wildlife, the Burmese python is most commonly found in South and Southeast Asian countries, such as Myanmar (Burma), Thailand, Indonesia, and Vietnam. These snakes were brought to the United States in the frame of the exotic pet trade, however, many of them were subsequently released into the wildlife by owners who found reptiles difficult to care, and turned into the invasive species in the unique ecosystems of South Florida [3, 4]. The python also lives in game reserves of India and Nepal [5].

The Burmese python has been listed as endangered in Appendix II of the Convention on International Trade in Endangered Species (CITES). It has been listed as vulnerable in the IUCN (International Union for Conservation of Nature) Red List since 2012, as the population of Burmese pythons in wildlife has declined by at least 30% in the first decade of the 21st century due to such factors as their skin being hunted for, use in the native medicine and food industry, the pet trade, habitat degradation, and other factors [6].

In the wildlife, pythons prefer damp areas of forests and marsh lands, as they like water and can swim well. Sometimes, due to living near water sources, where they feed on mammals, birds, reptiles, and amphibians, they are claimed to be semi-aquatic [7]. This is one of the largest snake species in the world, reaching lengths of up to 7 m or more, with an average length of about 3.5 m [8]. Burmese pythons are dimorphic, with females typically being longer, larger, and heavier than males. The weights recorded in mature females range from 14 to 75 kg, while in males — from 7 to 15 kg [9].

Burmese pythons are oviparous [10]. Males have larger cloacal appendages (vestigial appendages) than females. These are two projections, one on each side of the anus that are extensions of the hind limbs [11]. Burmese pythons have specialised heat-sensing organs on their heads, located on the upper and lower lips: these pits allow pythons to detect warm-blooded prey, especially in low-light conditions or complete darkness. Burmese pythons are ectothermic, they rely on the external heat sources to regulate their temperature. Like other reptiles, pythons are not particularly active and can remain motionless for several days in succession. They begin to move only when food becomes scarce or in the case of threatening danger [12].

Like other terrestrial snakes, Burmese pythons are carnivorous, feeding primarily on mammals and birds. Prey size depends on the snake's size: young pythons can feed on rodents, while adults can eat livestock, adult deer, and even alligators [13]. These non-venomous snakes have rows of recurved teeth specifically designated for capturing and immobilizing prey, while they are wrapping coils around it, contracting their muscles until the victim suffocates. Pythons are opportunistic carnivores (which means they will eat whenever food is offered), therefore, in captivity, they often suffer from obesity. When fasting, the snake has a normal heart volume, but their stomach volume, acidity level, and mass of intestines are reduced. Once food enters the stomach, the snake's heart ventricle increases in size by 40%, facilitating digestion. Its intestines gain mass, and the abdomen expands to produce more acid [14].

The Burmese python is a pet, which brings almost no troubles to its owners, as it requires little care and feeding and has kind temper. They are widely bred in Russia, both by specialists in zoos and at zoo exhibitions, as well as by amateurs. The population of these exotic animals in the country is growing, and they are becoming the patients of veterinary clinics more and more often, therefore studying the anatomical and morphological structure and any pathological changes in the organs and systems of these reptiles is a relevant objective. Unfortunately, despite the numerous cases of python deaths due to improper care, feeding and disease, the morphological status and the causes of their deaths are studied extremely rarely, often due to the reluctance of owners to provide the material for analysis. In this context, the aim of this study is to establish the anatomical and morphological characteristics of Burmese python specimens, as well as pathological changes in them upon death.

Materials and Methods. In 2024–2025, two corpses of Burmese python (male and female) from a private collection were delivered to "Khimera" veterinary clinic (Ussuriysk, Primorsky Territory) with the interval of 90 days. The deceased specimens were examined at Primorsky State Agrarian-Technological University (Ussuriysk) under artificial lighting using the autopsy method, during which the metric parameters of organs, as well as pathological changes in them were established.

The Burmese pythons under study were a two-year-old male and a one-year-old female. According to the owner, the reptiles were kept in the same room with a shallow pool, at an optimal temperature, and fed the euthanized rodents. On November 2, 2024, the male was found dead at the bottom of the pool, pressed down by the female. After 90 days, on January 31, 2025, the female was found dead.

Morphometric studies were carried out according to the proprietary methodology developed for describing internal organs (linear and weight parameters) [15]. The study began with an external examination of the pythons and recording main morphometric parameters. The names of body parts were listed, and the structural features were localized. The sex of the specimens was determined using a reptile sexing probe (*Nomoy Pet*, China).

Weight parameters of organs were obtained using a Delta KCE-40-21 electronic scale (*Delta*, China) with up to 0.001 g precision. Linear measurements of organs were performed using a measuring tape (up to 1 mm precision) and a Shock Proof electronic caliper (*Union Source Co*, China) with up to 0.1 mm precision. Internal organs were removed, examined and described according to the technique outlined in [16]. During the autopsy, photographs were taken using a Sony Cyber-shot DSC-W350 camera (*Sony*, Japan) to document certain phenomena and objects under study and obtain visual material. The ratio of internal organ masses to body mass was determined by calculating an average indicator of body mass and organs under study, and relative length was calculated by calculating the organ length to body length in a percentage.

Histological examination of lung lobe biopsy material was performed at CYTOVET Office of Veterinary Morphology (St. Petersburg) in compliance with the standard

techniques, including hematoxylin and eosin staining. Cytological examination was also performed at the same facility, using Pappenheim staining method. Bacteriological examination of respiratory tract contents was conducted in the bacteriology laboratory "TAFI-Diagnostics", LLC (Ussuriysk).

Due to the lack of scientific data on Burmese pythons, various literature and Internet sources were used to write the present article.

Results. External examination revealed that the skin of both specimens had an identical yellowish-brown mosaic pattern with asymmetrical, large, rectangular, dark brown spots of various shape along entire body length of both pythons. Dark stripes ran from the nostrils through the eye area, merging into spots on the neck (Fig. 1).

The body length of the male python was 215.0 cm with a weight of 19.8 kg; the length of the tail was 105.0 cm; the chest girth was 65.0 cm. The body length of the female was 177.0 cm with a body weight of 2.2 kg; the length of the tail was 21.0 cm; the chest girth was 14.5 cm. The nutritional status of both specimens was good: both the male and the female had significant fat deposits in the body cavities in the form of a garland of light pink fragments of moderately dense consistency (Fig. 2).

During the study of the internal organs, we have identified several morphometric features. The upper jaw of the male was attached to two bones — the lower and the mandibular bone. The teeth were thin, sharp, grey-pink in colour, curved caudally and directed toward the pharynx (Fig. 3). The length of the male's esophagus was 63.0 cm, the mucous membrane was grey-pink and moist. The stomach was elongated, 19.0 cm long, and the mucous membrane was dark red. The length of the small intestine was 60.0 cm, of the large intestine — 37.3 cm. At the junction of the small intestine and the large intestine, there was a cecum 3.5 cm long.

Fig. 1. Corpse of a male Burmese python

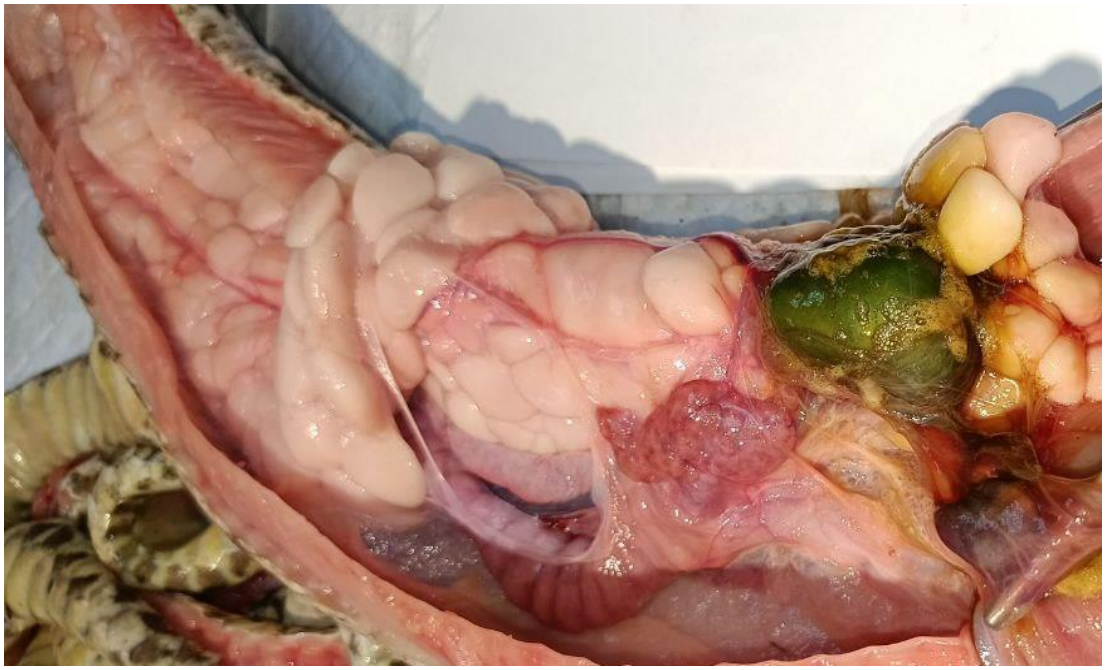


Fig. 2. Internal fat deposits in a male Burmese python

Fig. 3. Teeth of a male Burmese python

The anterior chamber of the male's cloaca (coprodeum) continued into the middle chamber (urodeum), where the ureters opened. The cloaca was 8.3 cm long. The size of pancreatic gland was measured 5.5 x 3.0 cm, was grey-pink, and connected by dense ligaments to the spleen capsule. The size of liver was measured 39.0 x 4.0 cm, it weighed 0.15 kg and was located along the lung. The ventral part of the liver contained the choledoch with the strongly-pronounced bile ducts. The gallbladder was 41.1 cm long and 25.7 cm wide. The kidneys were in the form of compact bodies, were located in the pelvic cavity and were dark brown in colour: the left kidney was 15.5 cm long and weighed 0.015 kg; the right kidney was 18.5 cm long and weighed 0.016 kg (Fig. 4).

The heart consisted of a ventricle and two atria separated by a septum. Both atria opened into the ventricle through separate openings. The ventricle was divided into two halves by an incomplete septum to prevent mixing of arterial and venous blood. The atria were 3.1 cm long, and the ventricle was 3.5 cm long. The heart's width at the base was 3.4 cm, and its mass was 10.0 g; the atria were filled with blood (Fig. 5).

The trachea was 48.0 cm long and divided into two bronchi at the entrance to the lungs. The right lung, 60.4 cm long, was delimited by the transition to the air sac, which was clearly visible. The left lung was shorter than the right (5.0 cm), was attached to the liver capsule by ligaments; the air sac was less visible. The lungs had a cellular structure and the shape of an elongated sac; the inner surface of the lungs had a folded, cellular structure (Fig. 6).

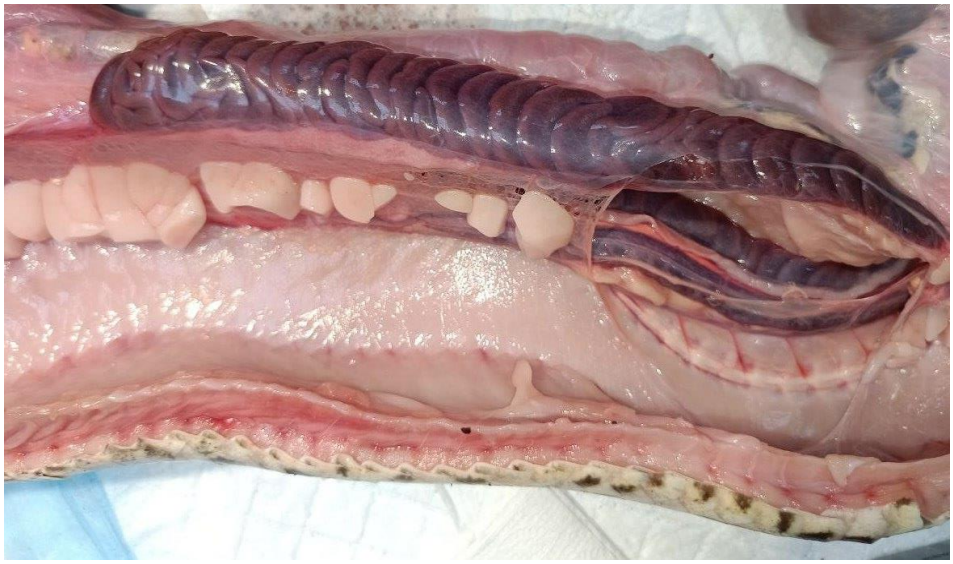


Fig. 4. Kidneys of a male Burmese python

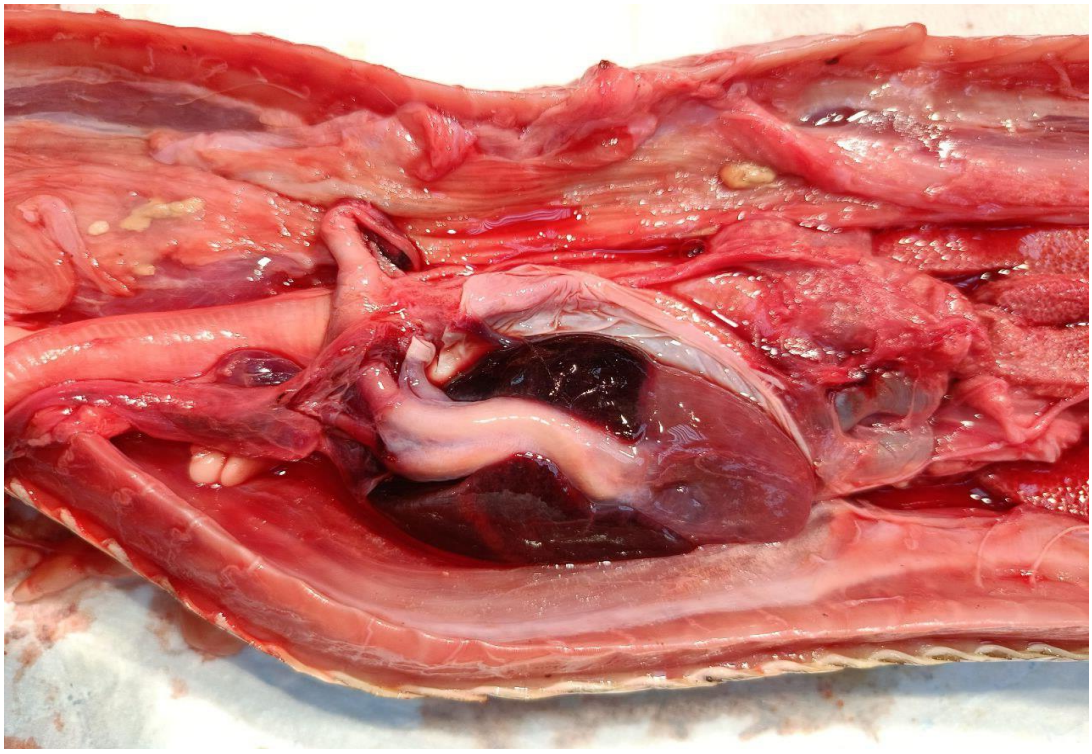


Fig. 5. Heart of a male Burmese python, sectioned through the ventricles

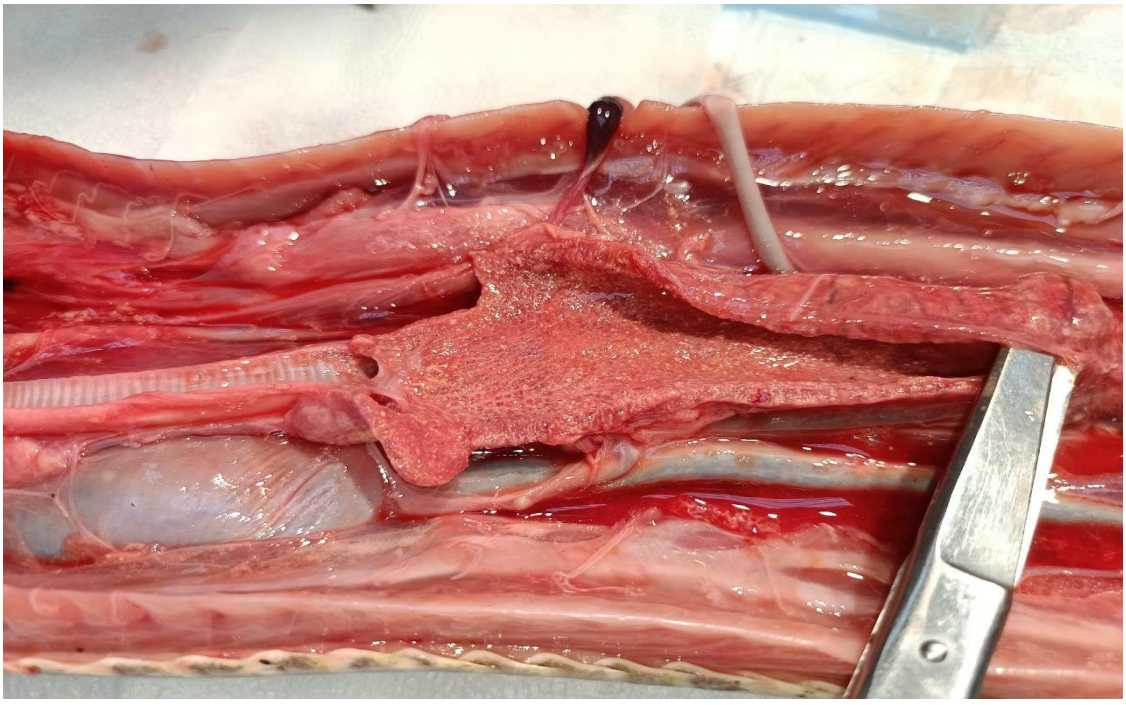
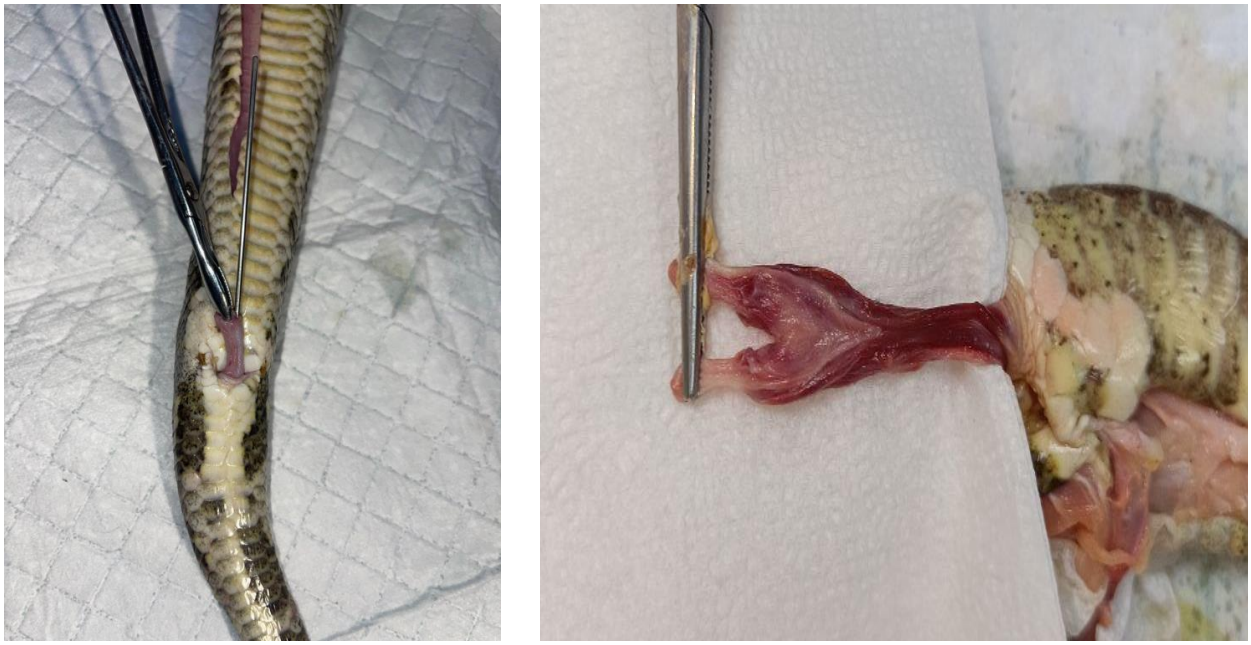


Fig. 6. Trachea bifurcation and lung of a male Burmese python after removal of fluid

The male's hemipenes were located in pockets on the ventral side of the tail near the cloaca. Along the edges of these pockets there were anal spurs — bony structures (pseudopods) in the form of small light-yellow claws that were not attached to the spine. The length of the left testicle was 15.5 cm, and of the right —16.0 cm. In female the paired hemiclitores were found on the ventral side of the tail near the cloaca and anal spurs in the form of small light-yellow claws, half the length of those in the male. The depth of sexing probe penetration, inserted into one of the female's paired hemiclitores, was 6.0 cm (Fig. 7).

Absolute and relative morphological parameters of the body and internal organs of the male and female Burmese python are presented in Table 1. The ratio of the mass of the internal organs to the body mass was determined, and the length of the organs to the body length, excluding the length of the tail.



a) *b*)

Fig. 7. a — female hemiclitores; b — male hemipenes in Burmese python

Table 1

Absolute and relative anatomical and morphological parameters of Burmese pythons

No	Parameters	Unit of measurement	Male	Relative value, %	Female	Relative value, %
1	Body length	cm	215		177	
2	Tail length	cm	105	48.8	21	11.8
3	Chest girth	cm	65	–	14.5	–
4	Body weight	kg	19.8	–	2.2	–
5	Length of the stomach	cm	19	8.83	16	9.32
6	Length of the small intestine	cm	60	27.9	62	35
7	Length of the large intestine	cm	37.3	17.3	41	23.2
8	Length of the cecum	cm	3.5	1.6	3.2	1.8
9	Length of the cloaca	cm	8.3	3.8	6.1	2.8
10	Length of the liver	cm	39	18.3	27	15.2
11	Liver mass	kg	0.15	0.75	0.05	2.36
12	Length of the pancreas	cm	8	2.5	5.5	4.51
13	Length of the left kidney	cm	15.5	7.2	10	5.6
14	Length of the right kidney	cm	18.5	8.6	12.2	6.9
15	Left kidney mass	kg	0.015	0.07	0.006	0.27
16	Right kidney mass	kg	0.016	0.08	0.007	0.31
17	Length of the atria	cm	3.1	1.4	2.2	1.2
18	Length of the heart ventricle	cm	3.5	1.6	2.7	1.5
19	Heart mass	kg	0.01	0.05	0.004	0.18
20	Length of the left lung	cm	5.0	2.3	3.0	1.7
21	Length of the right lung	cm	60.4	28	19	10.7
22	Length of the left testicle	cm	15.5	7.2	–	–
23	Length of the right testicle	cm	16	7.4	–	–

The analysis of absolute and relative morphological parameters in Burmese pythons revealed that the male's body size significantly exceeded that of the female's, despite the age difference between them was only one year. At the same time, the length of the intestine, stomach, cecum, and cloaca was nearly identical in both specimens, despite the difference in body length. At the same time, the length of the liver, pancreas, right and left lungs, and the weight and length of the liver was significantly greater in the male than in the female.

