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AIMS AND SCOPE

The mission of the publication is the delivery of information on basic development trends of veterinary science and practice and highlighting of vital issues and innovative developments in veterinary area for scientific community.

The journal is intended for scientists engaged in fundamental and applied research in the field of general and veterinary virology, epizootology, immunology, mycology, micotoxicology, bacteriology, as well as practicing veterinarians and doctors of veterinary laboratories and state veterinary services, university-level teachers for veterinary, biological, medical specializations, graduate and postgraduate students.

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Миссией издания является представление информации об основных направлениях развития российской и мировой ветеринарной науки и практики и привлечение внимания научной общественности к актуальным проблемам и инновационным разработкам в области ветеринарии.

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Science-based assurance of the disease freedom in reindeer herds of the Russian Arctic zone

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ABSTRACT
Reindeer husbandry takes a leading position in the agricultural sector of the Russian Federation Arctic zone. The purpose of the research is to analyze the science-based assurance of the freedom from highly dangerous infectious diseases in reindeer herds of the Arctic zone. It has been established that diseases such as anthrax, brucellosis, footrot, rabies are still relevant for the reindeer husbandry and can cause not only significant economic damage, but also diseases in humans. The analysis of the archival data and literary sources, as well as own research data lead to the following conclusions: the greatest risk of anthrax occurrence and spread is posed by old carcass sites; to eradicate brucellosis, vaccination of animals along with the general disease control measures is necessary; in case of footrot, special attention should be paid to the control of blood-sucking insects and warble flies; to prevent rabies in reindeer herds, it is important to avoid contacts between deer and wild carnivores and consider emergency vaccination. Undoubtedly, the eradication and prevention of the above-mentioned infectious diseases requires constant epidemiological surveillance, including its element – monitoring, with all necessary special management, animal health measures. There is an obvious need for constant surveillance of infectious diseases in the Arctic zone of the Russian Federation using GIS technologies. It is important to pay special attention to the generation of special information layers related to disease characteristics, including deer herd migration routes, sites where anthrax outbreaks were recorded, and the location of disease-infected facilities.

Keywords: review, reindeer, infectious diseases, epizootic situation, science-based assurance

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Научное обеспечение эпизоотического благополучия в оленеводческих стадах Арктической зоны Российской Федерации

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РЕЗЮМЕ
Северное оленеводство занимает ведущее место в сельскохозяйственной отрасли Арктической зоны Российской Федерации. Целью исследований является рассмотрение вопросов научного обеспечения эпизоотического благополучия по основным особо опасным инфекционным болезням в оленеводческих стадах Арктической зоны. Установлено, что такие болезни, как сибирская язва, бруцеллез, некробактериоз, бешенство, по-прежнему актуальны для оленеводческой отрасли и не только наносят существенный экономический ущерб, но могут быть причиной заболевания людей. В результате анализа архивных сведений и литературных источников, а также материалов собственных исследований сделаны следующие выводы: наибольшую угрозу возникновения и распространения сибирской язвы представляют старые падёжные места; при ликвидации бруцеллеза наряду с общехозяйственными мероприятиями необходима вакцинация животных; при некробактериозе следует особое внимание уделять борьбе с кровососущими насекомыми и оводами; для недопущения возникновения бешенства в оленеводческих стадах важно исключить контакты оленей с дикими плотоядными и рассмотреть возможность экстренной или вынужденной вакцинопрофилактики. Несомненно, ликвидация и профилактика вышеуказанных инфекционных

болезней требует постоянного эпизоотологического надзора, в том числе его элемента – мониторинга, проведения необходимых специальных общехозяйственных и лечебно-профилактических мероприятий. Очевидна необходимость постоянного надзора за инфекционными болезнями в Арктической зоне Российской Федерации с использованием ГИС-технологий. Особое внимание важно уделять формированию специализированных информационных слоев, связанных с эпизоотическими характеристиками, в том числе слоев маршрутов оленьих стад, территорий, на которых регистрировали вспышки сибирской язвы, слоев расположения неблагополучных по болезням объектов.