The autopsy revealed that the female had a significant amount of viscous, turbid mucus mixed with blood in its respiratory tract, and similar mucus was found in the lumen of its trachea (Fig. 8). The anterior segment of the right lung was filled with the grey-yellow catarrhal-purulent contents. The lung tissue was dark red. Four limited areas filled with the catarrhal-purulent contents were found in the left lung (Fig. 9).

The male's trachea contained foamy fluid with yellow flocculent inclusions. The lung sac contained a large amount of thick, turbid foamy fluid containing yellow flocculent inclusions. The lung parenchyma was thickened and red in colour (Fig. 10).

e

Fig. 8. Viscous mucus in the respiratory tract of a female Burmese python (photo by the authors)

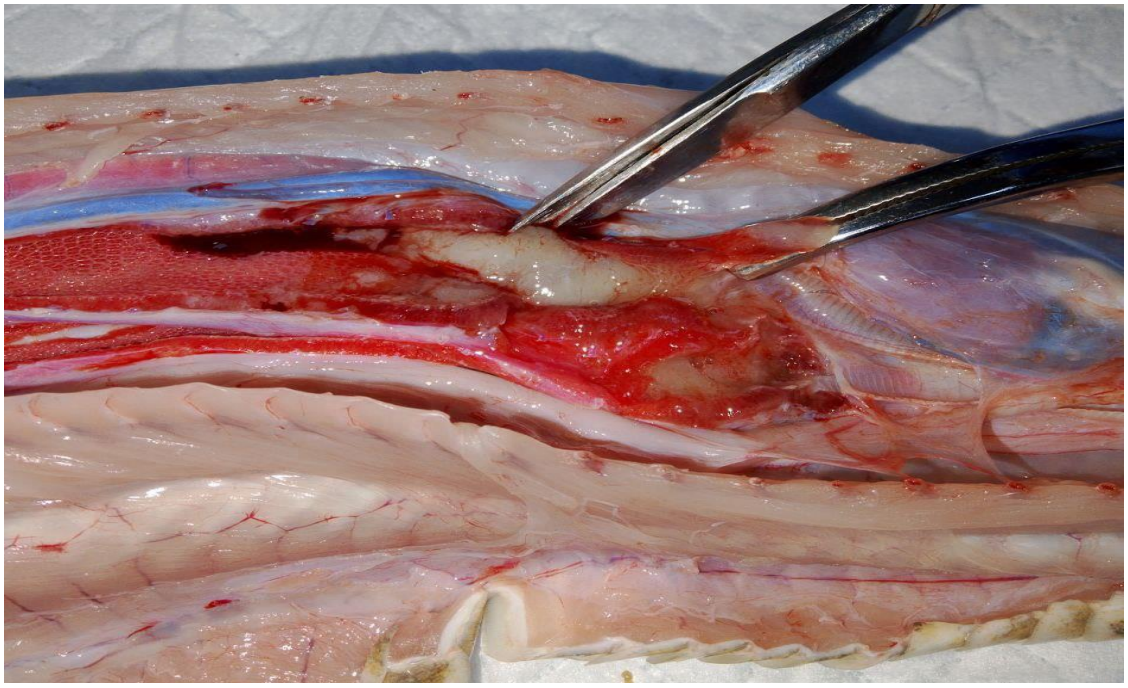


Fig. 9. Catarrhal-purulent contents of the right lung of a female Burmese python

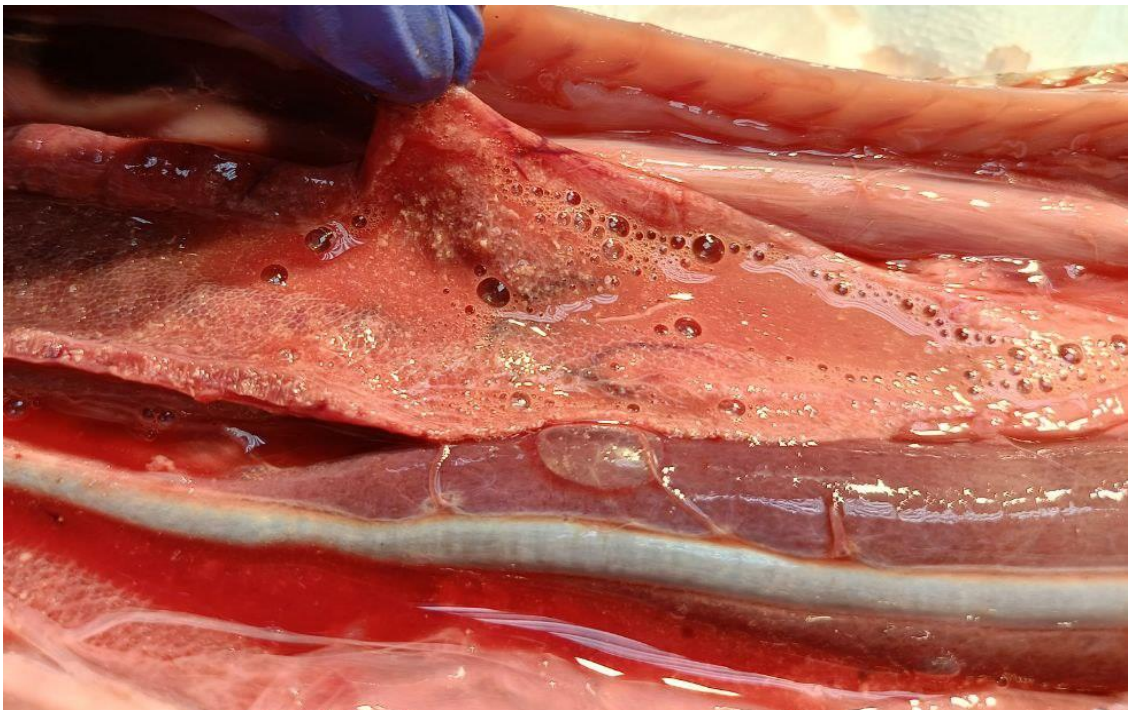


Fig. 10. Fluid in the lung sac of the male Burmese python

Bacteriological examination of sputum from the female's respiratory tract revealed the gram-negative bacterium Ochrobactrum anthropicus. Histological examination of the female's lung biopsy material revealed a severe focal inflammatory process in the faveoli, manifested as a moderate heterophilic inflammatory infiltrate associated with accumulation of interstitial fluid in the air spaces (Fig. 11). Bacteriological culture sampling in the male was not performed due to contamination of water in the pool.

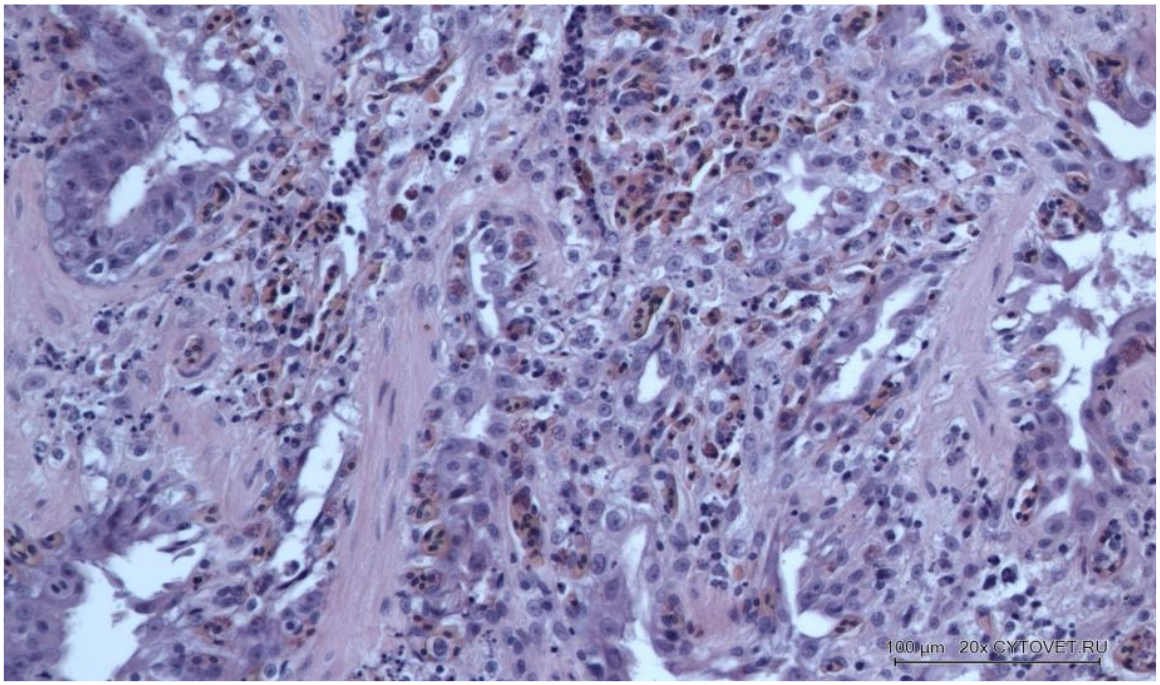


Fig. 11. Focal inflammatory process in the faveoli of the lungs of the female python (hematoxylin and eosin, ×200)

Analysis of the data obtained, brought us to the conclusion that death of the male python was caused by asphyxia due to drowning, pneumonia being a background reason. The female died from asphyxia due to bacterial pneumonia caused by Ochrobactrum anthropi.

Discussion and Conclusion. An anatomical and morphological study of the internal organs of two Burmese pythons revealed that the relative mass (index) of the liver in the male was 0.75%, the heart 0.05%, the left kidney 0.07%, and the right kidney 0.08%. With a body length

of 215.0 cm, the relative length of the left lung was 20.9%, and that of the right lung was 28.09%. The metric parameters of the female's internal organs were smaller than those of the male, which was due to its smaller body mass and size. This is clearly evident with the length of the liver, pancreas, right and left lungs, and the weight and length of the liver—these parameters were smaller in the female than in the male. The length of the intestines, stomach, cecum, and cloaca were nearly identical in both pythons.

Although the male was found in a pool, pressed by a female, drowning was not the only cause of the snake's death. According to various sources, the Burmese python is capable of holding its breath for 30 to 90 minutes, reducing its metabolism to 80% in underwater conditions, which allows oxygen reserves to be preserved for a much longer period. Furthermore, pythons have specialised valves that seal their trachea when submerged, larger air capillaries compared to mammals, which allows them to survive long periods of time without breathing, and a high concentration of myoglobin, which binds and stores oxygen, providing an internal reserve [17–19]. Taking into account this resistance to drowning, the male's death could only have occurred if it had been underwater for too long for its aerobic capacity limited by pneumonia, and was unable to get out of the pool in time due to general muscle weakness caused by the disease. Therefore, the presumed cause of death was drowning due to background pneumonia.

According to D.B. Vasiliev, pneumonia is common among reptiles because, due to the absence of a diaphragm, they lack a cough reflex. Evacuation in the trachea and bronchi in both directions is accomplished solely by the ciliated epithelium. During infections of any etiology, the cilia shorten and stick together losing their function almost completely. Therefore, the infectious agents tend to merge and adhere, which leads to the development of focal bronchopneumonia. The disease may progress without specific symptoms, limited to general depression [20]. According to A.M. Timmerman, the incidence of pneumonia increases when snakes are exposed to stress factors such as overcrowding, unsuitable temperature and humidity, transportation, parasites, etc. [21].

The study allowed us to establish that both Burmese pythons died from asphyxia: the male from drowning due to pneumonia, and the female from acute pneumonia, which may have developed due to a weakened immune system. Due to the saccular, elongated structure of the lungs, the presence of viscous fluid in the female's trachea led to the obstructive reduction of lung respiratory capacity and to limitation of aerobic metabolism.

Pathological changes in the lungs of the pythons studied were consistent with pneumonia, as confirmed by the bacteriological examination of sputum from the respiratory system of the female python. The probable cause of pneumonia was microbial colonization of the oropharynx and respiratory tract by the pathogen, the bacterium *Ochrobactrum anthropi*, and keeping in the confined space might have facilitated transmission of infection.

Ochrobactrum anthropi is a non-fermenting, gram-negative bacterium commonly found in the environment. According to published scientific data, it is a wide-spread soil-dwelling alphaproteobacterium that colonizes a wide range of organisms and is now often recognized as a potentially dangerous opportunistic and nosocomial pathogen that develops in patients with various types of immunodeficiency [22]. According to M.S. Mahmood, the consequences of *O. anthropi* pathogenicity appear to vary greatly in severity depending on the host. It causes bacteremia, which can lead to sepsis and septic shock [23].

We believe that the results we have presented will be useful for identifying the areas of further research in this field, which will undoubtedly be useful to both herpetologists and veterinary specialists.

References

1. Bowie D. *Burmese Python: How the Invasive Species Affects Ecosystems*. URL: https://animals.howstuffworks.com/snakes/burmese-python.htm#:~:text=The%20Burmese%20python%20(Python%20molurus,feet%20(3%20to%206%20meters) (accessed: 27.08.2024).

2. Alekseeva I. *Python Molurus.* (In Russ.) URL: https://wildfauna.ru/tigrovyj-piton?ysclid=m31gefib9w575995510 (accessed: 28.08.2025).

3. Lord I, Redinger J, Dixon J, Hart KM, Guzy J, Romagosa CM, et al. Telescoping Prey Selection in Invasive Burmese Pythons Spells Trouble for Endangered Rodents. *Food Webs*. 2023;37: e00307. https://doi.org/10.1016/j.foodweb.2023.e00307

4. Pittman SE, Bartoszek IA. Initial Dispersal Behavior and Survival of Non-Native Juvenile Burmese Pythons (*Python Bivittatus*) in South Florida. *BMC Zoology*. 2021;6:33. https://doi.org/10.1186/s40850-021-00098-2

5. Safi S, Abhishek, Maurya KK, Ojha G, Mall A, Roy H. Sightings of Burmese Pythons (Python Bivittatus) in and around the Valmiki Tiger Reserve, Bihar, India. *Reptiles and Amphibians*. 2020;27(3):519–521. https://doi.org/10.17161/randa.v27i3.14906

6. Stuart B, Nguyen TQ, Thy N, Grismer L, Chan-Ard T, Iskandar D, et al. Python Bivittatus. *The IUCN Red List of Threatened Species.* 2012:e.T193451A151341916. https://dx.doi.org/10.2305/IUCN.UK.2012-1.RLTS.T193451A151341916.en (accessed: 28.08.2025).

7. Korneev ID. *Burmese Pythone: Description and Maintenance Tips.* (In Russ.) URL: https://zveri.guru/zhivotnye/zmei/tigrovyy-temnyy-piton-opisanie-i-sovety-po-soderzhaniyu.html (accessed: 28.08.2025).

8. Nafus MG, Mazzotti FJ, Reed R.N. Estimating Detection Probability for Burmese Pythons with Few Detections and Zero Recaptures. *Journal of Herpetology*. 2020;54(1):24–30. https://doi.org/10.1670/18-154 (accessed: 28.08.2025).

9. Simon R. *Burmese Python, Snake, Facts.* (In Russ.) URL: https://ru2.socmedarch.org/burmese-python-snake-facts-4174983-15008 (accessed: 28.08.2025).

10. *Burmese Python.* URL: https://www.animalspot.net/burmese-python.html (accessed: 28.08.2025).

11. *Pythons. Interesting Facts*. (In Russ.) URL: https://www.livemaster.ru/topic/2357245-pitony-interesnye-fakty (accessed: 28.08.2025).

12. O'Malley B (Ed.). *Clinical Anatomy and Physiology of Exotic Species. Structure and Function of Mammals, Birds, Reptiles and Amphibians*. Elsevier, Saunders Ltd: 2005. 272 p. https://doi.org/10.1016/B978-0-7020-2782-6.X5001-7

13. Sarill M. *Burmese Pythons in the Everglades*. URL: https://iep.berkeley.edu/content/burmese-pythons-everglades (accessed: 28.08.2025).

14. Helmenstein AM. *Burmese Python Snake Facts*. URL: https://www.thoughtco.com/burmese-python-snake-facts-4174983 (accessed: 28.08.2025).

15. Lyubchenko EN, Korotkova IP, Ivanchuk GV, Kukharenko NS, Zhilin RA, Kozhushko AA. *Morphometric Studies of Wild Cats of the Far East: A Tutorial.* Ussuriysk: Primorsky State Agricultural Academy; 2019. 96 p. (In Russ.)

16. Kukharenko NS, Fedorova AO. *Pathological Anatomy. Organopathology: A Teaching manual for Laboratory and Practical Classes*. Blagoveshchensk: Far Eastern State Agrarian University; 2015. 38 p. (In Russ.)

17. *Snakes Can Hold Their Breath Longer than You Think.* URL: https://regardingreptiles.com/snakes-can-hold-their-breath-longer-than-you-think/ (accessed: 28.08.2025).

18. Thornberry M. *How Long Can Snakes Hold Their Breath?* URL: https://www.berrypatchfarms.net/how-long-can-snakes-hold-their-breath/ (accesssed: 28.08.2025).

19. *How Long Can Snakes Hold Their Breath Underwater*. URL: https://reptilecraze.com/how-long-can-snakes-hold-their-breath-underwater/ (accessed: 28.08.2025).

20. Vasiliev D.B. *Veterinary Herpetology.* Moscow: Aquarium Publ.; 2017. 477 p. (In Russ.)

21. Timmerman AM, Doolen MD. Common Reptilian Diseases. *Iowa State Veterinarian*. 1994;57(1):14–21. URL: https://dr.lib.iastate.edu/handle/20.500.12876/47690 (accessed: 28.08.2025).

22. Chain PSG, Lang DM, Comerci DJ, Malfatti SA, Vergez LM, Shin M, et al. Genome of *Ochrobactrum* Anthropi ATCC 49188^{T}, a Versatile Opportunistic Pathogen and Symbiont of Several Eukaryotic Hosts. *Journal of Bacteriology*. 2011:193(16). https://doi.org/10.1128/JB.05335-11

23. Saeed Mahmood M, Sarwari AR, Khan MA, Sophie Z, Khan E, Sami S. Infective Endocarditis and Septic Embolization with *Ochrobactrum Anthropi*: Case Report and Review of Literature. *Journal of Infection*. 2000;40(3):287–290. https://doi.org/10.1053/jinf.2000.0644

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ПАТОЛОГИЯ ЖИВОТНЫХ, МОРФОЛОГИЯ, ФИЗИОЛОГИЯ, ФАРМАКОЛОГИЯ И ТОКСИКОЛОГИЯ

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Original Empirical Research

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Subchronic Toxicity of D-Cyphenothrin-, Piperonyl Butoxide- and Pyriproxyfen-Based Insecticide-Acaricide upon Its External Use in Laying Chickens

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Abstract

Introduction. Implementation of safe and efficient insecticides-acaricides suitable for using in the presence of poultry infected with ectoparasites is particularly relevant for poultry farming specializing in egg production. The development and implementation of new medicinal products into veterinary practice is a complicated process requiring comprehensive preclinical studies. The objective of this research is to investigate the subchronic toxicity of a new D-cyphenothrin-, piperonyl butoxide-, and pyriproxyfen-based antiparasitic product and the effect of its external use on homeostasis in egg-laying chickens.

Materials and Methods. A subchronic toxicity study of the D-cyphenothrin-, piperonyl butoxide- and pyriproxyfen-based medicinal product was conducted in 2024 at Podolsk Experimental and Production Base of the All-Russian Research Institute of Fundamental and Applied Parasitology of Animals and Plants – Branch of the Federal State Budgetary Scientific Institution "Federal Scientific Center – All-Russian Research Institute of Experimental Veterinary Medicine (VIEV) of the Russian Academy of Sciences. Fifteen Hisex White chickens were divided into three groups of five birds each. Before each treatment, a 5.0% solution of the product was diluted in water at a ratio of 1:1000. A dose of 10.0 ml per 0.3 kg of body weight was assumed to be a therapeutic dose. Birds in the two experimental groups were treated in dosage of 33.3 ml/kg and 100.0 ml/kg, respectively, using a fine-mist spray pump. Chickens from the third control group were not treated. Treatment with a 0.005% aqueous emulsion of the medicinal product was carried out 6 times with an interval of 48 hours. The dynamics of changes in chicken weight, body temperature, some hematological and biochemical blood parameters was monitored, along with the features of behavior, feed and water intake.

Results. No significant changes in body weight in birds from the two experimental groups were recorded. Compared to the control group, no statistically significant changes in body temperature of chickens were revealed throughout the experiment. Six-fold application of the increased dose of the medicinal product resulted in destabilization of red blood cell parameters and decrease of protein metabolism in chickens from the second experimental group; however, these changes were reversible. Accordingly, a dose of 100.0 ml/kg can be assumed a threshold dose of no observed adverse effect level (NOAEL), and 33.3 ml/kg can be assumed a safe one of no observed effect level (NOEL).

Discussion and Conclusion. Statistically significant changes in some blood parameters in chickens were observed after six applications of a 0.005% aqueous emulsion of the new combined insecticide-acaricide at a dose of 100.0 ml/kg. However, these changes were reversible. Taking into account the threefold increase of the therapeutic dose in the experiment, the product proved to have a wide range of safe dosages for external use. Therefore, the antiparasitic treatment with the 0.005% aqueous emulsion of the combined product in dosage of 33.3 ml/kg can be ascertained safe for poultry.

Keywords: insecticide-acaricide product, subchronic toxicity, D-cyphenothrin, piperonyl butoxide, pyriproxyfen, chickens, preclinical studies

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Оригинальное эмпирическое исследование

Субхроническая токсичность инсектоакарицидного средства на основе D-цифенотрина, пиперонилбутоксида и пирипроксифена при наружном применении у яичных цыплят

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Аннотация

Введение. Внедрение безопасных и эффективных инсектоакарицидных средств с возможностью их применения в присутствии птиц при эктопаразитозах особенно актуально для яичного птицеводства. Разработка и внедрение новых препаратов в ветеринарную практику — сложный процесс, требующий всесторонних доклинических исследований. Цель работы — изучение субхронической токсичности нового противопаразитарного средства на основе D-цифенотрина, пиперонилбутоксида и пирипроксифена и его влияния на гомеостаз яичных цыплят при наружном применении.

Материалы и методы. Исследование субхронической токсичности средства на основе D-цифенотрина, пиперонилбутоксида и пирипроксифена было проведено в 2024 г. на Подольской опытно-производственной базе ВНИИП – филиала ФГБНУ ФНЦ ВИЭВ РАН (г. Москва). 15 цыплят кросса Хайсекс Уайт были разделены на три группы по пять голов в каждой. Перед каждой обработкой 5,0 %-ный препарат разводили водой в соотношении 1:1000. Условно за терапевтическую дозу принимали 10,0 мл на 0,3 кг массы тела птицы. В двух опытных группах птиц обрабатывали мелкокапельным опрыскиванием с помощью помпового опрыскивателя в дозах 33,3 мл/кг и 100,0 мл/кг соответственно. Цыплят из третьей контрольной группы не обрабатывали. Обработки 0,005 %-ной водной эмульсией лекарственного препарата проводили 6 раз с интервалом 48 ч. Контролировали у цыплят в динамике массу, температуру тела, некоторые гематологические и биохимические показатели крови, а также учитывали особенности поведения, приема корма и воды.

Результаты исследования. Достоверные изменения массы тела у птиц из двух опытных групп отсутствовали. Статистически значимых изменений не выявлено при анализе температуры тела у цыплят в течение всего эксперимента по сравнению с контролем. У цыплят из второй опытной группы в результате 6-кратного применения увеличенной дозы препарата выявлены дестабилизация показателей системы красной крови и снижение интенсивности белкового обмена, однако указанные изменения носили обратимый характер. Соответственно, дозу 100,0 мл/кг можно считать пороговой, а 33,3 мл/кг — недействующей (безопасной).

Обсуждение и заключение. На фоне 6-кратного применения 0,005 %-ной водной эмульсии нового комбинированного инсектоакарицидного средства в дозе 100,0 мл/кг описаны статистически значимые изменения некоторых показателей крови у цыплят, однако они носили обратимый характер. Учитывая трехкратное увеличение терапевтической дозы в эксперименте, у препарата имеется гарантия безопасного наружного применения в широком диапазоне доз. Исходя из этого можно утверждать, что при противопаразитарных обработках использование 0,005 %-ной водной эмульсии комбинированного препарата в дозе 33,3 мл/кг будет безопасно для птиц.

Ключевые слова: инсектоакарицидное средство, субхроническая токсичность, D-цифенотрин, пиперонилбутоксид, пирипроксифен, цыплята, доклинические исследования

Для цитирования. Индюхова Е.Н. Субхроническая токсичность инсектоакарицидного средства на основе D-цифенотрина, пиперонилбутоксида и пирипроксифена при наружном применении у яичных цыплят. *Ветеринарная патология.* 2025;24(3):26–33. https://doi.org/10.23947/2949-4826-2025-24-3-26-33

Introduction. The development of safe and efficient insecticides-acaricides for simultaneous disacarization and desinsection of livestock and poultry facilities is an important objective of modern parasitology. For example, the red poultry mite is widespread in industrial poultry farming [1–3]. These temporary hematophagous ectoparasites inhabit hard-to-reach places of cage equipment, joints, cracks, etc. During a parasitological examination of poultry houses, approximately 100–500 mites are usually found per linear meter, alongside, in the organisms of laying chickens numerous negative changes in the central metabolic processes, the development of oxidative stress, severe anemia syndrome, mixed-type hypoxia [4], feather loss, emaciation, anxiety and reduction of egg production capacity [5] are reported.

In 2024, the All-Russian Research Institute of Fundamental and Applied Parasitology of Animals and Plants (Branch of the All-Russian Research Institute of Experimental Veterinary Medicine (VIEV) of the RAS, Moscow) had developed a medicinal product based on three components claimed low toxic to poultry by the scientists [6–8]. The first component is D-cyphenothrin — the synthetic pyrethroid, which is active against fluff lice, argasid ticks, ixodic ticks and red poultry mites [9–10]. The second component is piperonyl butoxide belonging to pyrethroid synergists. The third substance is pyriproxyfen, a suppressor of ectoparasite embryogenesis. This combination of three components is classified as hazard class 3 (moderately hazardous substances) for oral use and as hazard class 4 (low-hazardous substances) for external use [11]. It should be noted that pyrethroids are less toxic to birds than to mammals, due to the higher rate of pyrethroid biotransformation in birds compared to mammals [12–14].

The implementation of new medicinal products into veterinary practice is a complicated process that requires numerous and comprehensive preclinical studies to confirm their safety and efficacy. One of the most important studies is investigation of toxic effects resulting from multiple use of the product in target animal species. The article aims to investigate the subchronic toxicity of an insecticide-acaricide based on D-cyphenothrin, piperonyl butoxide and pyriproxyfen and its effect on egg-laying chickens in case of external application.

Materials and Methods. The experiment was conducted in 2024 at Podolsk Experimental and Production Base of the All-Russian Research Institute of Fundamental and Applied Parasitology of Animals and Plants (Branch of the All-Russian Research Institute of Experimental Veterinary Medicine (VIEV) of the RAS, Moscow). Fifteen 30-day-old Hisex White chickens were divided into three groups (two experimental and one control) of five birds each. The birds were fed a complete feed respective to their age group. Access to water was restricted for chickens from two experimental groups only during periods of treatment. The poultry were housed in two-tiered cages: the control group was housed on the upper tier, while the chickens from the first and second experimental groups were housed separately on the lower tier.

The experiment was conducted in compliance with the guidelines for medicinal product preclinical studies published in 2012 and edited by A.N. Mironov[1]. The dosage regimen and frequency of administration were chosen to identify potential toxic effects on birds during long-term use of a 0.005% aqueous emulsion of the tested product, as well as in the event of its overdosage. The aqueous emulsion of the tested product is intended for antiparasitic treatment of poultry houses in the presence of chickens. The product was sprayed twice, with an interval of 5 days or more, in a form of fine mist using various technical means. According to the instruction for use, the consumption of the product aqueous emulsion was 50 ml/m^2; 10.0 ml per 0.3 kg of bird body weight was conventionally considered a therapeutic dose.

Before each treatment, a 5.0% product was diluted in water at a ratio of 1:1000 to obtain a 0.005% aqueous emulsion. In the first experimental group, birds were treated at a dose of 33.3 ml/kg using a fine-mist spray pump. In the second experimental group, the therapeutic dose was tripled (100.0 ml/kg). Before each treatment, the chickens were individually weighed to calculate the required product dose. Birds from the control group were not treated.

Treatments with an aqueous emulsion of the medicinal product were carried out six times at 48-hour intervals. Birds from three groups were weighed, their body temperature was measured, and blood samples were taken before treatment, the day after the sixth treatment, and 10 days after the sixth treatment. A range of hematological and biochemical parameters were determined in the blood samples according to generally accepted techniques [15]. The behaviour of the chickens, their motor activity, appearance, feed and water consumption were observed daily.

Statistical processing of digital data was performed using the Student's t-test in Microsoft Excel 2016. Results were considered statistically significant (reliable) if the significance level (P) was less than 0.05. The results of statistical data processing were presented in the following format: the mean value (M) is reported together with the standard error of the mean (± m).

Results. None of the chickens died during the entire experiment. During treatments, specimens from the first and second experimental groups bunched in a corner of the cage or moved actively around the cage with excessive vocalization. Feed and water intake by chickens from the experimental groups did not differ from that of the control group.

The dynamics of chicken body weight changes are presented in Table 1. There were no significant changes in body weight in birds from the two experimental groups. Furthermore, no statistically significant changes were detected when analysing the body temperature of chickens participating in the experiment compared to the ones from control group (Table 2).

[1] Guidelines for conducting preclinical studies of medicinal products. Part one. Moscow: Grif i K, 201. 944 p.. URL: https://rsmu.ru/fileadmin/templates/DOC/Zakon_RF/Mironov_Rukovodstvo_po_provedeniju_doklinicheskikh_issledovanii_lekarstvennykh_sredstv.pdf

Table 1

Dynamics of body weight changes in chickens (n=5), kg

Examination timeframes	Control group	First experimental group	Second experimental group
Before treatment	0.30 ± 0.00	0.29 ± 0.00	0.29 ± 0.01
After the 6th treatment	0.42 ± 0.01	0.41 ± 0.00	0.40 ± 0.01
10 days after the 6th treatment	0.52± 0.01	0.52± 0.01	0.51± 0.01

Note: P>0.05

Table 2

Temperature status of chickens (n=5), °C

Examination timeframes	Control group	First experimental group	Second experimental group
Before treatment	41.50± 0.15	41.70 ± 0.08	41.38± 0.24
After the 6th treatment	41.22 ± 0.17	41.58 ± 0.15	41.62 ± 0.17
10 days after the 6th treatment	41.54 ± 0.14	41.56 ± 0.14	41.44 ±0.17

Note: P>0.05

Upon analysis of some hematological and biochemical blood parameters, statistically significant changes were identified in chickens of the second experimental group compared to the control group. In egg-laying chickens, the number of erythrocytes, leukocytes and concentration of hemoglobin in the blood were assessed (Table 3).

Table 3

Some hematological parameters in the blood of chickens (n=5)

Indicator, unit of measurement	Examination timeframes	Control group	First experimental group	Second experimental group
Erythrocytes, $\times 10^{12}$/l	Before treatment	2.64 ± 0.14	2.59 ± 0.06	2.68 ± 0.12
	After the 6th treatment	2.94 ± 0.12	2.82 ± 0.11	2.73 ± 0.08
	10 days after the 6th treatment	2.86 ± 0.08	2.78 ± 0.06	2.92 ± 0.08
Hemoglobin, g/l	Before treatment	122.60 ± 2.99	121.60 ± 3.12	125.40 ± 2.73
	After the 6th treatment	126.40 ± 2.93	122.60 ± 2.38	113.80 ± 2.52*
	10 days after the 6th treatment	127.40 ± 2.66	128.60 ± 2.44	122.80 ± 2.75
Leukocytes, $\times 10^{9}$/L	Before treatment	7.34 ± 0.29	7.06 ± 0.58	6.88 ± 0.61
	After the 6th treatment	7.70 ± 0.26	7.66± 0.42	7.91 ± 0.39
	10 days after the 6th treatment	7.86 ± 0,29	8.30 ± 0.26	7.92 ± 0.29

Note: *P<0.05 compared to the control group

Cyanogen-containing pyrethroids, including D-cyphenothrin, are known to actively affect hematopoiesis [16]. Thus, a tendency towards a decrease in the number of erythrocytes by 7.1% was revealed in the specimens from the second experimental group after the 6th treatment compared to the control group. However, 10 days after the last treatment, the above mentioned tendency for this parameter was not observed. Also, in chickens from the second experimental group, a statistically significant decrease in concentration of the hemoglobin by 10.0% ($P < 0.05$) was found compared to the control group. After 10 days, no reliable changes of this parameter were found. Similar results have been presented in the publication studying changes of the hematological parameters in the blood of laboratory animals during use of a cyphenothrin-containing medicinal product [16].

All cyano-containing pyrethroids disrupt the transporting function of erythrocytes. Although, initially compensatory mechanisms maintain normal erythrocyte levels in the blood by stimulating erythropoietin synthesis, after some time, a decline in hematopoiesis is observed [16]. Furthermore, A. Khan et al. noted the inhibitory effect of synthetic pyrethroids on erythropoietin [17]. The above statement results in a decrease in the intensity of certain metabolic processes in chickens during multiple use of the combination product, as presented in Table 4.

Upon 6-fold application of the three-component product, a reliable decrease in the concentration of total protein by 9.1% ($P<0.05$) and globulins by 10.8% ($P<0.05$) was observed in the blood of chickens from the second experimental group, as well as a tendency towards a decrease in the albumin level by 7.4% compared to the control group. This indicates a disorder in the liver protein-synthesis function in chickens from the second experimental group and goes in line with other studies [17, 18]. However, 10 days after the application of the combination insecticide-acaricide, no statistically significant changes were observed, indicating the recovery of the protein-synthesizing function of liver in birds. Similar findings have been noted by peers in their publications [19]. It is known that the main target organ for piperonyl butoxide is the liver [20]. Studies conducted on laboratory animals have revealed an increase in the weight of this organ and increased activity of certain blood enzymes associated with liver pathologies [21]. However, many studies have confirmed the safety of piperonyl butoxide for birds, since even high doses of this drug did not cause death of animals [7, 21].

As a result of multiple external application of the medicinal product in chickens from the second experimental group, a decrease in the intensity of transamination processes was observed, which expressed in a reliable decrease in the activity of aspartate aminotransferase by 8.7% ($P < 0.05$), and a tendency towards a decrease in the activity of alanine aminotransferase by 15.5% compared to the control group. The current understanding of the diagnostic significance of these enzymes is presented in the work of A.S. Shidlovsky and A.I. Saltanov, in which a tendency towards a disruption of relationships within the carbohydrate, amino acid and energy metabolisms against the background of low activity of aminotransferases was noted [22]. Ten days after treatments were finished, no significant changes in the activity of these enzymes across the groups were detected.

During the experiment, no significant changes in creatinine concentrations in the blood of chickens from the two experimental groups were observed compared to the control group. It is known that one of the reasons underlying the decrease in creatine phosphokinase activity is the destabilisation of aerobic oxidation processes in the organism of animals. Thus, the day after the sixth treatment, chickens from the first experimental group showed a tendency towards a decrease in the activity of this enzyme by 7.9%, while birds from the second experimental group showed a significant decrease of its activity by 20.8% ($P<0.05$) compared to the control group. However, 10 days after the last treatment, these changes were not observed. This may be due to the normalization of energy metabolism in the birds' organisms against the background of stabilization of red blood cell parameters.

Analysis of some lipid metabolism parameters revealed no statistically significant changes in the concentration of triglycerides and cholesterol in the blood of chickens from the second experimental group compared to the control group.

No significant changes in blood glucose concentrations were detected in chickens, with levels within the physiological norm (11–15 mmol/l) in all experimental birds [23]. Furthermore, two tendencies were observed in the blood of chickens from the second experimental group after the sixth treatment: a 3.8% decrease in glucose concentration and a 3.3% decrease in α-amylase activity compared to the control group. After 10 days, no significant changes in carbohydrate and energy metabolism parameters were detected in chickens.

Thus, destabilization of red blood cell parameters and a decrease in protein metabolism were observed in chickens from the second experimental group after six external treatments of the medicinal product; however, these changes were reversible. Consequently, a dose of 100.0 ml/kg can be considered the threshold dose, and 33.3 ml/kg can be considered a dose of no observed effect, i.e. safe.

Table 4

Some biochemical parameters of chicken blood (n=5)

Indicator, unit of measurement	Examination timeframes	Control group	First experimental group	Second experimental group
Total protein, g/l	Before treatment	37.80 ± 0.92	37.40 ± 1.17	37.00 ± 1.10
	After the 6th treatment	39.40 ± 0,40	38.20 ± 0.58	35.80 ± 1.02*
	10 days after the 6th treatment	39.80 ± 0.37	39.60 ± 0.51	39.00 ± 0.71
Albumins, g/l	Before treatment	18.80 ± 0.49	18.60 ±0.40	18.20 ± 0.37
	After the 6th treatment	19.00 ± 0.63	18.60 ± 0.24	17.60 ± 0.60
	10 days after the 6th treatment	20.20 ± 0.20	19.40 ± 0.24	18.80 ± 0.49
Globulins, g/l	Before treatment	19.00 ± 0.45	18.80 ± 0.86	18.80 ± 0.86
	After the 6th treatment	20.40 ± 0.51	19.60 ± 0.40	18.20 ± 0.49*
	10 days after the 6th treatment	19.60 ± 0.40	20.20 ± 0.37	20.20 ± 0.37
Alanine aminotransferase, U/l	Before treatment	30.40 ± 1.89	32.20 ± 1.85	30.60 ± 2.18
	After the 6th treatment	25.80 ± 2.82	26.00 ± 2.45	21.80 ± 2.87
	10 days after the 6th treatment	14.20 ± 1.07	15.40 ± 0.68	15.00 ± 0.94
Aspartate aminotransferase, U/l	Before treatment	271.80 ± 7.81	273.00 ± 8.70	277.80 ± 9.01
	After the 6th treatment	269.00 ± 3.69	257.00 ± 5.16	245.60 ± 6.68*
	10 days after the 6th treatment	205.20 ± 7.77	210.00 ± 9.02	209.40 ± 9.21
Creatinine, μmol/l	Before treatment	27.60 ± 0.81	28.20 ± 0.97	27.80 ± 0.58
	After the 6th treatment	29.20 ± 0.80	27.80 ± 0.73	29.00 ± 0.71
	10 days after the 6th treatment	28.80 ± 0.80	29.20 ±1.16	29.00 ± 1.26
Creatine phosphokinase, U/l	Before treatment	1979.40 ± 70.89	2001.60 ± 80.69	2013.20 ± 88.46
	After the 6th treatment	2001.40 ± 60.58	1843.60 ± 26.02	1585.00 ± 67.44*
	10 days after the 6th treatment	1947.00 ± 44.47	2051.40 ± 47.98	1895.60 ± 68.71
Cholesterol, mmol/l	Before treatment	3.56 ± 0.12	3.60 ± 0.14	3.58 ± 0.14
	After the 6th treatment	3.92 ± 0.10	3.70 ± 0.15	3.88± 0.12
	10 days after the 6th treatment	3.98 ± 0.16	3.78 ± 0.11	3.70 ± 0.10
Triglycerides, mmol/l	Before treatment	0.57 ± 0.04	0.53 ± 0.05	0.59 ± 0.04
	After the 6th treatment	0.56 ± 0.04	0.52 ±0.04	0.55± 0.03
	10 days after the 6th treatment	0.66 ± 0.04	0.62 ± 0.04	0.59 ± 0.01
Glucose, mmol/l	Before treatment	12.46 ± 0.42	13.42 ± 0.21	13.06 ± 0.39
	After the 6th treatment	12.72 ± 0.30	12.58 ± 0.35	12.24 ± 0.27
	10 days after the 6th treatment	13.02 ± 0.26	13.18 ± 0.32	12.34 ± 0.29
Lactate dehydrogenase, U/l	Before treatment	1351.80 ± 35.03	1322.80 ± 42.71	1286.40 ± 31.20
	After the 6th treatment	1413.20 ± 52.41	1343.40 ± 79.28	1335.00 ± 26.61
	10 days after the 6th treatment	1395.00 ± 33.86	1361.20 ± 30.69	1439.40 ± 48.55
α-Amylase, U/L	Before treatment	326.80 ± 12.17	357.40 ± 24.02	320.20 ± 10.86
	After the 6th treatment	311.60 ± 11.99	318.00 ± 11.73	301.40 ± 21.85
	10 days after the 6th treatment	337.00 ± 13.67	349.00 ± 17.56	351.80 ± 10.68

Note: *P<0.05 compared to the control group

Discussion and Conclusion. The study results indicate

that the new combination product based on D-cyphenothrin, piperonyl butoxide, and pyriproxyfen is safe for laying chickens across a wide range of doses when applied externally. Statistically significant changes in some blood parameters were detected only in chickens from the second experimental group, which received the product at a dose of 100.0 ml/kg, and even these changes were reversible. Therefore, there is reason to believe that the use of a 0.005% aqueous emulsion of the combination product at a dose of 33.3 ml/kg for disacarization of premises (twice with an interval of 5 days or more) in the presence of poultry can be considered safe.

References

1. Küntüz T, Güneş Y, Sarı AB, Keleş OÜ. Navigating the Resistance: Current Perspectives on Ectoparasite Control in Veterinary Medicine. *Journal of Istanbul Veterinary Sciences*. 2023;7(2):56–67. https://doi.org/10.30704/http-www-jivs-net.1328872

2. Pavlićević A, Ratajac R, Stojanov I, Pavlovic I. The Control Program of Red Poultry Mite (*Dermanyssus Gallinae*), Today. *Arhiv Veterinarske Medicine (Archives of Veterinary Medicine)*. 2018;11(2):71–88. https://doi.org/10.46784/e-avm.v11i2.27

3. Sparagano OAE, Ho J. Parasitic Mite Fauna in Asian Poultry Farming Systems. *Frontiers in Veterinary Science.* 2020;7:400. https://doi.org/10.3389/fvets.2020.00400

4. Indyuhova EN, Arisov MV, Azarnova TO, Maximov VI. *Sanogenetic Basis for Correction of Physiological and Biochemical Status in Hens with Dermanyssosis.* Moscow: Publishing House Nauka, 2024. 242 p. (In Russ.) https://doi.org/10.31016/978-5-6050437-5-1-2024-242

5. Katsavou E, Vlogiannitis S, Karp-Tatham E, Blake DP, Ilias A, Strube C, et al. Identification and Geographical Distribution of Pyrethroid Resistance Mutations in the Poultry Red Mite *Dermanyssus Gallinae*. *Pest Management Science*. 2020;76(1):125–133. https://doi.org/10.1002/ps.5582

6. Gajendiran A, Abraham J. An Overview of Pyrethroid Insecticides. *Frontiers in Biology*. 2018;(13):79–90. https://doi.org/10.1007/s11515-018-1489-z

7. Toca C, Nica D, Panchiosu A., Gheorghe A. Determination of Piperonyl Butoxide in Honey Bees by Gas Chromatography Coupled With Mass Spectrometry. *Revista Română de Medicină Veterinară*. 2017;27(3):45–48. URL: https://agmv.ro/wp-content/uploads/2019/10/DETERMINAREA-PIPERONIL-BUTOXIDULUI.pdf (accessed: 01.09.2025).

8. Unlu I, Faraji A, Williams GM, Marcombe S, Fonseca DM, Gaugler R. Truck-Mounted Area-Wide Applications of Larvicides and Adulticides for Extended Suppression of Adult *Aedes Albopictus*. *Pest management science*. 2019;75(4):1115–1122. https://doi.org/10.1002/ps.5227

9. Arisov MV, Magomedshapiev GM, Kurochkina KG, Uspensky AV, Malakhova EI., Novik TS, et al. New Remedies for Preventive and Treatment Measures against Ixodidosis in Cattle in Livestock Farms of Dagestan. *Russian Journal of Parasitology*. 2015;(1):35–40. (In Russ.) URL: https://vniigis.elpub.ru/jour/article/view/130/133 (accessed: 01.09.2025)

10. Indyuhova EN, Arisov MV. Insectoacaricide Activity of 5% D-Cyphenothrin Emulsion against Argasid Ticks and Biting Lice. *Russian Journal of Parasitology*. 2024;18(2):211–218. (In Russ.) https://doi.org/10.31016/1998 8435-2024-18-2-211-218

11. Arisov MV, Indyuhova EN, Arisova GB, Poselov DS, Stepanov AA, Poselova EV. Acute Oral and Dermal Toxicity Parameters of a Drug Based on D-Cyphenothrin, Pyriproxyfen and Piperonyl Butoxide in Laboratory Animals. *Russian Journal of Parasitology*. 2024;18(4):410–418. (In Russ.) https://doi.org/10.31016/1998-8435-2024-18-4-410-418

12. Maund SJ, Campbell PJ, Giddings JM, Hamer MJ, Henry K, Pilling ED, et al. Ecotoxicology of Synthetic Pyrethroids. In book: *Matsuo N, Mori T (Eds.). Pyrethroids. Topics in Current Chemistry, Vol 314.* Berlin, Heidelberg: Springer; 2011. P. 137–165. https://doi.org/10.1007/128_2011_260

13. Ahamad A, Kumar J. Pyrethroid Pesticides: An Overview on Classification, Toxicological Assessment and Monitoring. *Journal of Hazardous Materials Advances*. 2023;10:100284. https://doi.org/10.1016/j.hazadv.2023.100284

14. Ruberti M. One Hundred Years of Pyrethroid Chemistry: A Still-Open Research Effort to Combine Efficacy, Cost-Effectiveness and Environmental Sustainability. *Sustainability*. 2024;16(19):8322. https://doi.org/10.3390/su16198322

15. Kondrakhin IP. *Methods of Veterinary Clinical Laboratory Diagnostics*. Moscow: Kolos Publishing House; 2004. 520 p. (In Russ.) URL: https://bioenc.ru/diagnostika-laboratornaya-klinicheskaya/metodyi-veterinarnoy-klinicheskoy-laboratornoy.html (accessed: 01.09.2025).