Ключевые слова: обзор, северные олени, инфекционные болезни, эпизоотическая ситуация, научное обеспечение

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INTRODUCTION

Reindeer husbandry takes a leading position in the agricultural sector of the Russian Federation Arctic zone. The economic significance of this industry is based on the sustainable management of the scarce food resources of the vast territories of tundra, forest tundra, and northern taiga. No livestock species, except for reindeer, can sustainably use about 300 million hectares of the Russian Arctic pastures [1].

According to the Russian Statistics Agency, the number of domesticated reindeer in the country is about 1.6 million animals in 2022. The largest number of reindeer are herded in the Yamalo-Nenets Autonomous Okrug, followed by the Nenets Autonomous Okrug, the Republic of Sakha (Yakutia) and the Chukotka Autonomous Okrug (Fig. 1).

The annual production capacity of reindeer husbandry is the following:

– about 20 thousand tons of high-quality dietary meat and offal;

– more than 400 thousand hides;

– more than 100 tons of antlers in velvet and other valuable by-products.

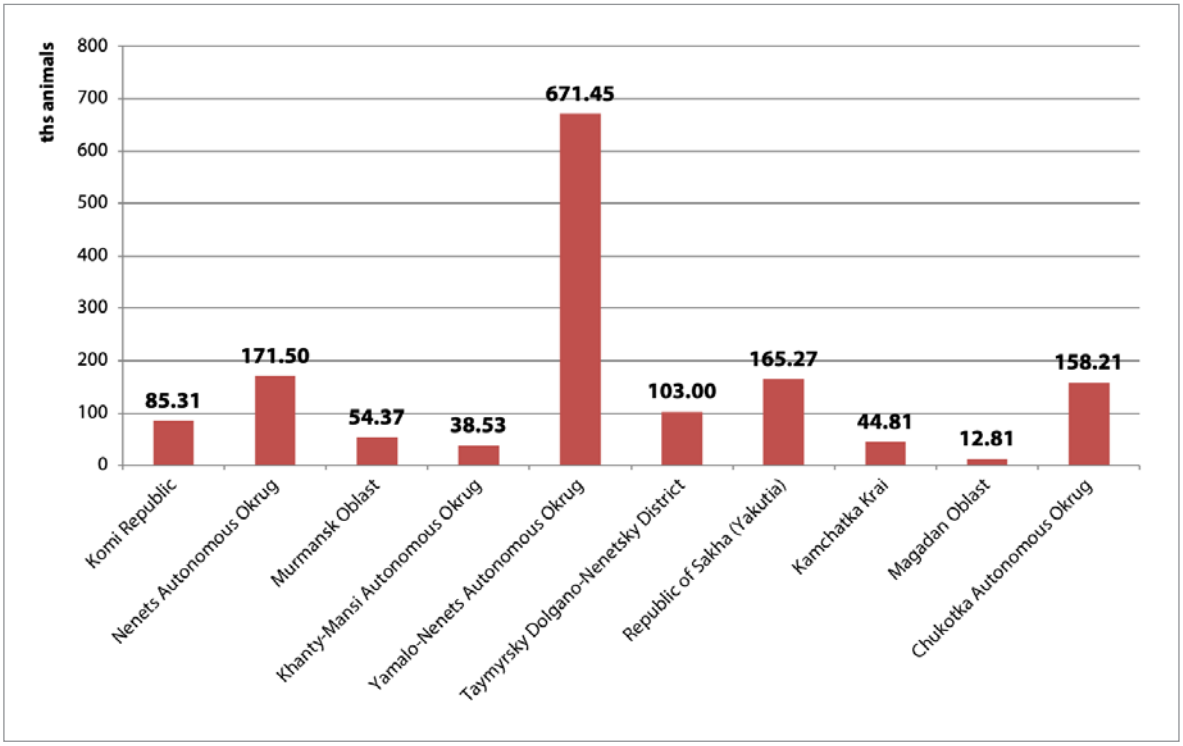

Fig. 1. The number of domesticated reindeer in the regions of the Russian Federation in 2022 (ths animals)

The prospects for the reindeer husbandry development are:

– deep processing of products (offal, glands, blood, hides), as well as promotion of reindeer products among consumers;

– export of products by large regional farms, optimization of supplies to the domestic market by small farms and private owners;

– development of ethnotourism and related activities (hunting, fishing, picking wild plants).