16. Srinivas BN, Muniswamy D. Effect Of Cyphenothrin-Induced Splenic Damage and Hematological Alterations in Male Wistar Rats (*Rattus Norvegicus*). *International Journal of Pharmacy and Pharmaceutical Sciences*. 2023;15(11):26–30. https://doi.org/10.22159/ijpps.2023v15i11.48970

17. Khan A, Ahmad L, Khan MZ. Hemato-Biochemical Changes Induced by Pyrethroid Insecticides in Avian, Fish and Mammalian Species. *International Journal of Agriculture and Biology*. 2012;14(5):834–842. URL: https://www.researchgate.net/profile/Ahrar-Khan/publication/235997317_IJAB-834-842-2012/links/0046351563b0282368000000/IJAB-834-842-2012.pdf (accessed: 01.09.2025).

18. Verma R, Pathak SK. Haemato-Biochemical Alteration in Chicks (*Gallus Domesticus*) Following Short Term Exposure of Synthetic Pyrethroid Type II Fenvalerate. *Environment Conservation Journal*. 2015;16(1&2):139–142. https://doi.org/10.36953/ECJ.2015.161221

19. Addy-Orduna LM, Zaccagnini ME, Canavelli SB, Mineau P. Formulated Beta-Cyfluthrin Shows Wide Divergence In Toxicity among Bird Species. *Journal of Toxicology*. 2011;(1):803451. https://doi.org/10.1155/2011/803451

20. Isshiki K, Tsumura S, Watanabe T. Residual Piperonyl Butoxide in Agricultural Products. *Bulletin of Environmental Contamination and Toxicology*. 1978;(19):518–523. https://doi.org/10.1007/BF01685835

21. *Reregistration Eligibility Decision for Piperonyl Butoxide (PBO) List B, Case No. 2525*. United States, Environmental Protection Agency. Prevention. Pesticides and Toxic Substances. (7508C). EPA 738-R-06-005. CiteSeer; 2006. 111 p. URL: https://www3.epa.gov/pesticides//chem_search/reg_actions/reregistration/red_PC-067501_14-Jun-06.pdf (accessed: 01.09.2025).

22. Shidlovsky AS, Saltanov AI. Variants of Mechanisms to Change the Transaminase Activity: Clinical Interpretation. *Annals of Critical Care.* 2015;(1):22–32. (In Russ.)

23. Buyko NV, Lizun RP, Nasonov IV, Zakharik NV. Biochemical and Hematological Parameters in Hysex White and Hysex Brown Laying Hens. *Ecology and Animal World*. 2014;(1):31–35. URL: https://bievm.by/gallery/Экология%20и%20животный%20мир%20№%201%202014.pdf#page=31 (accessed: 02.09.2025). (In Russ.)

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ПАТОЛОГИЯ ЖИВОТНЫХ, МОРФОЛОГИЯ, ФИЗИОЛОГИЯ, ФАРМАКОЛОГИЯ И ТОКСИКОЛОГИЯ

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Original Empirical Research

Morphological Features and Histogenesis of Canine Nodal Lymphoma

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Abstract

Introduction. One of the most common types of oncopathologies are lymphoproliferative disorders, in particular, lymphomas. The disorders are characterised by the complicated development mechanisms, multiple etiological factors and their diverse biological features. In addition, the common origin of lymphoid cells makes different types of lymphomas morphologically similar and complicates their diagnostics. The morphological study used to be, currently is and will continue to be a gold standard in the oncological pathology diagnostics. However, the lack of clear morphological criteria and histological classifications of the various types of tumours in animals prevents from adequate diagnostics of oncological diseases in them. The above urges to conduct a thorough study of various morphological types of lymphomas to identify their differential diagnostic features. The aim of the research is to study the morphological features and histogenesis of canine nodal lymphoma to facilitate the diagnostics and develop the efficient targeted therapy protocols.

Materials and Methods. The objects of the study conducted in the network of veterinary laboratories “VETLAB” (Moscow) in the period from January 2024 to January 2025 were the lymph nodes in dogs with the signs of nodal lymphoma. All samples (n = 30) were prepared according to the standard technique of histological sample preparation. Histological preparations stained with hematoxylin and eosin were obtained, and microscopy was performed. A comparative estimation and contrasting against the following morphological criteria were carried out: tumour growth pattern, spread of tumour in the tissue, tumour cell structure, size and location of nuclei, chromatin structure, presence of mitoses, and tumour microenvironment.

Results. By studying the canine nodal lymphoma features by means of microscopy, a number of significant differential diagnostic features were identified enabling to distinguish the following morphological variants of lymphomas: large-cell and small-cell lymphomas, immunoblastic, centroblastic and lymphoblastic lymphomas. Prognostic features included: proliferative index, diffuse growth pattern, cellular microenvironment, and tumour vascularization.

Discussion and Conclusion. The study had identified the main morphological variants and cell types of canine nodal lymphomas. Morphological features and differential diagnostic criteria for microscopic study of lymphomas were determined. The obtained data can be used in the every-day practices of a pathologist for morphological verification of canine lymphomas, and also become the basis for histological classification of tumours of lymphoid tissue in animals.

Keywords: lymphoma, nodal lymphoma, lymph node, micropreparation, tissue, lymphoblast, immunoblast, centrocyte, centroblast, dogs

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Оригинальное эмпирическое исследование

Морфологические свойства и гистогенез нодальной лимфомы у собак

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Аннотация

Введение. Одним из часто встречающихся видов онкопатологий являются лимфопролиферативные заболевания, в частности, лимфомы. Заболевания отличаются сложными механизмами развития, множеством этиологических факторов и разнообразием биологии их поведения. Кроме того, общность происхождения лимфоидных клеток делает различные типы лимфом морфологически сходными и затрудняет их диагностику. Золотым стандартом в диагностике онкологической патологии было, есть и будет морфологическое исследование. Однако для адекватной диагностики онкологических заболеваний у животных отсутствуют четкие морфологические критерии, гистологические классификации, характеризующие различные типы опухолей. Вышеизложенное диктует настоятельную необходимость в тщательном изучении различных морфологических типов лимфом и выявлении их дифференциально-диагностических признаков. Цель данного исследования — изучение морфологических признаков и гистогенеза нодальной лимфомы у собак для облегчения диагностики и разработки эффективных протоколов таргетной терапии.

Материалы и методы. Объектом исследования, проведенного на базе сети ветеринарных лабораторий «ВЕТЛАБ» (г. Москва) в период с января 2024 г. по январь 2025 г., стали лимфатические узлы собак с признаками поражения нодальной лимфомой. Все пробы (n = 30) прошли гистологическую пробоподготовку по стандартной общепринятой методике. Были получены гистологические препараты, окрашенные гематоксилином и эозином, и выполнена микроскопия. Проведена сравнительная оценка, и сопоставлены следующие морфологические критерии: форма роста опухоли, распространенность опухоли в ткани, строение опухолевых клеток, размер и расположение ядер, структура хроматина, наличие митозов, микроокружение опухолевых клеток.

Результаты исследования. Изучая особенности нодальных лимфом собак посредством микроскопии, выявили ряд существенных дифференциально-диагностических признаков, позволяющих разделять лимфомы на морфологические варианты: крупноклеточную и мелкоклеточную лимфому, иммунобластный, центробластный и лимфобластный клеточный тип. К прогностическим признакам отнесли: пролиферативный индекс, диффузную форму роста, клеточное микроокружение, васкуляризацию ткани опухоли.

Обсуждение и заключение. В ходе исследования выделили основные морфологические варианты и клеточные типы нодальных лимфом собак. Определили морфологические особенности и дифференциально-диагностические критерии для микроскопического изучения лимфом. Полученные данные могут быть использованы для морфологической верификации лимфом собак в повседневной практике патолога, а также лечь в основу гистологической классификации опухолей лимфоидной ткани у животных.

Ключевые слова: лимфома, нодальная лимфома, лимфатический узел, микропрепарат, ткань, лимфобласт, иммунобласт, центроцит, центробласт, собаки

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Introduction. Lymphoma is a widespread malignant neoplasm of haematopoietic and lymphoid tissues in animals and humans. The tumour develops in nodal and extranodal forms, and is not limited to any certain organ, which hinders timely diagnostics of the disease. In human medicine, there are classifications based on comparing clinical, morphological and immunological features resulting in distinguishing numerous variants of T- and B-cell lymphomas [1–3]. All tumours have a common lymphoid cell precursor, similar processes of activation or inhibition of signaling pathways, mutations in key genes, and are characterized by similar genetic disorders and structural abnormalities. All this enables using the new molecular diagnostic methods for clinical purposes to stratify patients into risk groups, develop prognostic and diagnostic models and search for new targets for drug therapy [4]. The common origin of various types of lymphomas complicates their morphological verification. Whereas, lymphoma diagnostic algorithm should begin with morphological examination of tumour cellular composition or its tissue. The objectives of morphological study also include identification and investigation of the prognostic factors; differential diagnostics of indolent and aggressive forms of lymphoma subject to histological transformations; studying the development of aggressive lymphoma during the evolution of an indolent clonal lymphoma [5].

In veterinary medicine, adequate diagnostics of oncological diseases in patients is complicated due to the lack of clear morphological criteria and histological classifications characterizing various tumour types. The development and identification of morphological criteria specific to various lymphoma types will not only improve the quality of differential diagnostics but also enhance the efficacy of subsequent therapeutic interventions. The aim of this study is to present a scientifically based approach to the morphological diagnostics of canine nodal lymphoma, based on the principle of the histogenetic origin of cells.

Materials and Methods. The study was conducted at the network of veterinary laboratories “VETLAB” (Moscow) from January 2024 to January 2025. The objects of the study were 30 lymph nodes in dogs affected by nodal lymphoma. Lymph nodes of various locations were examined; identification of the most common lymphoma locations was not the objective of the present study.

Specimens for morphological examination were selected based on the following criteria: medical history and clinical signs (lymph node enlargement and limited mobility, cachexia, dyspnea); the macroscopically visible signs of lymph node lesion caused by lymphoma (lymph node size greater than 2 cm in diameter, absence of a pattern on lymphoid tissue section, replacement of lymphoid tissue with structureless masses, lumpy surface of lymph nodes, ruptures of capsules); microscopy-based confirmation of nodal lymphoma.

Lymph nodes with macro- and microscopic signs of reactive inflammation, hyperplasia, or metastatic lesions caused by non-lymphoid tumours were excluded from the study. Macroscopic signs of reactive inflammation and hyperplasia in a lymph node include: lymph node size up to 2 cm, preservation of the pattern on the section, smooth lymph node surface and intact capsule. Macroscopic signs of metastatic lesions are as follows: partially preserved pattern on the section, limited tumour foci visible upon the section, and lumpy surface of a lymph node (in cases of multiple metastases).

For the study, a longitudinal incision was made across a lymph node, and up to 0.5 cm diameter section was removed from the tissue core and sent for examination [6].

Histological processing was performed according to the generally accepted protocol: 96% alcohol for 6 hours, xylene for 3 hours, paraffin for 3 hours [7, 8]. The specimens were embedded in a paraffin block using a Kedee KD-BMII automatic embedding system (*Kedee*, Russia). Microtomy of a paraffin block was performed using a Rotary 3004 M semiautomatic microtome (*Kedde*, Russia). Histological sections were cut at a thickness of 4 μm and fixed on glass slides.

The preparations were stained with hematoxylin and eosin according to the generally accepted protocol: xylene for 10 min, 96% alcohol for 15 min, Mayer’s hematoxylin for 20 min, hydrochloric acid for 10 sec, distilled water for 10 min, eosin solution for 10 min [9], using a Shandon Varistain 24–4 autostainer (*Leica*, Germany).

Microscopy was performed using a Microscreen microscope (“Minimed”, Russia) at ×100, 200, and 400 magnification.

The following factors were assessed: tumour growth pattern (diffuse, nodular, etc.); cell-related issues (small or large cells, polymorphic cell composition, anaplastic or

blastic/blastoid cell morphology, presence or absence of multinucleated cell forms, characteristics of nuclei structure); and the presence of reactive and residual components. The study adhered to the 5th edition of the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (2022).

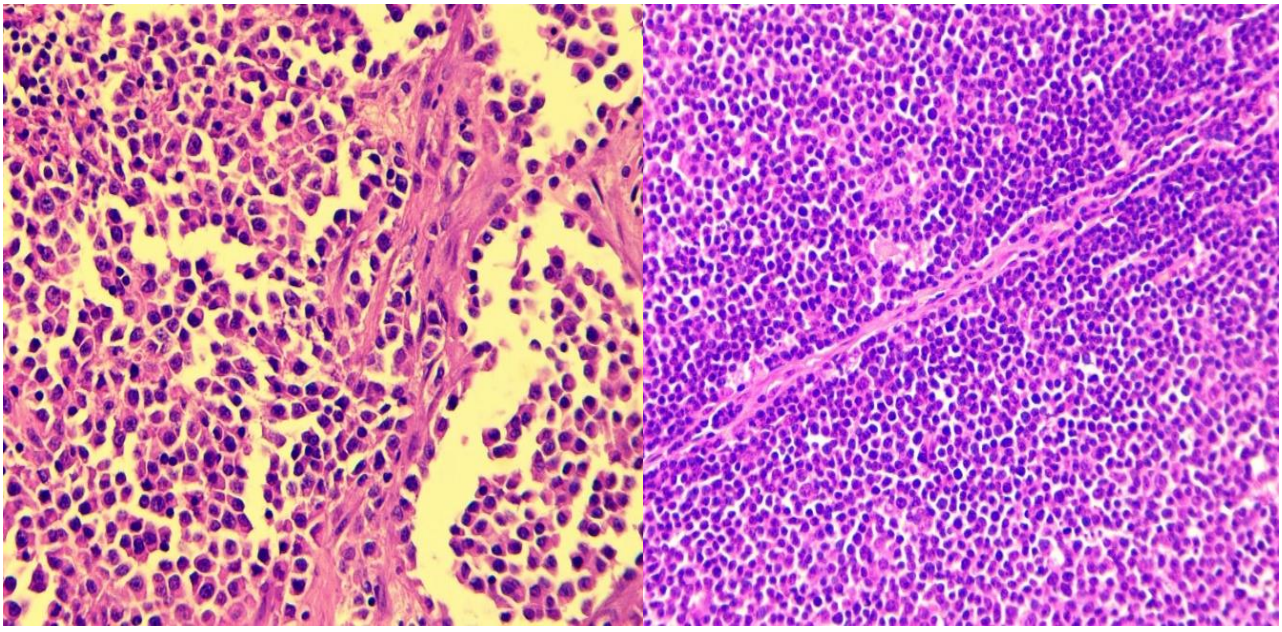
Results. The data we obtained during morphological examination of canine nodal lymphomas are presented in Table 1, which shows that the large-cell lymphoma variant was detected most frequently. All tumours had a diffuse growth pattern.

Large-cell tumours had large, eccentrically arranged nuclei. The condensed chromatin structure resulted in nucleoli being differentiated within the nuclei (Fig. 1). At 400x magnification, 5 to 10 mitoses were detected per field of view.

Table 1

Morphological classification of canine nodal lymphoma variants

Morphologic classification of canine nodal lymphomas	Number of cases	%
Small-cell lymphoma	6	20
Large-cell lymphoma	24	80



a) *b)*

Fig. 1. *a* — large-cell nodal lymphoma; *b* — fibrous tissue strand in the center of tumour. Hematoxylin and eosin staining, ×400 magnification

All large cell nodal lymphomas showed thin fibrous septa, which constitute the tumour stroma. These septa are likely to be the residues of the interfollicular septa of a lymph node (Fig. 1 *b*).

Nodal lymphomas, composed of small cells, had a number of features: small or medium-sized eccentrically arranged nuclei, tightly packed heterochromatin within the nuclei, and poor differentiation of nucleoli. At ×400 magnification, no more than 5 mitoses per field of view and a large number of apoptotic bodies were detected (Fig. 2).

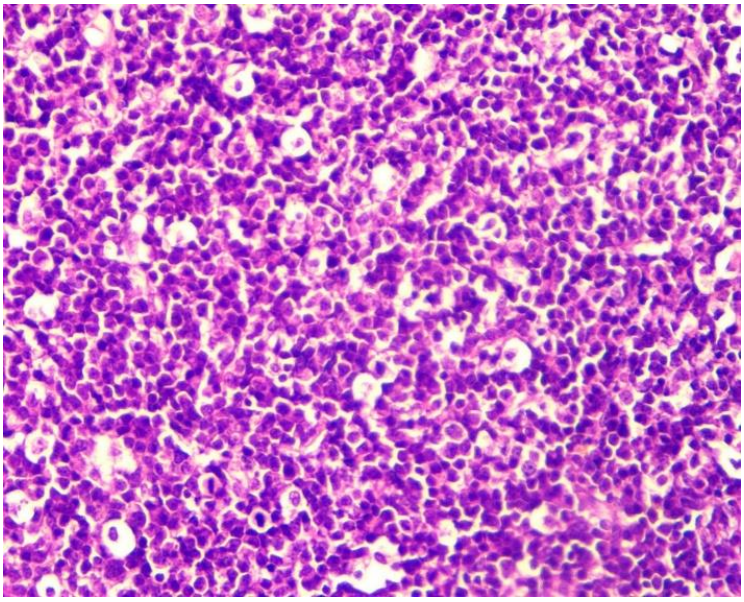


Fig. 2. Small-cell nodal lymphoma. Hematoxylin and eosin staining, ×400 magnification

The results of the study on the histogenetic origin of tumour cells indicate the presence of three cell types: lymphoblastic, immunoblastic and centroblastic (Table 2).

Table 2 shows that the centroblastic cell type of nodal lymphoma is the most common. It is worth noting that immunoblastic and centroblastic types were detected in large-cell variant of lymphoma.

The immunoblastic cell type was characterised by the presence of atypical immunoblasts in the tumour infiltrate — large cells with round, uneven, eccentric nuclei with a single large nucleolus being differentiated, and condensed chromatin loop pattern. The membrane of nucleolus was uneven, often with thin chromatin threads attached to it. The cytoplasmic rim of these cells was quite wide and pale (Fig. 3 *a*).

The centroblastic cell type was characterized by the presence of atypical centroblastic cells in the tumour infiltrate, which differed drastically from immunoblasts. The morphological features of centroblasts included large, round vesicular nuclei, vesicular chromatin pattern, and 2–3 nucleoli located near the nuclear membrane, as well as moderate amount of cytoplasm (Fig. 3 *b*).

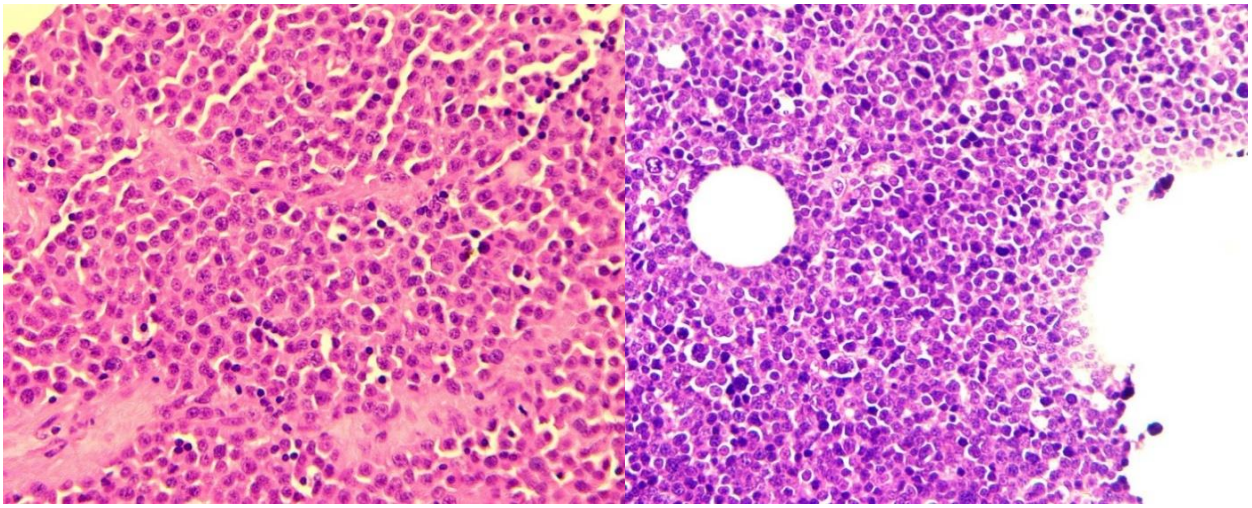
The lymphoblastic cell type was detected in small-cell tumours and was characterized by the presence of atypical lymphoblasts in the tumour infiltrate—small cells with round nuclei containing compact heterochromatin, undifferentiated nucleolus, uneven nuclear membrane surface and a narrow cytoplasmic rim (Fig. 4 *a*).

All tumours, regardless of cell type, had poor vascularization. Lymphocytes and plasma cells were present in the microenvironment. Small-cell tumours were infiltrated with macrophages. One case of a large number of eosinophils contained in the microenvironment (Fig. 4 *b*) was identified.