The main risk factors for the industry development include:

1) pastures:

– deliquination, overgrowth and decrease in plant community performance due to high grazing load;

– reduction of areas suitable for grazing due to commercial exploitation of lands, their use for hydrocarbon and mineral output, transport infrastructure;

– contamination of plant feeds with heavy metals at the local level (accidental pollution) and due to global atmospheric transport;

– changes in the species composition of the plant community due to global climatic changes, an increase in the proportion of herbaceous plants in the total phytomass;

2) organizational and economic measures:

– complicated logistics for the supply and export of products and high transportation costs (up to 50–60% of the product cost);

– lack of snowmobile maintenance services;

– low wages for reindeer herders, an acute shortage of qualified personnel (reindeer herders and veterinarians);

– low level of social protection and medical care for reindeer herders and their families, a shortage of women to start a family due to harsh living conditions;

3) diseases:

– epizootological and epidemiological risks associated with the endemic areas of anthrax and other infectious diseases;

– the emergence of new infectious diseases and pests due to global warming and occurrence of new vectors;

– decreased nonspecific resistance due to lack of feed.

The purpose of this analytical study was to summarize and analyze data on the situation related to major highly dangerous infectious diseases of reindeer, focusing on the main causes of their occurrence, as well as measures to prevent and control them.

MATERIALS AND METHODS

The results of the work are based on the analysis of archival data and literary sources, as well as on the own research data. Historical and comparative analysis, structural analysis, visualization, systematization, analog and generalization methods were used. The work was carried out at the St. Petersburg Federal Research Center of the Russian Academy of Sciences and on the reindeer farms of the Russian Arctic.

RESULTS AND DISCUSSION

Anthrax. The greatest danger for the occurrence and spread of the disease is represented by old carcass sites. In the past, during the outbreaks, in the absence of the disease control tools and methods, the reindeer herders left diseased and dead animals and migrated further and further on with a healthy herd until the outbreak stopped. Thus, a "trail" of unburied animals remained on the routes of reindeer herd migrations, forming the so-called carcass sites, covering the area of tens or even hundreds of square kilometers. There are more than 100 registered sites in the Arkhangelsk Oblast and the Komi Republic, more than 60 sites in Yamal, about 40 sites in Taymyr, more than 200 in Yakutia, there is a high number of them in other northern regions [2].

The particular danger of such infected territories is that they do not have concrete, well-defined registered borders. There are only landmarks, and the areas of sites are from 1 to 150 km^2 (Fig. 2).

The question "what to do with such a big number of carcass sites" has repeatedly been raised for a long time;