Table 2

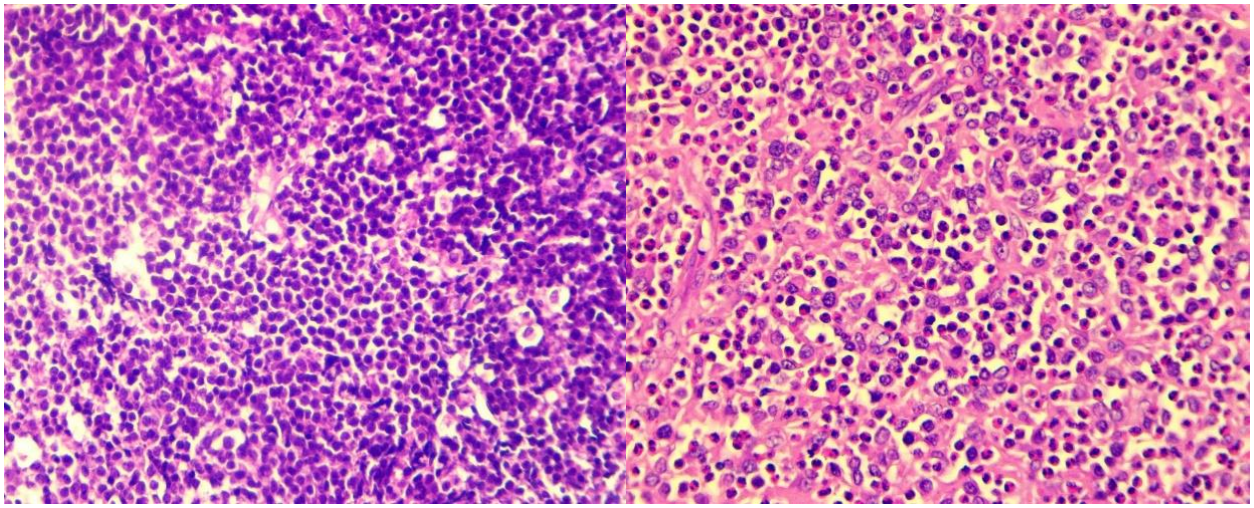
Cell types of canine nodal lymphomas

Cell type	Number of cases	%
Lymphoblastic	6	20.0
Immunoblastic	10	33.3
Centrablastic	14	46.7



a) *b)*

Fig. 3: *a* — immunoblastic cell type of large-cell nodal lymphoma;
b — centroblastic cell type of large-cell nodal lymphoma. Hematoxylin and eosin staining, ×400 magnification



a) *b)*

Fig. 4: *a* — lymphoblastic cell type of small-cell nodal lymphoma; *b* — immunoblastic cell type of large-cell lymphoma, pronounced tissue infiltration with eosinophils. Stained with hematoxylin and eosin, magnification ×400

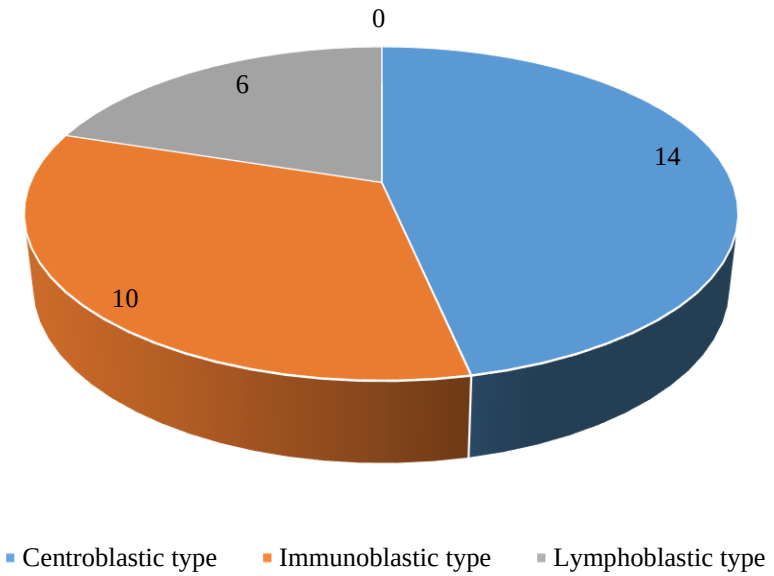


Fig. 5. Results of the study of cell types of canine nodal lymphomas (n = 30)

Discussion and Conclusion. A study of the pathological features of canine nodal lymphoma revealed that all tumours have a diffuse growth pattern. Large-cell and small-cell variants can be identified with regard to morphological classification, and according to histogenetic classification, the tumours are classified into lymphoblastic, centroblastic and immunoblastic. Morphological differential diagnostic criteria include: tumour cell size, size of nuclei, chromatin structure, differentiation of nucleoli, the number of mitoses per field of view and the presence of apoptotic bodies.

According to available literature [10, 11], the large-cell variant of lymphoma is most common in humans, which is consistent with the results of our study in dogs. This may be due to the predominance of large B-cells in the body, which are activated during antigenic stimulation.

Analysis of data obtained during the study of tumour cell types shows that the centroblastic cell type is identified most frequently (Fig. 5). It can be assumed that this happens within the process of antigen stimulation, due to B-cells initiating a reaction in the germinal center of the follicle, in which they get transformed into rapidly proliferating centroblasts. Whereas, proliferating cells are more often subject to tumour transformation. In human medicine, diagnostic criteria for lymphoblastic lymphoma include the predominance of atypical lymphoblasts in the tumour substrate and the presence of macrophages in the microenvironment [13–16]. Our data obtained in dogs confirm the above mentioned findings available in the scientific sources.

The centroblastic cell type of lymphoma is characterized by large, round nuclei containing several nucleoli near the nuclear membrane, whereas atypical immunoblasts, which make up the cellular substrate of immunoblastic lymphoma, have large, uneven nuclei containing a single nucleolus. The cytoplasmic rim of atypical immunoblasts is more abundant than that of centroblasts. Similar differential diagnostic features were mentioned by L.G. Babicheva and I.V. Poddubnaya in their description of nodal lymphoma in humans [17].

According to the 5th edition of the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (2022) currently used in human medicine, diffuse large-cell lymphoma has 14 clinical and biological forms [17–20]. These forms have not been studied or identified in animals; therefore, the results of our study may pave the way for the exploration of morphological features and differential diagnostic criteria for a larger number of nosological forms of lymphoid tissue tumours in veterinary pathology. Our study confirms the histogenetic origin of tumour cells, which should be reflected in the histological classification.

References

1. Kovrigina AM, Probatova NA. *Hodgkin's Lymphoma and Large Cell Lymphomas*. Moscow: Meditsinskoe informatsionnoe agentstvo; 2007; 216. (In Russ.)

2. Ursu D, Gudumac E, Bernic Ja, Tîbîrnă A, Railean S, LUPAN R., et al. Diagnostic Peculiarities of Lymphomas in the Cervical Region in Children. *Bulletin of the Academy of Sciences of Moldova. Medical Sciences*. 2023;76(2)36-41. https://doi.org/10.52692/1857-0011.2023.2-76.06

3. Kudacheva NA, Bespalova TYu. Clinical and Morphological Diagnosis of the Lymphoma among Dogs. *International Research Journal*. (In Russ.) 2017;(9–2(63)):14–17. https://doi.org/10.23670/IRJ.2017.63.006

4. Kovrigin AM. Does the Mutation in the Myd88 L265P Gene in Diffuse Large B-Cell Lymphomas Have Independent Diagnostic Significance? *Gematologiya i transfuziologiya (Russian Journal of Hematology and Transfusiology)*. 2024;69(4):499–501. (In Russ.) https://doi.org/10.35754/0234-5730-2024-69-4-499-501

5. Magomedova AU, Kovrigina AM, Mangasarova YaK, Kravchenko SK, Nikulina EE, Obukhova TN, et al. Transformation of Indolent Lymphomas into Diffuse Large B-Cell Lymphoma. *Gematologiya i transfuziologiya (Russian Journal of Hematology and Transfusiology).*2024;69(1):112–120. (In Russ.) https://doi.org/10.35754/0234-5730-2024-69-1-112-120

6. Yunusova YuR, Plokhova VA, Shuvalova TV, Poletaeva SV, Sukhachev PA, Kirichenko ND. *Handbook on Clinical Pathological Anatomy. A Textbook for Students of Higher Educational Medical Institutions*. Samara: Polygraphic Association “Standard”; 2020. 121 p. (In Russ.)

7. Asaulenko ZP, Chukhrai SM, Yakovleva AI, Alymova EV, Buchaka AS, Pavlinov GB. The Effect of a Shortened Standard Specimen Preparation Procedure on the Quality of Histological Preparations. In: *Collection of Abstracts of the Second Russian Congress of Laboratory Histotechnology with International Participation “Histotechnology”*. Moscow; 2024. P. 31–34. (In Russ.)

8. Peshkov MV, Dygalo II. Histological Tissue Processing Procedure, by Using Isopropanol and Mineral Oil. *Arkhiv patologii (Russian Journal of Archive of Pathology).* 2009;71(3):39–41. (In Russ.)

9. Bezrukov AV, Belanov ME, Borisoglebsky AA, Bukharov GA, Kravtsov PA, Kuznetsov MV, et al. New Family of Automated Slide Stainers. *Polyclinic*. 2023;(4(1)):28–31. (In Russ.)

10. Kalenik OA. Results of Chemoimmunotherapy in Patients with GCb-Subtype Diffuse B-Cell Non-Hodgkin Lymphoma. *Journal of the Grodno State Medical University*. 2022;20(6):599-602. (In Russ.) https://doi.org/10.25298/2221-8785-2022-20-6-599-602

11. Vadasz B, Wolniak K, Sukhanova M, Chen YH, Behdad A. Leukemic Presentation of Anaplastic Large Cell Lymphoma: A Diagnostic Challenge Mimicking T-Cell Prolymphocytic Leukemia. *AJSP Reviews & Reports*. 2022;27(3):119–122. https://doi.org/10.1097/pcr.0000000000000511

12. Rukavitsyn OA (Ed.). *Hematology: National Guidelines*. 2nd Edition, Revised and Supplemented. Moscow: GEOTAR-Media Publ.; 2024. 916 p. (In Russ.) https://doi.org/10.33029/9704-8188-2-GEM-2024-1-916

13. Lipartia MG, Ashurova DT, Daminova MN. Non-Hodgkin's Lymphoma in Children. *Eurasian Bulletin of Pediatrics*. 2022;1(12):39–42. URL: https://goo.su/zko6XF (accessed: 01.09.2025)

14. Lipartia MG, Daminova MN. Optimization of Diagnostics of Anaplastic Large Cell Lymphoma in Children. *Journal a New Day in Medicine*. 2022;1(39):119–122. URL: https://newdayworldmedicine3d.com/upload_files/journal_article/65b23c7d9dee3.pdf (accessed: 01.09.2025)

15. Krylova YaV, Mikhailova NB, Lepik EE, Lepik KV, Beynarovich AV, Smirnova AG, et al. T-Cell Prolymphocytic Leukemia (T-PLL): A Clinical Case and Review of the Literature. *Cellular Therapy and Transplantation*. 2023;12(4):43–49. https://doi.org/10.18620/ctt-1866-8836-2023-12-4-43-49

16. Quintanilla-Martinez L, Sander B, Chan JKC, Xerri L, Ott G, Campo E, et al. Indolent Lymphomas in the Pediatric Population: Follicular Lymphoma, IRF4/MUM1+ Lymphoma, Nodal Marginal Zone Lymphoma And Chronic Lymphocytic Leukemia. *Virchows Archiv*. 2016;468:141–157. https://doi.org/10.1007/s00428-015-1855-z

17. Babicheva LG, Poddubnaya IV. Heterogeneous Diffuse Large B-Cell Lymphoma: Accurate Diagnosis as a Key to Successful Therapy. A review. *Journal of Modern Oncology*. 2023;25(2):168–177. (In Russ.) https://doi.org/10.26442/18151434.2023.2.202237

18. Igonin YuA, Dolgov OYu, Khavaneva OV. *Lymphomas: A tutorial*. Cheboksary: Chuvash State University Named after I.N. Ulyanov; 2020. 104 p. (In Russ.)

19. Krivolapov YuA, Leenman EE. *Morphological Diagnostics of Lymphomas*. St. Petersburg: "Kosta" Publ.; 2006. 208 p. (In Russ.)

20. Baram DV, Asaulenko ZP, Spiridonov IN, Krivolapov YuA. WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues, 2022 (5th Edition): Lymphoid Tumors. *Russian Journal of Archive of Pathology*. 2023;85(4):24–31. (In Russ.) https://doi.org/10.17116/patol20238504124

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ANIMAL PATHOLOGY, MORPHOLOGY, PHYSIOLOGY, PHARMACOLOGY AND TOXICOLOGY

ПАТОЛОГИЯ ЖИВОТНЫХ, МОРФОЛОГИЯ, ФИЗИОЛОГИЯ, ФАРМАКОЛОГИЯ И ТОКСИКОЛОГИЯ

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Development of a Methodology for Obtaining Connective Tissue Bioequivalent from Rabbits

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Abstract

Introduction. The potential of cell therapy and tissue engineering technologies in veterinary medicine is quite high. However, the use of these technologies in the Russian Federation is currently limited due to the absence of standardized protocols for cell isolation, donor selection and creation of tissue equivalents. Development of a methodology for obtaining connective tissue bioequivalent is particularly relevant for clinical veterinary medicine, as connective tissue constitutes up to the half of the body weight and ensures the normal functioning of skin, mucous membranes, and internal organs of animals. The aim of this study is to develop a methodology for obtaining connective tissue equivalent from rabbits.

Materials and Methods. The study was conducted at Don State Technical University (DSTU) from November 13, 2023 to March 17, 2025. The objects of the study were multipotent mesenchymal stem cells (MMSCs) and fibroblasts from adult male rabbits. Enzymatic methods were used to isolate MMSCs from the greater omentum and fibroblasts from the animal skin. Stable cell lines were obtained, and their differentiation potential was studied *in vitro* during myogenic and lipogenic induction. Connective tissue equivalents were created using 3D extrusion bioprinting, their morphological properties were studied by means of light, confocal, and electron microscopy.

Results. Application of the sets of factors during induction ensured the adipogenic and myogenic differentiation of MMSCs. Adipogenic differentiation came along with formation of lipid droplets, while myogenic differentiation — with formation of myotubes. 3D bioprinting enabled creation of connective tissue equivalents with maintained cell viability, developing intercellular contacts, and active secretion for at least 72 hours.

Discussion and Conclusion. A new approach to obtaining connective tissue equivalents from rabbits was developed by optimizing MMSCs isolation and differentiation techniques. The resulting constructs demonstrated morphological and functional activity, thus, confirmed their potential for using in clinical veterinary medicine for regeneration of connective tissue and for experimental studies.

Keywords: tissue engineering, cell culture, mesenchymal stem cells, MMSCs, tissue equivalent, bioequivalent, rabbit, lipoblasts, fibroblasts, transmission electron microscopy

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Оригинальное эмпирическое исследование

Разработка методики получения биоэквивалента соединительной ткани кролика

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Аннотация

Введение. Клеточная терапия и тканевая инженерия обладают значительным потенциалом применения в ветеринарии, однако использование данных технологий в Российской Федерации в настоящее время ограничено из-за отсутствия стандартизированных протоколов выделения клеток, подбора доноров и создания тканевых эквивалентов. Особую актуальность для клинической ветеринарии имеет разработка методики получения биоэквивалента соединительной ткани, ведь она составляет до половины массы тела и является основой для нормального функционирования кожи, слизистых оболочек и внутренних органов животных. Цель исследования — разработать методику получения тканевого эквивалента соединительной ткани кролика.

Материалы и методы. Исследование проведено на базе ДГТУ в период с 13 ноября 2023 г. по 17 марта 2025 г. Объектом исследования выступили мультипотентные мезенхимальные стволовые клетки (ММСК) и фибробласты взрослых самцов кролика. Ферментными методами были выделены ММСК из большого сальника и фибробласты из кожи животных. Получены стабильные культуры клеток, исследован их дифференцировочный потенциал при миогенной и липогенной индукции *in vitro*. С применением экструзионной 3D-биопечати созданы эквиваленты соединительной ткани, морфологические свойства которой изучены с помощью световой, конфокальной и электронной микроскопии.

Результаты исследования. Индукция наборами факторов обеспечила дифференцировку ММСК в адипо- и миогенном направлении. Адипогенная дифференцировка сопровождалась образованием липидных капель, миогенная — формированием миотрубочек. 3D-биопечать позволила сформировать эквиваленты соединительной ткани с сохранением жизнеспособности клеток, развитием межклеточных контактов и активной секрецией в течение не менее 72 ч.

Обсуждение и заключение. Разработан новый подход к получению тканевых эквивалентов соединительной ткани кролика благодаря оптимизации методов выделения и дифференцировки ММСК. Сформированные конструкты продемонстрировали морфофункциональную активность, что подтверждает перспективность их применения в клинической ветеринарии для регенерации соединительной ткани и в экспериментальных исследованиях.

Ключевые слова: тканевая инженерия, культура клеток, мезенхимальные стволовые клетки, ММСК, тканевой эквивалент, биоэквивалент, кролик, липобласты, фибробласты, трансмиссионная электронная микроскопия

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Introduction. Nowadays, cell technologies based on the use of cell cultures and tissue equivalents are being actively implemented in veterinary medicine. This can be explained by the need to reduce the ethical burden within the experimental research, by improvement of the *in vitro* experimental models, as well as by development of the regenerative medicine and implementation of the human medicine achievements into veterinary medicine [1, 2]. The benchmark for the development of this scientific field is implementation of the 3R bioethical principles — Replacement, Reduction, Refinement [3] — aimed at optimisation of the use of animals in experiment to stimulate transition to *in vitro* models [4]. Cell cultures and tissue equivalents used as experimental models represent practical implementation of these principles enabling not only to replace animals in research and reduce their number, but also to enhance accuracy and reproducibility of results through standardization [5].

A tissue equivalent (bioequivalent) is a bioengineered construct — an analogue of a tissue or organ. The key properties of a tissue equivalent are its cellular composition, the composition and properties of the intercellular substance, and a specific histotypic architecture [6].

Connective tissue constitutes approximately 50% of animal body weight and performs critical functions: supporting-motor, reparative and metabolic. Its cells influence the differentiation of epithelial and muscle cells and angiogenesis. Connective tissue is involved in the most functions of the body in the normal condition, and is always involved in pathological processes and participates in regeneration [7].

Therefore, the development and clinical implementation of connective tissue bioequivalents for the temporary or permanent replacement of defects and damages of native tissues is highly requested in the regenerative medicine.

Since 2025, the U.S. Food and Drug Administration (FDA) has officially recommended the use of *in vitro* platforms for toxicologal studies and evaluation of the efficacy of new pharmacological agents emphasising that cell models, organoids, and tissue equivalents more accurately reproduce the molecular and cellular mechanisms of pathogenesis, reduce inter-experimental variability, and increase the predictive capacity of the data obtained [8, 9]. The integration of *in vitro* systems at the preclinical stage of drug development makes it possible to reduce the number of rejections at later stages of clinical trials and optimize risk management when testing new therapeutic strategies [5, 9]. Modern research confirms that the use of cell cultures provides reproducible and statistically reliable data using smaller groups of animals compared to the traditional *in vivo* models, which minimizes bioethical risks and increases the rationale of scientific approaches [5]. Alongside, the direction of modifying cellular systems is developing to create highly accurate models of specific pathologies, including infectious, oncological and metabolic ones, which enables in detail study of the molecular mechanisms of pathogenesis and search for new targets for potential medicinal compounds [10, 11].

In veterinary regenerative medicine, the use of multipotent mesenchymal stem cells (MMSCs) is being actively implemented for the treatment of osteoarthritis in horses and spinal injuries in dogs and cats [12]. The clinical efficacy of MMSCs has been verified in studies in dogs, where intraarticular administration of autologous MMSCs improved the function of a joint and reduced pain [13, 14]. Similar results have been obtained in the treatment of horses with ligament injuries [14–16]. Stem cell therapy proved to be efficient in the treatment of acute and chronic skin lesions in dogs [12].

3D bioprinting technology is getting improved, this enables creation of multilayer tissue constructs from cells and biocompatible polymers that are similar in architecture and mechanical properties to the native body tissues. The use of bioprinted implants for the restoration of bone defects in pets improves osseointegration and accelerates healing [17]. 3D bioprinted tissue constructs are used in screening and research within antitumor and radiation therapy in animals. Phantoms made from tissue equivalents can be used to measure and verify radiation dose distribution, ensuring the accuracy of radiation treatment plans for pets and calibrating equipment [18].

In veterinary medicine, three-dimensional tissue equivalents allow creating the personalized animal tumor models using various cells and biomarkers for the selection of targeted chemotherapy and immunotherapy in the treatment of oncological diseases [12]. Also, tissue equivalents of skin and connective tissue, constructed from the appropriate cell cultures and matrix, can be used to treat animal burns and are a promising direction in the treatment of wounds in animals in general [19, 12]. Significant experience in this field has been accumulated worldwide [20, 12], however, in the Russian Federation, there is still no production of affordable tissue equivalents with cells for the treatment of wounds in both humans and animals, since standardized protocols for cell isolation, donor selection, and obtaining bioequivalents have not been yet developed. Thus, the aim of the study is to develop a methodology for creating connective tissue equivalents, which performs vital functions in the body, from rabbit biomaterials.

Materials and Methods. The study was conducted from November 13, 2023, to March 17, 2025, at the Faculty of Bioengineering and Veterinary Medicine, DSTU (Rostov-on-Don). All cell culture experiments were performed in the Cell Technologies Laboratory; the laboratory animals were maintained and handled in the faculty's vivarium in accordance with the acting regulations.

Adult male Soviet Chinchilla breed rabbits (*Oryctolagus cuniculus domesticus*) served as donor animals (n=3). To isolate MMSCs, 2 g of adipose tissue fragments were collected from the greater omentum via percutaneous biopsy; abdominal skin biopsies were used to isolate fibroblasts. To isolate MMSCs, fragments of greater omentum adipose tissue were incubated for 60 min in a 0.2% collagenase solution in Dulbecco's phosphate-buffered saline (DPBS) at 37°C with constant stirring. After filtering the resulting suspension through a cell strainer, the cell fraction was pelleted by centrifugation. The pellet was transferred to culture flasks containing DMEM/F12 medium supplemented with 10% fetal bovine serum (FBS) and 1 ng/ml recombinant basic human fibroblast growth factor-2. The isolated cell culture was subcultured for five passages and then cryopreserved.

Fibroblasts were isolated from skin biopsies. After antiseptic treatment, the biopsies were aseptically excised with scissors and transferred to a trypsin solution preheated to 37°C for 15 minutes to dissociate the cells. The cells were then pelleted by centrifugation, placed in culture flasks containing DMEM medium supplemented with 10% FBS, antibiotics, and antifungals, and incubated at 37°C with 5% CO2. Taking advantage of fibroblasts' ability to rapidly adhere to the flask surface, to separate them from other cell types, the flasks were washed after 4 hours to remove unattached cells and replaced with fresh nutrient medium. The procedure was repeated after 2 hours, after which the isolated cells were cultured to a subconfluent monolayer. Fibroblast subculture was performed without the addition of antibiotics or antifungals.

MMSCs were subjected to lipogenic and myogenic differentiation. The first was used to obtain lipoblasts, which

are necessary for the formation of a bioequivalent of connective tissue. Myogenic differentiation served as a marker of the multipotency of the isolated stem cells.

DMEM medium containing 4 mg/l glucose, 10% FBS, 1 μmol dexamethasone, 100 μmol indomethacin, 500 μmol 3-isobutyl-1-methylxanthine, and 10 μg/ml insulin were used as adipogenic differentiation inducers [21]. The cells were exposed to the inducers for 10 days, after which they were maintained in standard DMEM medium. As a negative differentiation control, MMSCs were cultured in complete DMEM medium without inducers. The formation of lipid droplets in the cell cytoplasm served as a differentiation marker [22].

DMEM medium containing 4 mg/l glucose, 10% FBS, 10 μmol 5-azacytidine, and 50 μmol hydrocortisone were used to induce myogenic differentiation. The cells were exposed to inducers for 15 days, after which they were maintained in standard DMEM medium. As a negative differentiation control, MMSCs were cultured in complete DMEM medium without inducers. Cell fusion to form myotubes was considered a marker of myogenic differentiation [23].

An improved AI-based algorithm, YOLOv8S-seg, developed and described previously by us [24], was used to count cells at all stages of culture.

A 3D bioprinting method was used to create connective tissue equivalents. To prepare the bioink, sodium alginate powder was sterilized under ultraviolet light in a biological safety cabinet for 40 minutes. The components were then dissolved in calcium- and magnesium-free DPBS at a concentration of 30 mg/ml. Three ml of the resulting hydrogel was transferred to a 5 ml syringe, and fibroblasts and lipoblasts were added at a 1:1 ratio, at a rate of 2 x 10^6 cells per 1 ml of hydrogel, by mixing them between two syringes containing the hydrogel, connected by an adapter.