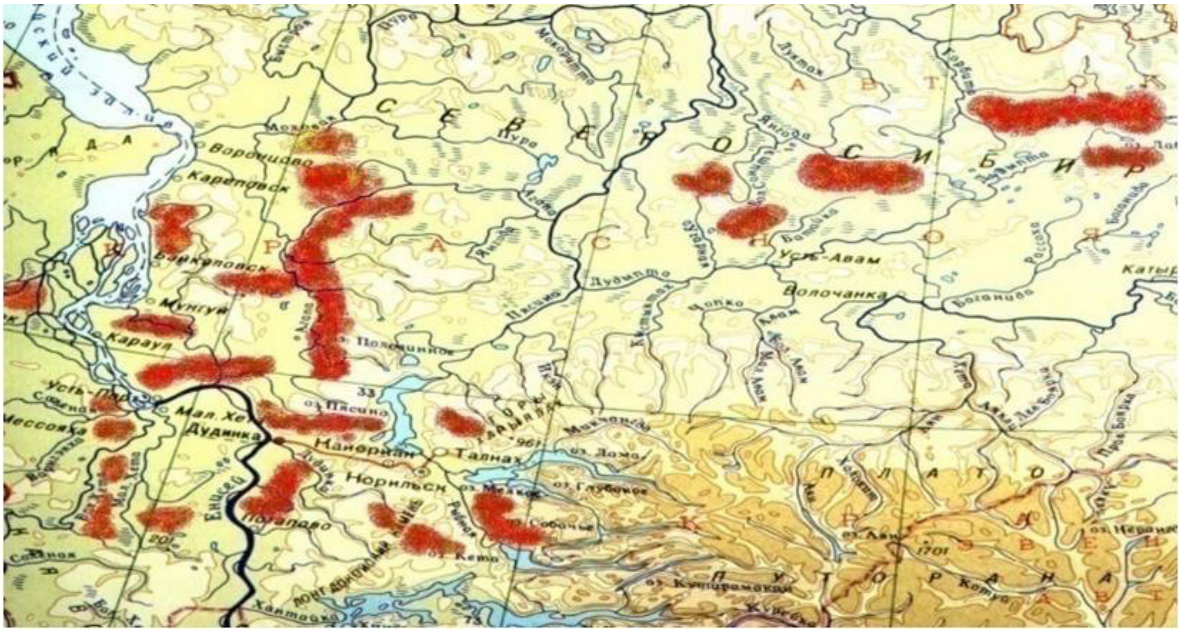

Fig. 2. Location of old carcass sites in the Taymyr Peninsula

discussions about the feasibility of blanket reindeer vaccination in the setting of transport difficulties, high labor intensity and costs of veterinary and preventive measures to be taken in reindeer husbandry have been held.

On the one hand, anthrax spores are known to survive and remain pathogenic in the soil for more than 70 years, therefore, carcass sites can still be potentially dangerous [3]. This is confirmed by anthrax cases among deer in the state farms "Popigaisky" (1969) and "Oktyabrsky" (1977), who grazed their herds in old carcass sites.

On the other hand, N. F. Gamaleya noted that anthrax spores can lose virulence and even be lysed under unfavourable conditions [4]. Ipatenko N. G. et al. emphasized that *Bacillus anthracis* spore germination heavily depends on a number of conditions: minimum temperature of 15 °C, organic matter content up to 14%, maximum humidity of 50% and minimum pH of 7.0 [5].

It should also be noted that domesticated reindeer herds are constantly grazing on the carcass sites, and they are not always vaccinated against anthrax, while a large number of constantly migrating wild animals are present in the region, earthworks and mining are actively carried out [6], however, with the exception of sporadic cases in the Taymyr Peninsula and in the Republic of Sakha (Yakutia), the disease has not been reported for more than 80 years.

Based on the data available and comprehensive studies conducted in the Yamalo-Nenets Autonomous Okrug, it was suggested that anthrax cases in deer herds are of external origin. In this context, it was decided to exclude the anthrax vaccination of reindeer from the routine vaccination plan in this region in 2007. However, in 2016, an anthrax outbreak occurred in one of the herds of domesticated deer in the Yamalo-Nenets Autonomous Okrug. The occurrence was explained by the exposure to the bacteria preserved in soil after unusual heat waves, which melted the permafrost. The animals were grazed on pastures where anthrax was reported in 1938–1941. 2,650 reindeer got diseased; 36 people got infected and one died after coming into contact with diseased and dead animals [7, 8, 9, 10]. Currently, vaccination against anthrax in the Yamalo-Nenets Autonomous Okrug is performed to the full extent.

To prevent anthrax infection and induce active artificial immunity in anthrax-risk areas, a lyophilized vaccine, containing live spores of strain 55 or STI is widely used. Immunity is formed 10 days after immunization and lasts for at least 12 months [2].

Speaking about anthrax prevention in reindeer, the focus should be made on the current research by scientists from the Pechora Veterinary Department of the Research Institute of Agriculture of the Komi Republic under the guidance of professor E. S. Kazanovsky and the All-Russian Scientific Research Institute of Veterinary Virology and Microbiology on the combined prevention of anthrax and infestations with warble flies. The results of the studies showed the good compatibility and the possibility of using iver-, avermectins and anthrax vaccine based on 55-VNIIVViM strain in one injection. It has been established that the pharmaceutical composition has a high preventive effectiveness against warble fly parasitizing larvae and induces stable immunity against anthrax with high antibody titers. Currently, unfortunately E. S. Kazanovsky's et al. methodological recommendations have not been approved and are not applied in practice [11].

Brucellosis. The first suspicion of brucellosis in reindeer herding farms in the Chukotka Peninsula were expressed in 1939 by A. V. Rudakov, but in natural conditions, brucellosis was first diagnosed in reindeer in Taymyr by serological and allergic skin tests in 1948 by I. M. Golosov, and in 1955 V. A. Zabrodin was the first to isolate *Brucella* strains from reindeer. For the first time, *Brucella* species specific for reindeer were isolated from wild animals by V. A. Zabrodin in the Taymyr Peninsula from the affected limb of a wild reindeer. Subsequent studies established a high brucellosis prevalence in wild reindeer in the Taymyr Peninsula, in some years reaching 35–40%. Later, when studying the epizootology of brucellosis, *Brucella* strains were isolated from other animal species (wolf, blue and white arctic fox, wolverine, ermine, sable, silver fox). Herewith, based on their morphological, tinctorial and biochemical properties, these strains were identical to *Brucellas* isolated from domesticated and wild reindeer, and belonged to *Brucella suis* biovar 4. These studies confirm that brucellosis endemic areas have established in certain Arctic territories of the Russian Federation [12, 13].

In the 60–80s of the last century, brucellosis was widespread in the reindeer farms of the Taymyr Peninsula (Dolgano-Nenets), Evenki, Yamalo-Nenets and Chukotka Autonomous Okrugs, the Kamchatka Oblast and the Yakut ASSR. The brucellosis prevalence in some herds reached 30–40%, and the number of clinically diseased animals was 20–25% [14].

The current brucelosis situation in domesticated reindeer herds of the Russian Arctic zone is described in the table.

When analyzing the economic damage to reindeer husbandry from brucellosis infection, it should be borne in mind that it includes losses from increased infertility and

Table
Number of reported reindeer brucellosis-infected localities in 2015–2021

RF Subject	2015	2016	2017	2018	2019	2020	2021
Khanty-Mansi Autonomous Okrug	0	0	1	0	0	0	0
Yamalo-Nenets Autonomous Okrug	7	6	6	9	9	7	5
Taymyrsky Dolgano-Nenetsky Municipal District	0	1	1	2	3	1	0
The Republic of Sakha (Yakutia)	45	42	42	37	35	26	21
Chukotka Autonomous Okrug	1	1	1	1	1	1	1
Total	53	50	51	49	48	35	27

abortions in females, weak, often dead new-born calves, emergency culling and slaughter of animals showing clinical signs of the disease and positive reactors in diagnostic tests, irregular economic activities due to quarantine restrictions in infected herds, additional costs for diagnostic and health improvement measures.

Particularly noteworthy is the risk of reindeer brucellosis to the health of humans, especially those who consume products from diseased animals, mainly non heat-treated (traditional cuisine of indigenous minorities of the northern regions), as well as those involved into reindeer herding or primary processing of reindeer products [15, 16].

Initially, to prevent and control brucellosis many researchers recommended using only general animal health measures in reindeer husbandry, however, experience has shown that this is not enough to eradicate brucellosis in reindeer, since there are brucellosis endemic areas, and the technological features of this industry do not allow taking proper and comprehensive actions.

Currently, health status is improved using vaccines based on *Brucella abortus* 82 strain in accordance with the approved guidelines [17].

In the light of my own experience with reindeer brucellosis, I would like to note the following:

1. It is impossible to solve the brucellosis problem in reindeer herds without vaccination. Long-term efforts were made to eradicate brucellosis without using vaccines in the Magadan Oblast, but no particular achievements have been gained.