The resulting bioink was transferred to the injector of a 3D bioprinter assembled on the basis of Anet A8 (*Anet*, China) and modified in-house as described previously in [21], and the printing process was carried out according to a given digital model with the following parameters: object dimensions 30 × 40 × 3 mm, layer height — 0.2 mm, number of perimeters — 0, number of solid layers at the bottom — 0, number of solid layers at the top — 0, fill — 8%, fill type — rectilinear, fill angle — 90°, fill speed — 10 mm/s, nozzle diameter — 0.2 mm, rod diameter — 1.2 mm, extruder feed multiplier — 1.3. Printing was carried out in a microbiological safety cabinet at room temperature in Petri dishes. After printing, the resulting scaffolds were filled with CaCl2 solution (100 mg/ml) for 10 min to stabilize the alginate. After hardening, the scaffolds were transferred to Petri dishes with DMEM medium and placed in a CO2 incubator at 37 °C for 72 h with the medium changed every 12 h.

After 72 hours of incubation, samples were collected from random sections of the tissue equivalents for microscopic analysis and prepared accordingly. The morphological properties of the resulting tissue equivalents were examined using an Olympus BX53 light microscope (*Olympus*, Japan), an Infinity Line confocal laser scanning microscope (*Abberior Instruments*, Germany), and a JEM-1011 transmission electron microscope (*Jeol*, Japan).

Results. The cell isolation and differentiation protocols used allowed us to obtain stable cell cultures that retained their phenotype and growth dynamics during subculture for 25 passages for fibroblasts and lipoblasts and 12 passages for myosatellite cells (Fig. 1).

The appearance of the Ca2+-stabilized construct after 3D bioprinting is shown in Fig. 2.

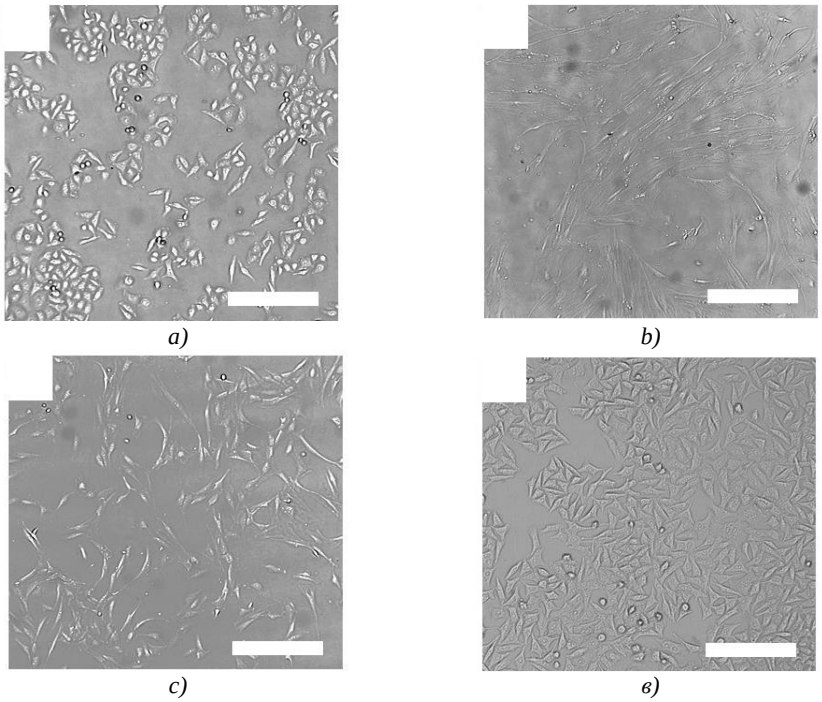


Fig. 1. Light microscopy of the obtained cell cultures, scale bar represents 200 μm: *a* — MMSCs; *b* — myosatellite cells; *c* — fibroblasts; *d* — lipoblasts

Fig. 2. Appearance of the 3D-bioprinted construct stabilized by Ca^{2+}

Figure 3 shows a micrograph of semi-thin sections of a fragment of the construct after 72 hours of cultivation. It contains round and spindle-shaped cells uniformly distributed within the matrix, with elongated processes and cytoplasmic outgrowths.

Three-dimensional reconstruction of a SYTOX® Green-stained tissue equivalent fragment after 72 h of cultivation (Fig. 4) demonstrates the integrity of cell nuclei and normal chromatin density

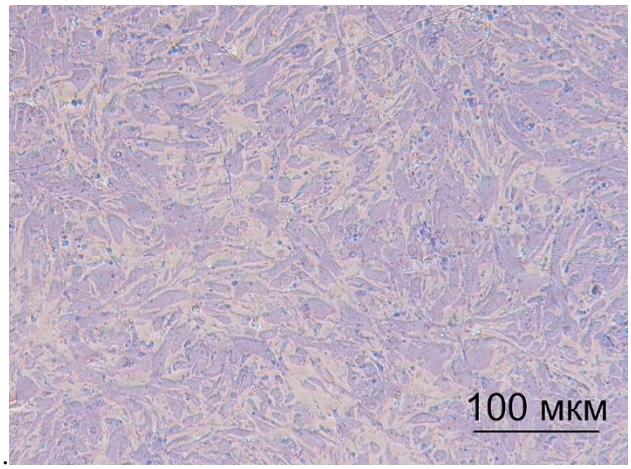


Fig. 3. Semi-thin section of tissue equivalent after 72 hours of cultivation, stained with methylene blue

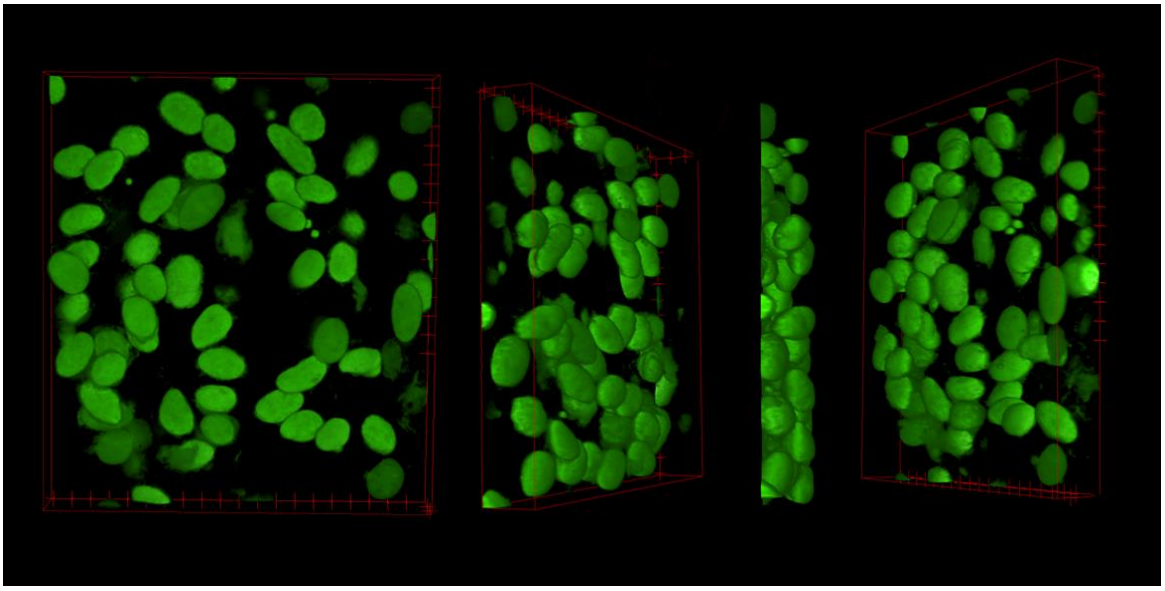


Fig. 4. Confocal laser scanning microscopy of a fragment of the construct stained with SYTOX® Green. Three-dimensional reconstruction was performed using ImageJ (Rasband, 1997–2018)

Transmission electron microscopy data reveal the ultrastructure of cells within the tissue equivalent and their intercellular interactions (Figs. 5–6). Electron diffraction patterns reveal two cell types with ultrastructural characteristics of fibroblasts and lipoblasts. Both cell types are spindle- or triangular-shaped, with branched, thin processes directed primarily along the cell bodies. In addition to these processes, the cells have irregularly shaped cytoplasmic outgrowths. A high cell density within the construct is also noted, indicating an increase in their number over 72 hours of cultivation, as such a density is unattainable at the initial concentration used during printing (2 × 106 cells per 1 ml of hydrogel). The cells within the construct are tightly adjacent to each other and are in contact with the membranes of their cell bodies and processes (Fig. 5). The cells contain regularly shaped nuclei with finely dispersed chromatin, with one or two large nucleoli visible within the nuclei—a sign of fibroblast activity [25–27]. In some cases, the cell nuclei had invaginations and protrusions of the karyolemma. The fibroblast cytoplasm is rich in organelles, reflecting a high level of intracellular metabolism. Numerous mitochondria, rough endoplasmic reticulum, the Golgi complex, lysosomes, centrioles, and multivesicular bodies are visible within the cytoplasm. The lipoblast cytoplasm contains numerous inclusions in the form of rounded osmophilic droplets, further confirming the lipogenic differentiation of MMSCs. Cells have a well-developed network of endoplasmic reticulum, which occupies most of the cytoplasm and is visualized as stacks of convoluted tubes and elongated cisterns studded with ribosomes on the side facing the cytoplasm. Ribosomes not attached to the reticulum are also evenly distributed throughout the cytoplasm.

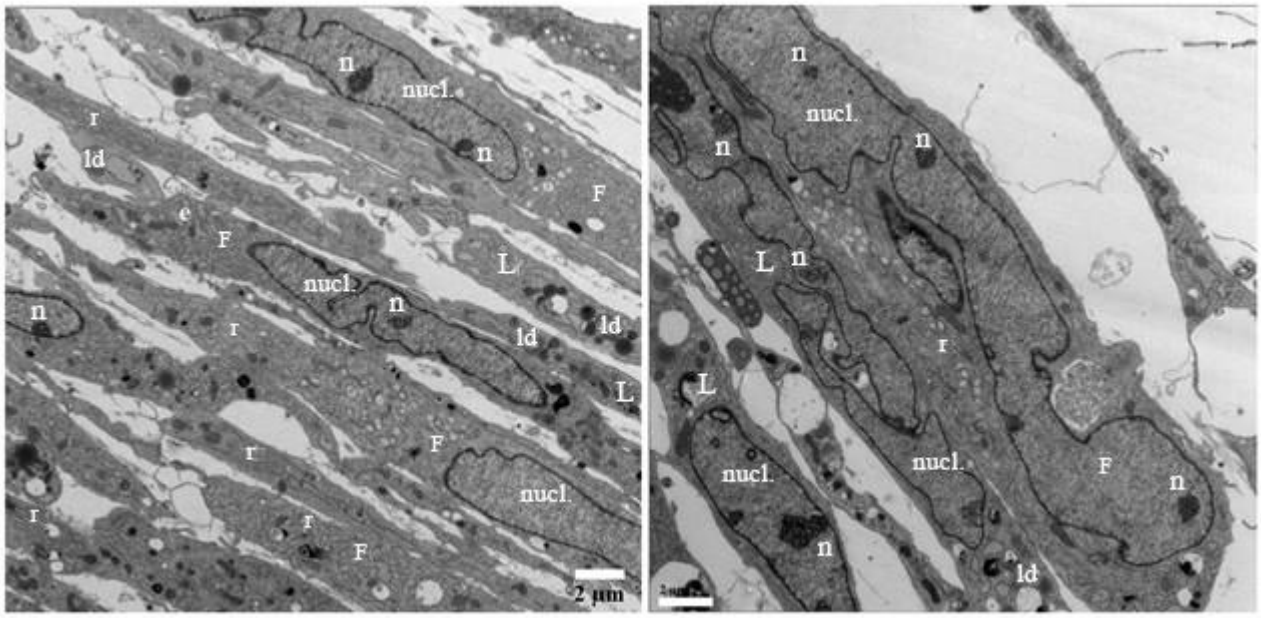
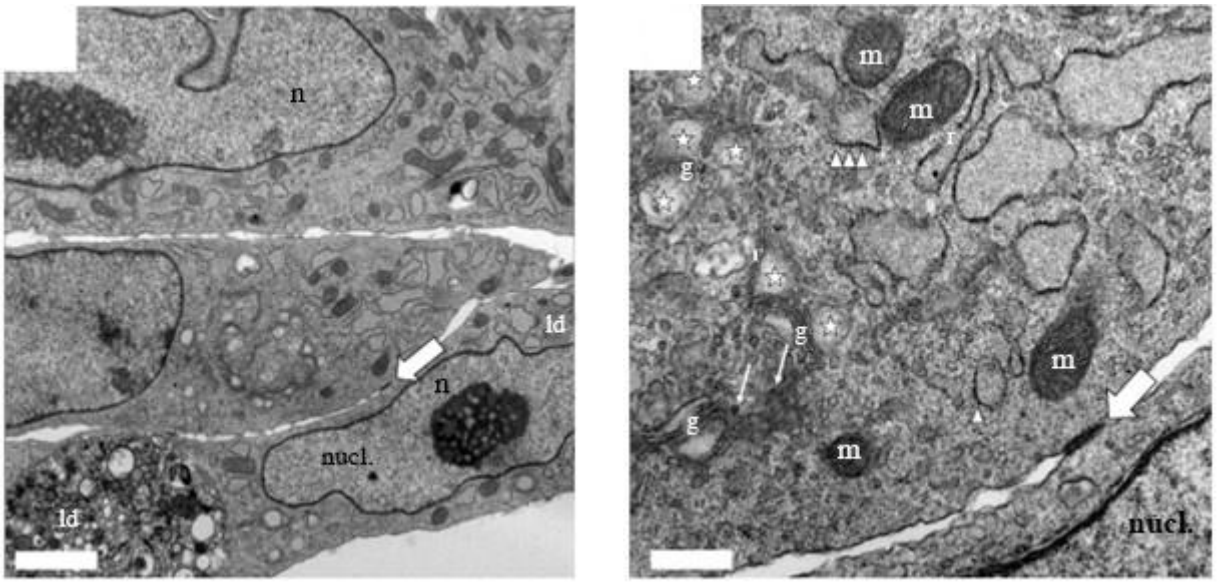


Fig. 5. Transmission electron microscopy of cells within a tissue equivalent, contrasted with osmium tetroxide, lead citrate, and uranyl acetate, scale bar represents 2 μm: F — fibroblast, L — lipoblast, nucl. — nucleus, n. — nucleolus, r —rough endoplasmic reticulum, ld— lipid droplets

Electron diffraction patterns of fibroblasts at higher magnification reveal activity of the Golgi complexes synthesizing procollagen (Fig. 6 *a*). Dictyosomes, transport vesicles, and cisternae are visible within the Golgi complexes. Some cisternae are dilated and filled with an osmium-dense, electron-dense substance resembling tangled threads, likely procollagen molecules. The contents of the vesicles gradually condense and transform into transport vacuoles, which collect into secretory granules on the side of the Golgi complex facing the plasma membrane. This pattern of Golgi complex activity also indicates high secretory activity of the cells within the construct for 72 hours.

Another important visualized element of intercellular interaction is the presence of tight junctions (Fig. 6 *a, b*), which appear as two osmium-filamentous seals with an intercellular cleft at the junction of the membranes of adjacent cells. A tight junction is a highly specialized, selective barrier that ensures direct contact between the proteins of two adjacent plasma membranes and allows groups of cells to function as structural units. Moreover, in the tissue equivalent sample, tight junctions were detected both between cells of the same type (fibroblast/fibroblast and lipoblast/lipoblast) and between cells of different types: for example, Fig. 6 *a, b* shows a tight junction between a fibroblast and a lipoblast.



a) *b)*

Fig. 6. Transmission electron microscopy showing signs of cell proliferation within the printed tissue equivalent after 72 hours of cultivation, contrasted with osmium tetroxide, lead citrate, and uranyl acetate, the *a* scale corresponds to 2 μm, the *b* scale to 0.5 μm. *a* — ultrastructure of the active fibroblast region. The center of the electron diffraction pattern is occupied by the Golgi complex region. Asterisks mark spherical expansions. The Golgi complex cisterns contain osmium-dense material in the form of tangled threads, which may be procollagen molecules. Vesicles with condensed contents are indicated by thin arrows: nucl. — nucleus, n — nucleolus, g — Golgi complex, m — mitochondrion, r — rough endoplasmic reticulum, ld — lipid droplets

A well-developed endoplasmic reticulum network [28], high activity of the Golgi apparatus [29], and numerous ribosomes indicate active protein synthesis processes and confirm the viability and good survival of cells within the tissue equivalent for at least 72 hours of cultivation. Transmission electron microscopy data also confirm the presence of cell differentiation markers: lipid droplets in lipoblasts and procollagen molecules in fibroblasts.

Discussion and Conclusion. This study developed a novel tissue-engineering method for producing cultured 3D connective tissue equivalents using differentiated rabbit MMSCs and 3D bioprinting. *In vitro* exposure of rabbit MMSCs to a set of inducers, including DMEM medium containing 4.5 g/l glucose, 10% FBS, 1 μM dexamethasone, 100 μM indomethacin, 500 μM 3-isobutyl-1-methylxanthine, and 10 μg/mL insulin, resulted in their adipogenic differentiation, the functional marker of which was the formation of lipid droplets. *In vitro* exposure of rabbit MMSCs to a set of inducers, including DMEM medium containing 4.5 g/l glucose, 10% FBS, 10 μM 5-azacytidine, and 50 μM hydrocortisone, induced their myogenic differentiation, the functional marker of which was myotube formation. The 3D extrusion bioprinting method ensured the formation of three-dimensional connective tissue equivalents, in which differentiated rabbit cells maintained viability and metabolic activity, forming tight junctions and secretory vesicles for at least 72 hours. Morphological analysis of the resulting bioequivalent, including confocal and transmission electron microscopy, confirmed the viability and high metabolic activity of the cells, indicating their potential to maintain the functionality of the construct.

The proposed method for creating tissue equivalents can be widely used: in clinical veterinary medicine for the treatment of connective tissue defects and injuries; in experimental veterinary medicine, as a model for studying the patterns of morphogenesis, cyto-, histo-, and organogenesis, cell differentiation and intracellular structures, intercellular interactions, regenerative processes in individual development, and their adaptation to the effects of exogenous and endogenous factors in animals at the macro-, micro-, and ultrastructural levels using morphological and other research methods; screening of new medicinal compounds; and as phantoms for developing approaches in radiation therapy and diagnostics. Furthermore, this method can be transferred to the development of cultured meat products, as it enables production of a final product with properties identical to those of productive animal tissues, including structural integrity and organoleptic properties.

A clear disadvantage of this technology is its high cost and the technological complexity of large-scale cell cultivation in standard laboratories. This high cost is primarily due to the most expensive component of the culture medium—fetal bovine serum. Despite numerous attempts to produce serum-free media and find serum substitutes, their efficiency in cultivation is currently inferior to that of serum. To reduce costs and optimize large-scale cell cultivation, it is advisable to use bioreactors, which, combined with the use of a 3D bioprinter, increases the skill requirements of operators.

The technological advantages of this method include the possibility of standardization and high reproducibility, which are ensured by the conditions of 3D bioprinting: the cell concentration in the hydrogel and their distribution within the construct will be consistent throughout reproduction, since printing is performed according to a predetermined digital template and its parameters are controlled at the hardware and software levels. The use of artificial intelligence-based algorithms for cell cultivation control significantly mitigates the influence of the human factor on the final result.

References

1. Sharun K, El-Husseiny HM, Muthu S. Editorial: Advances In Veterinary Tissue Engineering: Unlocking Potential with Cell-Free and Cell-Based Methods. *Frontiers in Veterinary Science*. 2025;12:1591272. https://doi.org/10.3389/fvets.2025.1591272

2. Yun SH, Lee DY, Lee J, Mariano EJ, Choi Yeongwoo, Park Jinmo, et al. Current Research, Industrialization Status, and Future Perspective of Cultured Meat. *Food science of animal resources*. 2024;44(2):326–355. https://doi.org/10.5851/kosfa.2024.e13

3. Hubrecht RC, Carter E. The 3Rs and Humane Experimental Technique: Implementing Change. *Animals*. 2019;9(10):754. https://doi:10.3390/ani9100754

4. Sulakhiya K, Paliwal R, Kisku A, Sahu M, Aditya S. Soni P, et al. Experimental Tools as an "Alternative to Animal Research" in Pharmacology. In book: Singh D, Tiwari P (Eds.). *Software and Programming Tools in Pharmaceutical Research*. Bentham Science Publishers; 2024. P. 170–206. https://doi.org/10.2174/9789815223019124010 1

5. Amelian A, Wasilewska K, Megias D, Winnicka K. Application of Standard Cell Cultures and 3D in Vitro Tissue Models as an Effective Tool in Drug Design and Development. *Pharmacological Reports*. 2017;69:861–870. https://doi.org/10.1016/j.pharep.2017.03.014

6. Meleshina AV, Bystrova AS, Rogovaya OS, Vorotelyak EA, Vasiliev AV, Zagaynova EV. Skin Tissue-Engineering Constructs and Stem Cells Application for the Skin Equivalents Creation (Review). *Sovremennyetehnologii v medicine (Modern Technologies in Medicine).* 2017;9(1):198–218. https://doi.org/10.17691/stm2017.9.1.24

7. Shkurupy VA, Kim LB, Kovner AV, Cherdantseva LA. Connective Tissue and the Problems of Its Pathological Conditions. *Bulletin of Siberian Medicine*. 2017;16(4):75–85. (In Russ.) https://doi.org/10.20538/1682-0363-2017-4-75–85

8. Zushin PH, Mukherjee S, Wu JC. FDA Modernization Act 2.0: Transitioning beyond Animal Models with Human Cells, Organoids, and AI/ML-Based Approaches. *The Journal of Clinical Investigation*. 2023;133(21):e175824. https://doi.org/10.1172/JCI175824

9. Ravikumar B, Cichońska A, Sahni N, Aittokallio T, Rahman R. Advancements in Rational Multi-Targeted Drug Discovery: Improving the Efficacy-Safety Balance of Small Molecule Cancer Therapeutics. In book: Peters JU (Ed.). *Polypharmacology: Strategies for Multi-Target Drug Discovery*. John Wiley & Sons, Inc.; 2025. P. 109–125. https://doi.org/10.1002/9781394182862.ch9

10. Hu J, Lin YY, Chen PJ, Watashi K, Wakita T. Cell and Animal Models for Studying Hepatitis B Virus Infection and Drug Development. *Gastroenterology*. 2019;156(2):338–354. https://doi.org/10.1053/j.gastro.2018.06.093

11. Harb A, Fakhreddine M, Zaraket H, Saleh FA. Three-Dimensional Cell Culture Models to Study Respiratory Virus Infections Including COVID-19. *Biomimetics*. 2021;7(1):3. https://doi:10.3390/biomimetics7010003

12. Golovin SN, Kirichenko EYu, Sedova DA, Ermakov AM. Prospects of Cell Therapy Application in Veterinary Medicine. *International Bulletin of Veterinary Medicine*. 2025;4. (In Russ.) (in print).