2. The vaccine based on *Brucella abortus* 19 strain has been undeservedly neglected. It is the most stable and most potent strain. Therefore, it is necessary to recommend the use of this vaccine, especially in the regions where brucellosis endemic areas have formed (Taymyr, north-west of the Republic of Sakha (Yakutia) and north-east of Yamal). The effectiveness of such vaccination has been confirmed by the positive results of reindeer immunization in the Taymyr Peninsula. This vaccine is criticized for its long-term post-vaccination titers, but if administered in small doses, the titers disappear within 6–9 months, and long-term post-vaccination titers in reindeer were reported after the use of vaccines based on other strains.

3. Immunization doses of brucellosis vaccines for reindeer should be reviewed and approved. Reindeer are significantly smaller than cattle, and it is not feasible to administer 1/2, 1/4 of the full cattle dose, since it has been proved in theory and practice that high antigen doses, on the contrary, suppress the immune system, and consequently, the development of strong immunity.

4. The issue of diagnostic reactions and diagnostic antibody titer in reindeer requires additional studies. Now some specialists use ELISA to diagnose brucellosis in reindeer, and they do not know what to do with positive reactors. Are positive titers equal to 1:25, 1:50 so dangerous if there are no clinical signs and the brucellosis agent has not been isolated by bacteriological testing?

Footrot. Footrot of reindeer in the Russian North conditions was firstly studied by N. I. Ekkert, who described its major clinical signs in 1898 [18]. In 1909 E. N. Pavlovsky wrote that digital suppurative inflammation causing great damage to the nomads of circumpolar tundra was reported every year in the tundra and forests of the Arkhangelsk governorate [19]. The disease can affect 1.5 to 50% of reindeer in herds.

According to I. M. Golosov and B. V. Maslukhin [20], and state statistical reports, 42,834 reindeer died from footrot in the Taymyr National District over 16 years (1950–1965). As noted by I. G. Machakhtyrov [21], in the 90s of the last century, 10 to 50 thousand reindeer got diseased in the Republic of Sakha (Yakutia) as a whole, and the mortality rate reached 32.1%.

Currently, footrot of reindeer is not included into official statistical data, in our opinion, this is due to the need to impose quarantine restrictions in case of a positive result, which is not beneficial to animal owners, but this means, the causative agent is still circulating in reindeer herds. This is confirmed by studies of the reindeer rumen microbiome: *Fusobacterium necrophorum* species were isolated from more than 50% of animals [22, 23].

Speaking about the prevention of this disease in reindeer herds, it should be noted that many scientists are working on the creation of the specific means to control this disease in reindeer. Currently, footrot vaccines have been developed, but they are rarely used in reindeer husbandry, since they either cause post-vaccination complications or do not fit into the reindeer husbandry technology. However, the development of the vaccines to prevent footrot of reindeer is an important and relevant issue.

Currently, the most effective treatment is the wound debridement and subsequent antimicrobial therapy with drugs mainly based on oxytetracycline (for example, Nitox). Combined systemic drugs are now widely used: Tetracine, Fusobaksan-2 and Fusobarin, Necrofar-C, as well as a topical drug – Necrogel, which can increase the effectiveness of footrot treatment compared with a conventional therapy by more than 2–3 times. We believe that work in this area needs to be continued. Research on antibacterial aerosols to treat wounds and improve tissue regeneration as well as long-term protection of wounds from re-infection are promising.

General animal health and hygiene measures aimed at increase of natural resistance of the body and protecting from harmful environmental factors are of paramount importance in the disease control strategy.

First of all, the footrot issue, of course, should be considered in association with blood-sucking insect and warble fly control. Together with the scientists from the All-Russian Scientific Research Institute of Veterinary Entomology and Arachnology (Tyumen), we have tested new drugs to control gnats and warble flies in reindeer herding farms of the Yamalo-Nenets Autonomous Okrug and the Taymyrsky Dolgano-Nenets Municipal District. The production tests proved that the footrot incidence in herds in which reindeer were treated against gnats and warble flies was 6.5 times lower than in non-treated herds [24]. Research in this area should be continued, and first of all they must be aimed at the development of insecticides and repellents providing longer protection.

In our opinion, the proper winter grazing and mineral supplementation of animals during winter and early spring seasons are important for the prevention of footrot and other diseases of reindeer. Together with the staff of the Naryan-Mar agricultural research station, a concentrated feed containing carbohydrates, vitamins and minerals was developed, which can be successfully used for feeding reindeer in winter and spring. Economic efficiency of the feed additive use in a 1,800 reindeer herd

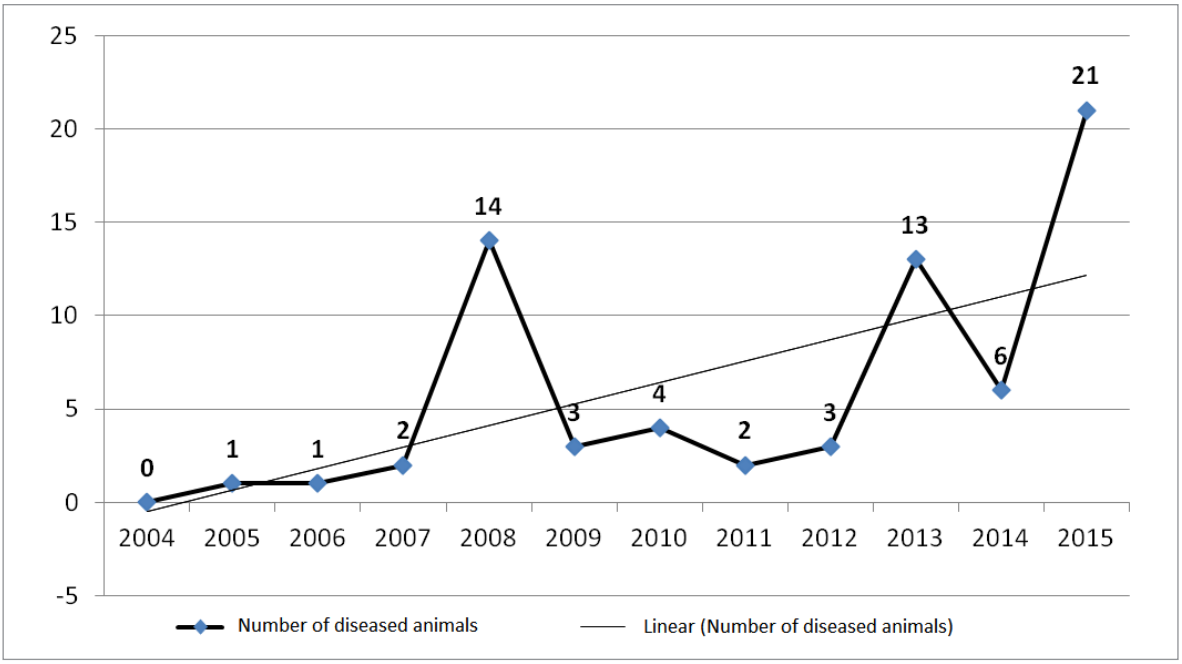

Fig. 3. The dynamics of rabies incidence in the Nenets Autonomous Okrug in 2004–2015

within 40 days is more than 1.2 million rubles. Together with Biotrof+ LLC, research is being conducted on the topic "Microbiome of *Rangifer tarandus* rumen in the Russian Arctic". In the course of the studies microorganisms from reindeer rumen were obtained, being a potential source of cellulases and biodegraders of toxins, produced by micromycetes. Currently, a range of therapeutic and prophylactic drugs are being developed on the basis of these microorganisms [25].

Rabies. Previously, animal rabies in the Russian Arctic zone was reported quite rarely and most often it was called "dikovanie" (the state of being furious). The peculiarities of rabies manifestation in the high latitudes contributed to the formation of the concept that an independent animal disease exists in the tundra zones known as "dikovanie", or, in other words, Arctic rabies, but currently the causative agent of "dikovanie" is recognized as a geographical variant of the classic rabies virus [26]. In recent years, rabies has been periodically reported in the Nenets and Yamalo-Nenets Autonomous Okrugs, and the Republic of Sakha (Yakutia).