13. Armitage AJ, Miller JM, Sparks TH, Georgiou AE, Reid J. Efficacy of Autologous Mesenchymal Stromal Cell Treatment for Chronic Degenerative Musculoskeletal Conditions in Dogs: A Retrospective Study. *Frontiers in Veterinary Science*. 2023;9:1014687. https://doi.org/10.3389/fvets.2022.1014687

14. Goel S, Gandhi S, Dubey S, Shah M, Saini S, Arora P, et al. Stem Cell Therapy: Promises and Challenges in Treating Animal Diseases. In book: Mukhopadhyay CS, Choudhary RK, Panwar H, Malik YS (Eds.). *Biotechnological Interventions Augmenting Livestock Health and Production. Livestock Diseases and Management*. Singapore: Springer; 2023. P. 13–38. https://doi.org/10.1007/978-981-99-2209-3_2

15. Voga M, Kovač V, Majdic G. Comparison of Canine and Feline Adipose-Derived Mesenchymal Stem Cells/Medicinal Signaling Cells with Regard to Cell Surface Marker Expression, Viability, Proliferation, and Differentiation Potential. *Frontiers in Veterinary Science*. 2021;7:610240. https://doi.org/10.3389/fvets.2020.610240

16. Fraile AP, González-Cubero E, Martínez-Flórez S, Olivera ER, Villar-Suárez V. Regenerative Medicine Applied to Musculoskeletal Diseases in Equines: A Systematic Review. *Veterinary Sciences*, 2023;10(12):666. https://doi.org/10.3390/vetsci10120666

17. Vickram AS, Infant SS, Manikandan S, Sowndharya BB, Gulothungan G, Chopra H. 3D Bio-Printed Scaffolds and Smart Implants: Evaluating Functional Performance in Animal Surgery Models. *Annals of Medicine and Surgery* 2025;87(6):3618–3634. https://doi.org/10.1097/MS9.0000000000003333

18. Gallivanone F, D'Ambrosio D, Carne I, D'Arcangelo Micol, Montagna P, Giroletti E, et al. A tri-modal tissue-equivalent anthropomorphic phantom for PET, CT and multi-parametric MRI radiomics. *Physica Medica*. 2022;98:28–39. https://doi.org/10.1016/j.ejmp.2022.04.007

19. Tello LH. *Burns in Small Animals. Proceedings of 38th World Congress of World Small Animal Veterinary Association (WSAVA), Auckland, New Zealand. March 2013.* https://www.vin.com/apputil/content/defaultadv1.aspx?id=5709840&pid=11372& (accessed: 16.08.2023).

20. Chogan F, Chen Y, Wood F, Jeschke MG. Skin Tissue Engineering Advances in Burns: A Brief Introduction to the Past, the Present, and the Future Potential. *Journal of Burn Care & Research*. 2023;44(Suppl_1):S1–S4. https://doi.org/10.1093/jbcr/irac127

21. Golovin SN, Kirichenko EY, Khanukaev MM, Logvinov AK. 3D Bioprinting of Hybrid Cultured Meat from Rabbit Cells and Sunflower Protein. *Foods and Raw Materials.* 2026;14(1):52–60. https://doi.org/10.21603/2308-4057-2026-1-659

22. Lund P, Pilgaard L, Duroux M, Fink T, Zachar V. Effect of Growth Media and Serum Replacements on the Proliferation and Differentiation of Adipose-Derived Stem Cells. *Cytotherapy*. 2009;11(2):189–197. https://doi.org/10.1080/14653240902736266

23. Freshney RI. *Culture of Animal Cells: A Manual of Basic Technique and Specialized Applications.* John Wiley & Sons, Inc. 2010; 732 p. https://doi.org/10.1002/9780470649367

24. Nabiullina R, Golovin S, Kirichenko E, Petrushan M, Logvinov A, Kaplya M, et al. 3D Bioprinting of Cultivated Meat Followed by the Development of a Fine-Tuned YOLO Model for the Detection and Counting of Lipoblasts, Fibroblasts, and Myogenic Cells. *Frontiers in Bioscience (Landmark Edition)*. 2025;30(3):36266. https://doi.org/10.31083/FBL36266

25. Hernandez-Verdun D, Roussel P, Thiry M, Sirri V, Lafontaine DL. The Nucleolus: Structure/Function Relationship in RNA Metabolism. *Wiley Interdisciplinary Reviews: RNA*. 2010;1(3):415–431. https://doi.org/10.1002/wrna.39

26. Grummt I. The Nucleolus—Guardian of Cellular Homeostasis and Genome Integrity. *Chromosoma*. 2013;122(6):487–497. https://doi.org/10.1007/s00412-013-0430-0

27. Dubois ML, Boisvert FM. The Nucleolus: Structure and Function. In book: Bazett-Jones D, Dellaire G (Eds.). *The Functional Nucleus*. Cham: Springer; 2016. P. 29–49. https://doi.org/10.1007/978-3-319-38882-3_2

28. Csala M, Bánhegyi G, Benedetti A. Endoplasmic Reticulum: A Metabolic Compartment. *FEBS Letters*. 2006;580(9):2160–2165. https://doi.org/10.1016/j.febslet.2006.03.050

29. Wang H, Zhang X, Xiu T, Wang H, Li P, Tang B. Fluorescence Probes for Sensing and Imaging within Golgi Apparatus. *Coordination Chemistry Reviews*, 2024;502:215618. https://doi.org/10.1016/j.ccr.2023.21

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INFECTIOUS DISEASES AND ANIMAL IMMUNOLOGY
ИНФЕКЦИОННЫЕ БОЛЕЗНИ И ИММУНОЛОГИЯ ЖИВОТНЫХ

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Original Empirical Research

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Preclinical Trials in White Mice and Rabbits of the Improved "Associated Inactivated Emulsion Vaccine against Infectious Bovine Rhinotracheitis (IBR), Bovine Viral Diarrhea/Mucosal Disease (BVD), Bovine Parainfluenza Virus 3 (BPIV-3) and Chlamydia in Cattle"

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Abstract

Introduction. Infectious respiratory and intestinal diseases in cattle are multifactorial diseases, which explains the associated viral or viral-bacterial etiology of the infection among productive livestock. Combating associated respiratory and intestinal infections in cattle is carried in the vast majority of countries in the world and requires efficient measures and medications. The aim of the present research is conducting the preclinical trials in white mice and rabbits of the experimental series of the "Associated inactivated emulsion vaccine against IBR, BVD, BPIV-3 and chlamydia in cattle" improved by expanding the chlamydial antigen spectrum.

Materials and Methods. The trials were conducted in the Animal Viral Disease Laboratory of the Federal Center for Toxicological, Radiation, and Biological Safety (Kazan) from February to November 2024. Two versions of the biopharmaceutical were produced: a standard associated vaccine and an experimental vaccine with the AMK-16 and MZ-89 strains added to the chlamydial antigen. White mice and rabbits served as laboratory animals. The vaccines were evaluated for sterility, safety, tolerability, antigenic activity, impact on antiviral humoral immunity and immunogenicity.

Results. Both versions of the associated vaccine had proved to be sterile, harmless, and well-tolerated by laboratory animals. Changing the chlamydial antigen composition of the associated vaccine did not have an adverse effect on the development of antiviral humoral immunity in laboratory animals. The level of specific anti-chlamydial antibodies in rabbits vaccinated with the improved vaccine was higher than in the group of rabbits vaccinated with the standard technology vaccine. The protection index in the group of white mice vaccinated with the improved vaccine was 1.3 times higher than that with the standard vaccine.

Discussion and Conclusion. Based on the data obtained, it can be concluded that the improved associated vaccine, the same as the standard one, is well tolerated by laboratory animals. Expansion of the chlamydial antigen spectrum of the "Associated vaccine against IBR, BVD, BPIV-3 and Chlamydia in cattle" by adding additional strains did not have any negative effect on the development of antiviral humoral immunity in laboratory animals, but on the contrary had stimulated the development of a humoral response to the chlamydial antigen, thus, boosting the vaccine immunogenicity by 1.3 times.

Keywords: preclinical trials, associated vaccine, antigen, immunogenicity, strains, chlamydia, IBR, BPIV-3, BVD, cattle, white mice, rabbits

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Оригинальное эмпирическое исследование

Доклинические испытания усовершенствованной «Ассоциированной вакцины против ИРТ, ВД-БС, ПГ-3 и хламидиоза крупного рогатого скота инактивированной эмульсионной» на белых мышах и кроликах

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Аннотация

Введение. Инфекционные респираторно-кишечные болезни крупного рогатого скота являются многофакторными заболеваниями, что обуславливает ассоциированную вирусную или вирусно-бактериальную этиологию течения инфекционного процесса среди различного поголовья продуктивных животных. Борьба с респираторно-кишечными ассоциированными инфекциями крупного рогатого скота ведется в подавляющем большинстве стран мира и требует эффективных мер и препаратов. Целью исследования явились доклинические испытания экспериментальной серии «Ассоциированной вакцины против ИРТ, ВД-БС, ПГ-3 и хламидиоза крупного рогатого скота инактивированной эмульсионной», усовершенствованной за счет расширения спектра хламидийного антигена, на белых мышах и кроликах.

Материалы и методы. Испытания проведены в лаборатории вирусных заболеваний животных ФГБНУ «ФЦТРБ-ВНИВИ» (г. Казань) в период с февраля по ноябрь 2024 г. Были изготовлены два варианта биопрепарата: стандартная ассоциированная вакцина и экспериментальная, в состав хламидийного антигена которой были добавлены штаммы «АМК-16» и «МЗ-89». В качестве лабораторных животных выступили белые мыши и кролики. Вакцины оценивались на стерильность, безвредность, переносимость, антигенную активность, влияние на формирование гуморального противовирусного иммунитета, иммуногенность.

Результаты исследования. Было доказано, что оба варианта ассоциированной вакцины стерильны, безвредны и хорошо переносятся лабораторными животными. Изменение состава хламидийного антигена ассоциированной вакцины не оказало негативного влияния на формирование противовирусного гуморального иммунитета у лабораторных животных. Уровень специфических противохламидийных антител у кроликов, иммунизированных усовершенствованной вакциной, был выше, чем в группе кроликов, привитых препаратом, изготовленным по стандартной методике. Индекс защиты в группе белых мышей, привитых усовершенствованным препаратом, был в 1,3 раза выше, по сравнению со стандартным образцом.

Обсуждение и заключение. На основании полученных данных можно заключить, что усовершенствованная ассоциированная вакцина, как и стандартная, хорошо переносится лабораторными животными. Расширение спектра хламидийного антигена «Ассоциированной вакцины против ИРТ, ВД-БС, ПГ-3 и хламидиоза крупного рогатого скота» дополнительными штаммами не только не оказало негативного эффекта на формирование гуморального противовирусного иммунитета у лабораторных животных, но более того — стимулировало выработку гуморального ответа в отношении хламидийного антигена, способствуя повышению иммуногенности вакцины в 1,3 раза.

Ключевые слова: доклинические испытания, ассоциированная вакцина, антиген, иммуногенность, штаммы, хламидии, ИРТ, ПГ-3, ВД-БС, крупный рогатый скот, белые мыши, кролики

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Introduction. Infectious respiratory and intestinal diseases in young cattle represent a serious problem for livestock farms throughout Russia. The etiology of these pathologies typically involves multiple pathogens simultaneously, representing stable associations of viral and bacterial agents that cause diseases of various manifestations and severity [1–3]. Combating associated respiratory and intestinal infections in cattle is carried out in the livestock industry in the vast majority of countries worldwide [3–5]. An important factor determining the need to improve methods of combating, as well as preventing the respiratory and intestinal infections, is the economic reason: livestock complexes suffer

enormous financial losses due to the widespread prevalence of associated infections among livestock [6, 7].

Viral pathogens typically cause a primary infection, which initially proceeds in a mild form: at this stage, the immune system is suppressed, which subsequently leads to increased susceptibility to secondary bacterial infections [6, 8–10]. However, in some cases, the infectious process development algorithm may be changed, and the pathogens causing the primary infection are represented by the bacteria, which typically are capable of long-term persistence in the infected organism without causing pronounced clinical signs of infection, e.g. chlamydia [11].

Chlamydia are obligate intracellular parasites with a unique two-stage development cycle [12]. These microorganisms are capable of infecting a huge number of animal species. The course of the infectious process in chlamydiosis, even in different populations of the same animal species, is usually not systematic [13], which is due to the evolutionary ability of this type of microorganism to affect various organ systems of animals, causing various clinical signs of the disease (pneumonia, arthritis, conjunctivitis, encephalitis, etc.). In addition, chlamydia can be transmitted from one animal species to another, which also plays a significant role in widespreading this infection among domestic and wild animals [14, 15].

The most common viral pathogens in the Russian Federation are those causing infections such as infectious bovine rhinotracheitis (IBR), bovine parainfluenza virus 3 (BPIV-3), bovine viral diarrhea (BVD) [16, 17]. Previously, a team of scientists from the Federal Center for Toxicological, Radiation, and Biological Safety (Kazan) developed an "Associated Inactivated Emulsion Vaccine against Infectious Bovine Rhinotracheitis (IBR), Bovine Viral Diarrhea/Mucosal Disease (BVD), Bovine Parainfluenza Virus 3 (BPIV-3) and Chlamydia in Cattle" [17]. The antigenic composition of this biopreparation included one strain each of the IBR, BPIV-3, and BVD viruses, and the *Chlamydia psittaci "250"* strain isolated from cattle.

As a result of long-term applied and fundamental research into chlamydial infections in animals it was established that different strains of chlamydia of the same species, isolated from the same animal species or from other farm animals with various pathologies, differ from each other antigenically. These differences in the biochemical and genetic structure of chlamydia directly correlate with the immunogenicity of different strains relative to each other [18]. Chlamydia's capacity for horizontal gene transfer among different species of this pathogen has also been established, allowing some chlamydial strains to contain in their biochemical composition the antigenic epitopes specific to other species of this pathogen [19].

We previously studied the antigenic and immunogenic properties of various chlamydia strains isolated on the territories of different subjects of the Russian Federation [18, 20, 21]. In the frame of our research, we constructed a new antigenic composition consisting of the three most immunogenic and antigenically distinct chlamydia strains isolated from different animal species. Furthermore, following whole-genome sequencing and subsequent bioinformatics analysis of the nucleotide sequence of the chromosome of one of the strains included in the new antigenic composition, it was established that this strain contains antigenic epitopes specific to two chlamydia species at once—*Chlamydia psittaci* and *Chlamydia abortus* [22]. Therefore, using a new chlamydial antigen composition in a new associated vaccine including three chlamydia strains should have good prospects and was implemented.

Thus, the composition of the "Associated vaccine against IBR, BVD, BPIV-3 and Chlamydia in cattle" was supplemented with antigens from two additional chlamydia strains: "AMK-16" (causative agent of arthritis and abortion in goats) and "MZ-89" (causative agent of meningoencephalitis in calves). However, the quantitative ratio of antigens from different pathogens in the vaccine remained unchanged. Preclinical trials in laboratory animals were aimed at determining the effect of the chlamydial antigen composition change on the properties of the "Associated vaccine against IBR, BVD, BPIV-3 and Chlamydia in cattle".

Materials and Methods. The study was conducted at the Animal Viral Diseases Laboratory of the Federal Center for Toxicological, Radiation, and Biological Safety (Kazan) from February to November 2024.

Strains. The following virus and chlamydia strains were used:

– VK-1 strain of the BVD virus, infectious titer $10^{-6.87}TCID_{50}/ml$;

– TK-A (VIEV)-B-2 vaccine strain of the IBR virus of cattle;

– PTK-45/86 reference strain of the BPIV-3 virus of cattle;

– *Chlamydia psittaci* strain "AMK-16", isolated from pathological material of an aborted goat;

– *Chlamydia psittaci* strain "250", isolated from pathological material of an aborted cow;

– Chlamydia psittaci strain "MZ-89", isolated from the brain of a calf with the encephalitic form of chlamydial infection;

– Chlamydia psittaci strain "RS-85", isolated from pathological material from an aborted sow.

Nutrient media. The sterility of the biopreparations was assessed by plating them on the following nutrient media: meat-peptone agar (MPA); meat-peptone broth (MPB); meat-peptone liver broth and Sabouraud medium. The following nutrient media were used for culturing and maintaining the cell culture: synthetic medium 199; Hanks' balanced solution; fetal bovine serum manufactured by the All-Russian Research Institute of Veterinary Medicine; Eagle's medium MEM (Minimum Essential Medium) with glutamine, pH 7.5–7.

Cell cultures. Viruses were cultured using continuous bovine kidney cell culture (MDBK).

Biological models. Chlamydia biomass was obtained by infecting chicken embryos with chlamydia strains into the yolk sac.

Laboratory animals. White mice and rabbits.

During experimental animal experiments, the requirements of 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 were observed. Animals were maintained in optimal conditions and had free access to feed and water.

Reagents and Test Systems. Serological studies were conducted using the following diagnostic test systems:

– "Set of Antigens and Sera for the Serological Diagnosis of Chlamydia in Farm Animals" (ROSS RU.FV01.N00022) manufactured by the Federal Center for Toxicological, Radiation, and Biological Safety of the All-Russian Research Institute of Veterinary Medicine (Kazan);

– "Kit for the Diagnosis of Bovine Parainfluenza-3" (Kursk Biofactory – "BIOK" Firm);

– "Kit for the Detection of Antibodies to the Infectious Bovine Rhinotracheitis Virus by the Enzyme Immunosorbent Assay "IBR-SEROTEST" (LLC "Vetbiokhim", Moscow);

– "Kit for the Enzyme Immunosorbent Assay of Bovine Viral Diarrhea – Mucosal Disease (BVD) in Cattle" (VIEV, Moscow).

Vaccine composition. Two variants of the associated vaccine with different antigen compositions were prepared for the study. The first (standard) batch of the biopreparation included the following virus strains: BPIV-3 — PTK-45/86", BVD — "VK-1", IBR — "TK-A (VIEV)-V-2", and chlamydia — "250". The second (experimental) batch of the vaccine used similar virus strains, and two more strains — "AMK-16" and "MZ-89" — were added to the chlamydial antigen. The antigens of all strains in the chlamydial antigen were in equal proportions. In both finished vaccine variants, the virus and chlamydia antigens were presented in an equal ratio: BPIV-3, IBR, BVD, and chlamydia in a ratio of 1:1:1:1. Oil-lanolin adjuvant (OLA) was used as an auxiliary component in the production of each vaccine batch. The vaccine emulsion was a water-in-oil system.

Methods. Vaccine sterility was determined in accordance with "OFS 1.2.4.0003.15 General Pharmacopoeia Monograph. Sterility" (Section 2.3) using the direct inoculation method.

The safety of the experimental preparations was determined in accordance with GOST 31926. For each vaccine batch, groups of 15 white mice aged 2 to 3 months and weighing 18 to 25 g were formed. Prepared samples of the test preparations were administered intraperitoneally to the animals in a volume of 0.25 cm^3. Subsequently, for 10 days after vaccine administration, daily clinical examinations of the vaccinated animals were conducted to identify sick or dead animals. The vaccine was considered safe if no deterioration in the general condition of the animals or deaths were recorded during the entire observation period.

To evaluate the vaccine tolerance and antigenic activity, 12 rabbits were divided into three groups of four animals each. The first group of animals was vaccinated with the standard vaccine series, while the second group was vaccinated with the experimental vaccine series. Animals in the third group were not vaccinated and served as controls. The animals were administered 0.5 cm^3 of the biological preparations intramuscularly into the thigh.

The following parameters were taken into account when assessing the vaccine tolerance of the rabbits:

– General condition of the animals;
– General body temperature after vaccination;
– Presence of a local reaction at the injection site;
– Changes in appetite after vaccination;
– Behavioral reactions.

Vaccine tolerance was assessed during the first 10 days after immunization. The above-mentioned parameters were recorded and recorded daily during clinical examinations of the animals. Body temperature was measured using a mercury thermometer. General condition was assessed visually, focusing on the animals' posture, coat, and gait. Animal appetite was assessed by the presence or absence of food in the feeders after feeding. Vaccine tolerance was assessed by the absence of local or systemic reactions to the administration of the biopreparation.

The antigenic activity of the vaccines was assessed using serological tests. Serum samples were collected systematically from the animals studied over a period of 6 months (on days 30, 60, 90, and 180 post-vaccination). The concentration of antibodies specific to the BPIV-3 virus was determined using the hemagglutination inhibition test (HIT). The level of anti-chlamydial antibodies in the sera of immunized animals was determined using the complement fixation test (CFT). Specific antibodies to the IBR and BVD viruses were determined using an enzyme-linked immunosorbent assay (ELISA).

The ability of the vaccines to induce anti-chlamydial immunity in immunized animals was determined in an acute experiment on white mice (n=180). For each vaccine series, four experimental (eight experimental groups in total) and four control groups of laboratory animals were formed, each containing 15 animals. Animals in the experimental groups were administered the test drugs subcutaneously in a volume of 0.2 cm3. On the 30th day after immunization, animals in all groups were infected with various chlamydia strains. The first experimental groups of mice were infected with the "AMK-16" strain, the second with the "RS-85" strain, the third with the "250" strain, and the fourth with the "MZ-89" strain. The control groups were infected in a similar manner.

The immunogenicity of biopreparations was assessed by the protection index, which was calculated using the formula:

$$X = \frac{A}{B} \quad (1)$$

where X — Protection index; A — number of dead animals in control groups; B — number of dead animals in vaccinated groups.

To confirm the chlamydial etiology of the deaths of infected mice, smears were examined using a light immersion microscope (*Nikon Eclipse*, Japan). Smears were prepared from the internal organs of dead animals and stained using a modified Stemp method.

Results. Inoculation of two vaccine samples (improved and standard) onto MPA, MPB, MPPPB, and Sabouraud nutrient media revealed no microbial growth on the media during the observation period, confirming the sterility of the test preparations.

Administration of the experimental and standard vaccine samples to white mice did not cause any adverse reactions or negative pathological processes during the observation period, indicating the safety of the preparations.

Observations of immunized and intact rabbits during the vaccine tolerance study revealed that the average body temperature of the animals in the two experimental groups, vaccinated with different variants of the associated vaccine, and the control group remained virtually unchanged throughout the study, ranging from 38.8°C to 39.0°C. All parameters were within normal physiological limits.

Daily clinical examinations of immunized rabbits revealed no pathological conditions. The animals' general condition was satisfactory, their appetite was maintained, and there were no abnormal behavioral reactions. A slight swelling was observed at the injection site of the biological preparations in animals in the experimental groups. This swelling resolved within 10–15 days after vaccination, which is acceptable for immunization with emulsion vaccines.

To determine the effect of the vaccine on the development of post-vaccination humoral immunity, serological studies were conducted to determine the levels of specific antibodies to the antigens used in the experimental and standard vaccines in the blood of rabbits at various times after immunization. The serological results are presented in Table 1.

Table 1 shows that vaccination of rabbits with both biopreparations induced the production of both antiviral and antichlamydial antibodies. On the 30th day after vaccination, the level of immunoglobulins specific to the parainfluenza-3 virus in animals immunized with the standard preparation varied within titers from 1:80 to 1:320. In all rabbits immunized with the experimental preparation, titers of antibodies to the BPI-3 virus were equal to 1:160 at this time point. The average titers of immunoglobulins to the BPI-3 virus in the two groups were equal to a titer of 1:160. The concentration of antiviral antibodies specific to the infectious rhinotracheitis virus in the two groups on the 30th day of the study was within titers from 1:400 to 1:1600.

No significant differences were observed in the development of humoral immunity to the BVD virus. On day 30 of the study, immunoglobulins specific to this virus ranged in titers from 1:800 to 1:1600. A slight difference was observed on day 30 post-vaccination in the development of humoral anti-chlamydial immunity. In the group immunized with the standard preparation, the concentration of complement-fixing immunoglobulins in all animals was at a titer of 1:20. In the group of animals immunized with the experimental preparation, the mean antibody titer was slightly higher, at 1:30.

It should be noted that the lowest concentration of both antiviral and antichlamydial immunoglobulins was detected on the 30th day after vaccination. Over the next two months, an increase in the concentration of antibodies specific to all antigens included in the associated vaccine was observed in the blood serum of immunized animals. Thus, on the 90th day, average antibody titers to the BPIV-3 virus in the HI assay were established at 1:1440 and 1:1600 for the standard and experimental vaccine variants, respectively. Average titers of specific antibodies to the IBR virus in the ELISA during this period were within the titers of 1:5600 for the standard vaccine sample and 1:5200 for the experimental one. On the 90th day after vaccination, average titers of antiviral antibodies to the BVD virus in both groups equaled a titer of 1:3600.

A somewhat different picture was observed when studying blood sera with chlamydial antigen, where a significant difference was found between antibody levels in the two groups of vaccinated rabbits. Thus, the average antibody titer in the serum with chlamydial antigen in the group of animals vaccinated with the standard biopreparation sample was 1:60, while in the group of laboratory animals immunized with the experimental preparation, the average titer was higher, at 1:100. It should be noted that this pattern was also observed at other study time points, on days 60 and 180.

By day 180, the concentration of antiviral and antichlamydial antibodies in the blood serum of immunized animals began to decline in both groups, but was still higher in the experimental group. However, no significant difference was detected between antibody levels to specific antigens across the groups, with the exception of the chlamydial antigen, for which higher antibody levels were detected in the group of animals vaccinated with the experimental variant.

Figures 1, 2, 3, and 4 show the dynamics of average specific antibody titers to viral and chlamydial antigens over the entire observation period (for clarity, the titers are shown separately for each antigen).

Table 1

Testing in rabbits the antigenic activity of the standard and experimental versions of the "Associated vaccine against IBR, BVD, BPIV-3 and Chlamydia in cattle"

Antigen / Test	Vaccine variant	Animal number	Antibody titers			
			30th day	60th day	90th day	180th day
BPIV-3/HI test	Standard	1	1:80	1:1280	1:1280	1:1280
		2	1:80	1:640	1:1280	1:1280
		3	1:160	1:640	1:640	1:640
		4	1:320	1:640	1:2560	1:2560
		Mean titer	**1:160**	**1:800**	**1:1440**	**1:1440**
	Experiment	1	1:160	1:1280	1:1280	1:1280
		2	1:160	1:1280	1:2560	1:2560
		3	1:160	1:640	1:1280	1:640
		4	1:160	1:320	1:1280	1:640
		Mean titer	**1:160**	**1:880**	**1:1600**	**1:1280**
IBR /ELISA	Standard	1	1:1600	1:3200	1:6400	1:6400
		2	1:400	1:3200	1:3200	1:3200
		3	1:800	1:1600	1:6400	1:3200
		4	1:800	1:1600	1:6400	1:6400
		Mean titer	**1:900**	**1:2400**	**1:5600**	**1:4800**
	Experiment	1	1:400	1:400	1:1600	1:1600
		2	1:400	1:1600	1:6400	1:3200
		3	1:1600	1:3200	1:6400	1:6400
		4	1:1600	1:3200	1:6400	1:6400
		Mean titer	**1:1000**	**1:2100**	**1:5200**	**1:4400**
BVD /ELISA	Standard	1	1:1600	1:6400	1:6400	1:3200
		2	1:1600	1:1600	1:3200	1:6400
		3	1:800	1:800	1:1600	1:1600
		4	1:800	1:1600	1:3200	1:1600
		Mean titer	**1:1200**	**1:2600**	**1:3600**	**1:3200**
	Experiment	1	1:800	1:1600	1:3200	1:800
		2	1:1600	1:3200	1:3200	1:3200
		3	1:1600	1:3200	1:6400	1:6400
		4	1:800	1:800	1:1600	1:1600
		Mean titer	**1:1200**	**1:2200**	**1:3600**	**1:3000**
Chlamydia /CF- test	Standard	1	1:20	1:20	1:80	1:80
		2	1:20	1:20	1:40	1:40
		3	1:20	1:20	1:80	1:80
		4	1:20	1:40	1:40	1:40
		Mean titer	**1:20**	**1:20**	**1:60**	**1:60**
	Experiment	1	1:20	1:40	1:80	1:80
		2	1:40	1:80	1:160	1:80
		3	1:20	1:20	1:80	1:80
		4	1:40	1:40	1:80	1:80
		Mean titer	**1:30**	**1:45**	**1:100**	**1:80**

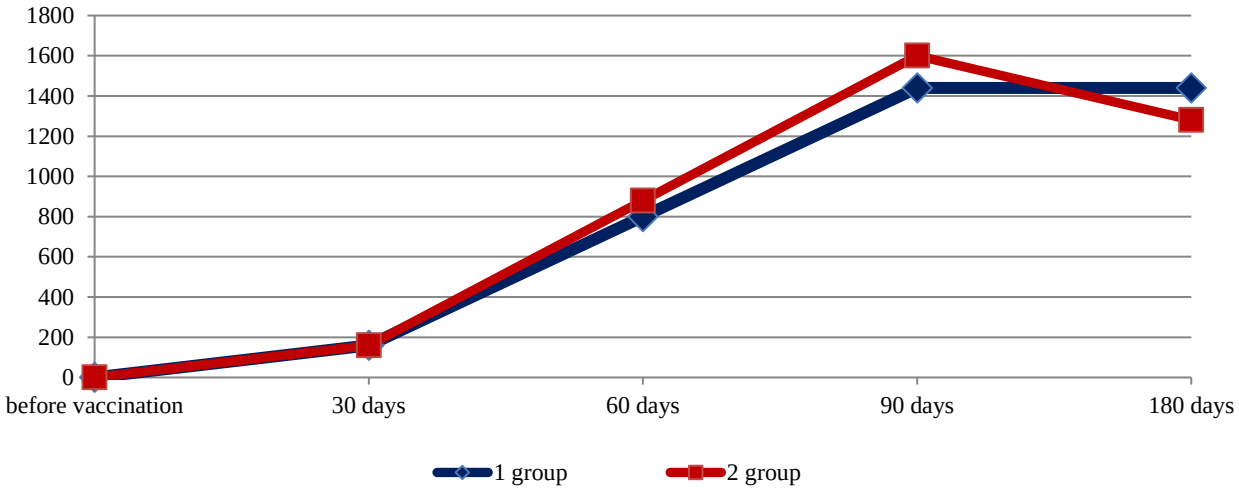

Fig. 1. Mean titers of antiviral antibodies specific to the causative agent of BPIV-3

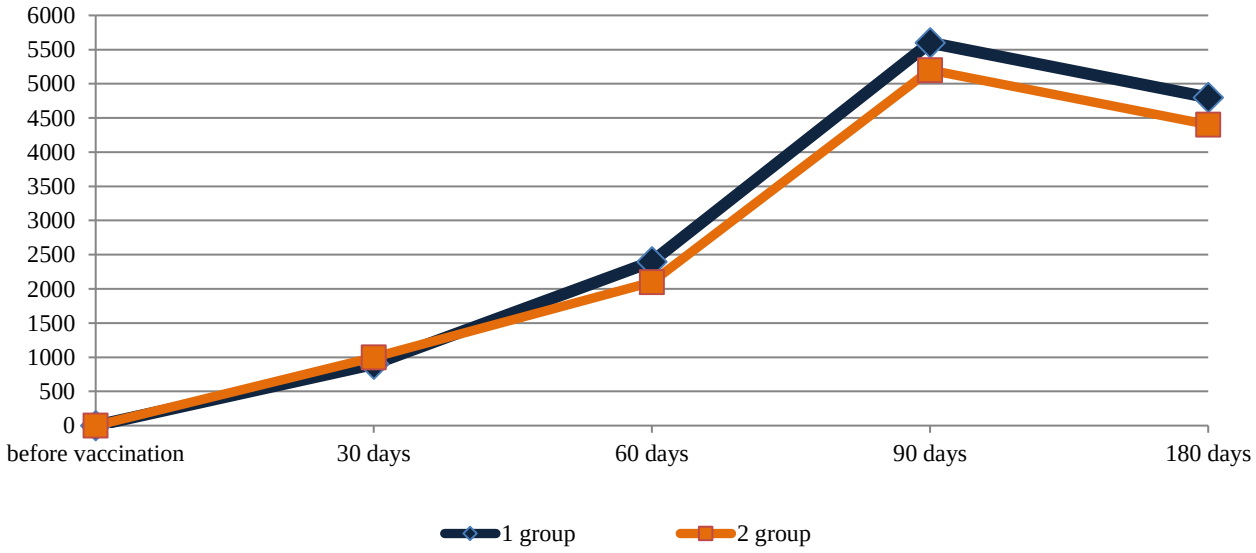

Fig. 2. Mean titers of antiviral antibodies specific to the causative agent of IBR

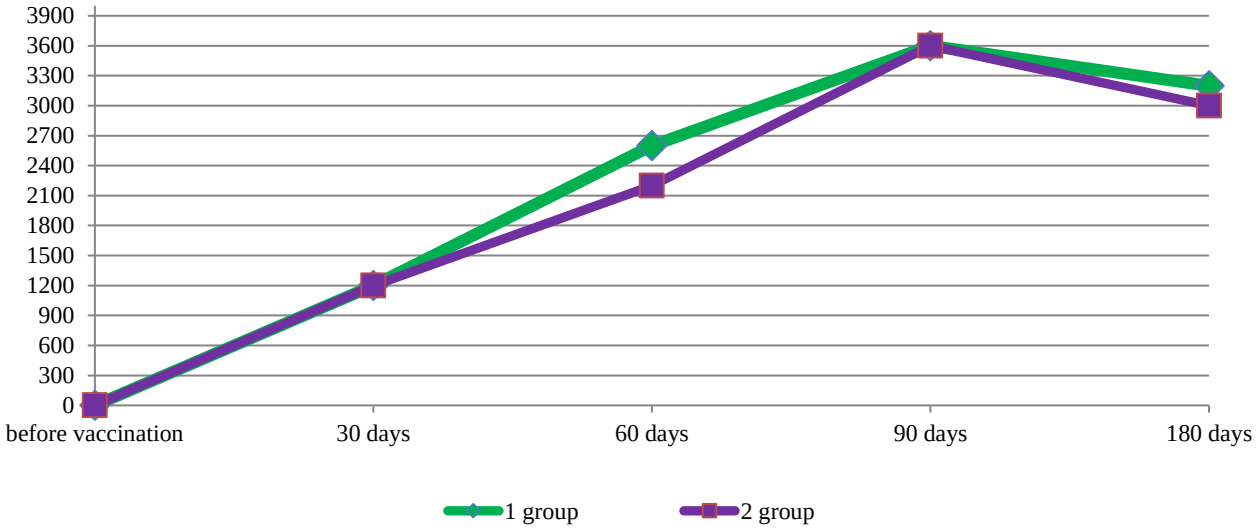

Fig. 3. Mean titers of antiviral antibodies specific to the causative agent of BVD

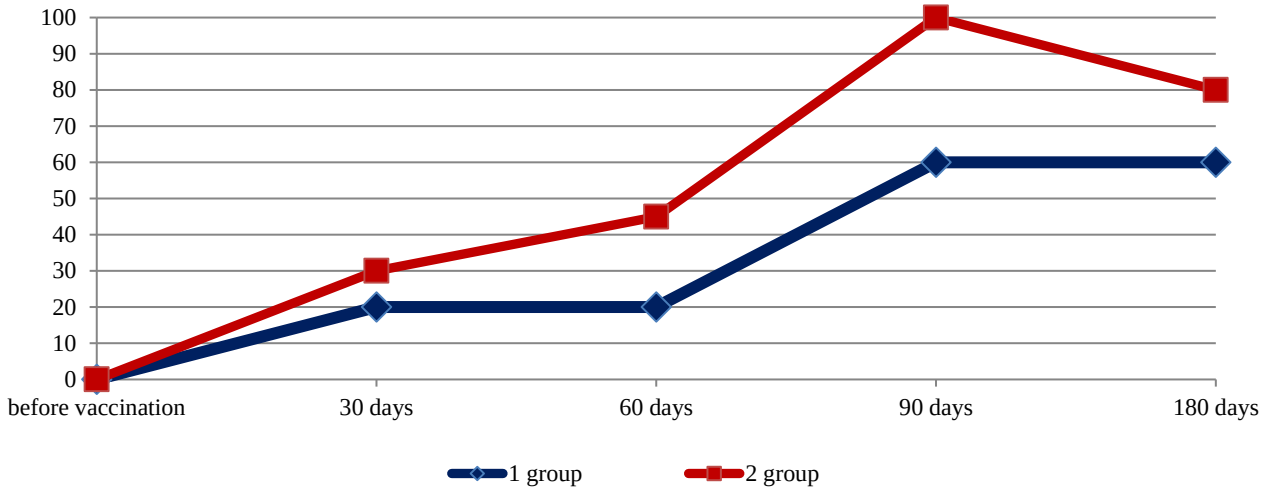

Fig. 4. Average/Mean titers of antichlamydial antibodies

The presented data (Figs. 1, 2, 3, and 4) indicate that altering the chlamydial antigen composition in the associated vaccine did not negatively impact the development of humoral antiviral immunity, but did increase the production of specific antichlamydial antibodies in the blood sera of vaccinated animals.

To determine the effect of the improved vaccine on immunogenicity, an acute experiment was conducted on laboratory white mice. The first and second groups of animals were immunized with the modified vaccine and the standard sample, respectively. All groups of animals were then challenged with four commercial chlamydia strains. The results of these studies are presented in Table 2.

Table 2

Immunogenicity study of standard and experimental vaccine samples in an acute experiment in white mice

Batch number of the vaccine for immunization	Strain for infection	Number of dead animals	Surviving animals	Protection index
Standard	"250"	4	11	3,5
	"PS-85 (PC-85)"	3	12	4,3
	"AMK-16"	4	11	3,75
	"MZ-89 (M3-89)"	3	12	4,7
Total accross groups		**14**	**46**	**4**
Experiment	"250"	3	12	4,7
	"PS-85 (PC-85)"	2	13	6,5
	"AMK-16"	2	13	7,5
	"MZ-89 (M3-89)"	2	13	7
Total accross groups		**9**	**51**	**6,2**
Control	"250"	14	1	–
	"PS-85 (PC-85)"	13	2	–
	"AMK-16"	15	–	–
	"MZ-89 (M3-89)"	14	1	–
Total accross groups		**56**	**4**	**-**

As shown in Table 2, 46 of the 60 mice vaccinated with the standard vaccine survived infection with four

chlamydia strains. The protection indices in the groups of white mice after infection with chlamydia pathogens ranged from 3.5 to 4.7. The average protection index across the four groups of white mice vaccinated with the standard vaccine was 4.

In the groups of mice vaccinated with the improved associated vaccine, the number of surviving animals was significantly higher (51). The protection indices for infection with different chlamydia strains ranged from 4.7 to 7.5. The average protection index across all groups infected with different chlamydia strains and vaccinated with the improved vaccine was 6.2, which is 1.3 times higher than that of the standard vaccine.

In the control groups, after infection with a virulent culture of chlamydia of different strains, only 4 (6.7%) of 60 white mice remained alive.

Discussion and Conclusion. Data obtained during preclinical trials of the improved "Associated inactivated emulsion vaccine against IBR, BVD, BPIV-3 and Chlamydia in cattle", make it possible to assertain that it is as well tolerated by laboratory animals as the standard vaccine. Altering the chlamydial antigen composition of the vaccine does not adversely affect the development of humoral antiviral immunity in laboratory animals. On the contrary, adding two chlamydia strains to the vaccine stimulates the development of a humoral response to the chlamydial antigen and increases the vaccine's immunogenicity by 1.3 times compared to the standard vaccine.

References

1. Murray GM, Cassidy JP, Clegg TA, Tratalos JA, McClure J, O'Neill RG, et al. A Retrospective Epidemiological Analysis of Risk Factors for a Primary Necropsy Diagnosis of Bovine Respiratory Disease. *Preventive Veterinary Medicine*. 2016;132:49–56. https://doi.org/10.1016/j.prevetmed.2016.08.009

2. Callaby R, Toye P, Jennings A, Thumbi SM, Coetzer JA, Conradie Van Wyk IC, et al. Seroprevalence of Respiratory Viral Pathogens of Indigenous Calves in Western Kenya. *Research in Veterinary Science.* 2016;108:120–124. https://doi: 10.1016/j.rvsc.2016.08.010

3. Prytychenko AV, Krasochko IA. Immunogenicity of the Inactivated Associated Vaccine against Virus Respiratory Infections of Cattle. In: *Proceedings of the International Scientific and Practical Conference Dedicated to the Day of Belarusian Science and the 95th Anniversary of the Department of Epizootiology and Infectious Diseases "Current Problems of Infectious Pathology in Animal and Ways to Solve Them"*. Vitebsk: Vitebsk State Academy of Veterinary Medicine; 2023. P. 95–97. (In Russ.)

4. Murray GM, O'Neill RG, More SJ, McElroy MC, Earley B, Cassidy JP. Evolving Views on Bovine Respiratory Disease: An Appraisal of Selected Control Measures – Part 2. *The Veterinary Journal*. 2016;217:78–82. https://doi.org/10.1016/j.tvjl.2016.09.013

5. Bell RL, Turkington HL, Cosby SL. The Bacterial and Viral Agents of BRDC: Immune Evasion and Vaccine Developments. *Vaccines*. 2021;9(4):337. https://doi.org/10.3390/vaccines9040337

6. Grissett GP, White BJ, Larson, RL. Structured Literature Review of Responses of Cattle to Viral and Bacterial Pathogens Causing Bovine Respiratory Disease Complex. *Journal of Veterinary Internal Medicine*. 2015;29(3):770–780. https://doi.org/10.1111/jvim.12597

7. Brodersen BW, Kelling CL. Effect of Concurrent Experimentally Induced Bovine Respiratory Syncytial Virus and Bovine Viral Diarrhea Virus Infection on Respiratory Tract and Enteric Diseases in Calves. *American Journal of Veterinary Research.* 1998;59(11):1423–1430.

8. Woolums AR, Ames TR, Baker JC. The Bronchopneumonias (Respiratory Disease Complex of Cattle, Sheep, and Goats). In: *Large Animal Internal Medicine*; Louis, MO: Mosby Elsevier; 2015. P. 584–603.

9. Krasochko PA, Krasochko PP, Ivashchenko IA. Harmlessness Study of Different Variants of the Associated Virus-Bacterial Vaccine. In: *Proceedings of the International Scientific and Practical Conference Dedicated to the 95th Anniversary of the Birth of Professor N. I. Smirnova and the Day of Belarusian Science "Topical Issues of Veterinary Virology, Microbiology and Bee Diseases in Modern Conditions".* Vitebsk: Vitebsk State Academy of Veterinary Medicine; 2024. P. 79–84. (In Russ.).

10. Glotov AG, Glotova TI, Nefedchenko AV, Koteneva SV, Budulov NR, Kungurtseva OV. Etiological Structure of Mass Respiratory Diseases in Young Cattle in Dairy Farms. *Siberian Herald of Agricultural Science.* 2008;3(183):72–78.

11. Fedorova VA, Lyapina AM, Khizhnyakova MA, Zaitsev SS, Saltykov YuV, Subbotina IA, et al. *Chlamydia of Animals and Humans.* Moscow: Nauka; 2019. 135 p.

12. Mustafayeva NA, Safarova SA, Jumshudova FA, Babanly LT, Mammadova MA. Chlamydia of Farm Animals. *Prikaspiiskii vestnik veterinarii (Caspian Bulletin of Veterinary Medicine)*. 2023;(1):24–28.

13. Ravilov AZ, Gaffarov KhZ, Ravilov RKh. *Animal Chlamydia*. Kazan: "Fehn" Publishing House of the Academy of Sciences of the Republic of Tatarstan; 2004. 368 p.

14. Borel N, Sachse K. Zoonotic Transmission of *Chlamydia* Spp.: Known for 140 Years, but Still Underestimated. In book: Sing A. (Ed.) *Zoonoses: Infections Affecting Humans and Animals.* Cham: Springer; 2023. P. 1–28. https://doi.org/10.1007/978-3-030-85877-3_53-1

15. Akbashev IR. *Improvement of Means of Specific Prevention of Viral Chlamydia Infections in Cattle.* Cand. Sci. (Veterinary Sciences) Dissertation. Kazan; 2021. 129p

16. Evstifeev VV, Gumerov VG, Khusainov FM, Kharimullina IG, Akbashev IR, Yakovlev SI, et al. Development of Associated Vaccine against PIV-3, IBR, BVD and Chlamidiosis of Cattle. *Veterinarny Vrach (Veterinarian).* 2020;(6):21–28. (In Russ.) https://doi.10.33632/1998-698X.2020-6-21-28

17. Yakovlev SI. *Improvement of Means of Specific Prevention of Chlamydia in Animals*. Cand. Sci. (Veterinarian Sciences) Dissertation. Moscow; 2022. 138 p. (In Russ.).

18. Feodorova VA, Zaitsev SS, Khizhnyakova MA, Saltykov YuV, Evstifeev VV, Khusainov FM, et al. Data of de Novo Genome Assembly of the Chlamydia Psittaci Strain Isolated from the Livestock in Volga Region, Russian Federation. *Data Brief*; 2020;29:105190. https://doi.10.1016/j.dib.2020.105190

19. Evstifeev VV. *Development and Improvement of Biological Preparations for the Diagnosis and Specific Prevention of Animal Chlamydiosis*. Dr.Sci.(Biology) Dissertation. Kazan; 2015. 417 p. (In Russ.).

20. Zaitsev S, Khizhnyakova M, Saltykov Yu, Evstifeev V, Khusainov F, Ivanova S, et al. Complete Genome Sequence of *Chlamydia Psittaci* AMK-16, Isolated from a Small Ruminant in the Middle Volga Region, Russia. *Microbiology Resource Announcements*: 2024;13(5):e0054323. https://doi.10.1128/mra.00543-23

Feodorova VA, Zaitsev SS, Lyapina AM, Kichemazova NV, Saltykov YuV, et al. Whole Genome Sequencing Characteristics of *Chlamydia Psittaci* Caprine AMK-16 Strain, a Promising Killed Whole Cell Veterinary Vaccine Candidate against Chlamydia Infection. *PLoS ONE*. 2023;18(10):e0293612. (In Russ.) https://doi.10.1371/journal.pone.0293612

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SI Yakovlev: collecting and processing the material, formulating conclusions and refining the text.

RZ Khamidullina: assistance in refining the text.

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