Thus, in the territory of the Nenets Autonomous Okrug, rabies in carnivorous animals and reindeer is reported annually, creating a complicated epizootological and epidemiological situation in the regions. The dynamics of the rabies incidence in this district in 2004–2015 reflects the problem in 2008, 2013 and 2015 (Fig. 3) [27].

Microscopic examinations using fluorescent antibodies (FA) technique of pathological samples (brain) from various animal species, which died in the Okrug, 70 (54%) of 130 samples were positive, among them 37% were samples from domesticated reindeer, 53% from wild animals (Arctic foxes – 33%, foxes – 20%), 10% from stray dogs. Animal cases were reported in the winter-spring periods (February – March) during active migration and breeding seasons of wild carnivores. In the same period or somewhat later (April – May), taking into account the latency period, cases among domesticated reindeer were reported.

CONCLUSION

Thus, it has been established that diseases such as anthrax, brucellosis, footrot, rabies are still relevant for the reindeer husbandry and can cause not only significant economic damage, but also diseases in humans. Summarizing the above, the following conclusions can be drawn: the risk of anthrax occurrence and spread is mostly associated with old carcass sites; brucellosis eradication requires animal vaccination along with general animal health measures; speaking about footrot, special attention should be paid to the control of blood-sucking insects and warble flies; to prevent rabies in reindeer herds, it is necessary to exclude contacts between reindeer and wild carnivores and consider the possibility of vaccination. Undoubtedly, the eradication or prevention of the above-mentioned infectious diseases requires constant epizootological supervision, appropriate special management and animal health measures.

There is an obvious need for constant surveillance of infectious diseases in the Arctic zone of the Russian Federation using GIS technologies. It is important to pay special attention to the generation of special information layers related to disease characteristics, including deer herd migration routes, areas where anthrax outbreaks were recorded, and locations of disease-infected facilities. For example, if the layers of locations of carcass sites, cyclone sites infected with a pathogen, migration routes of wild animals, etc. are known, it is possible to plan the droving routes that reduce the risk of certain infectious disease occurrence in deer and, most importantly, to actively introduce comprehensive veterinary and preventive measures.

In conclusion, it should be noted that the review takes into account only the most significant infectious diseases of reindeer. Of course, reindeer herding establishments should take into account foot-and-mouth disease, paratuberculosis and other infectious and emergent diseases, depending on the region. For example, in Taymyr and Yamal, a head disease has been repeatedly reported, the etiology of which is still understudied.

REFERENCES

1. Zabrodin V. A., Layshev K. A., Dubovik I. K. The development northern of reindeer husbandry in the framework of the Arctic interests of Russia. *Izvestiya Saint-Petersburg State Agrarian University*. 2015; (40): 108–112. https://elibrary.ru/svmmgc (in Russ.)

2. Kazanovsky E. S., Karabanov V. P., Klebenson K. A. Disease of reindeer (veterinary guide). Syktyvkar: Poligraf-Servis; 2011. 36 p. (in Russ.)

3. Carlson C. J., Getz W. M., Kausrud K. L., Cizauskas C. A., Blackburn J. K., Bustos Carrillo F. A., et al. Spores and soil from six sides: interdisciplinarity and the environmental biology of anthrax (*Bacillus anthracis*). *Biological reviews of the Cambridge Philosophical Society*. 2018; 93 (4): 1813–1831. https://doi.org/10.1111/brv.12420

4. Bakulov I. A., Gavrilov V. A., Seliverstov V. V. Anthrax: new pages in the study of the "old" disease. Vladimir: Posad; 2001. 283 p. (in Russ.)

5. Ipatenko N. G., Gavrilov V. A., Zelepukin V. S., Manichev A. A., Bakhtarov S. I., Saiitkulov B. S., Tatarintsev N. T. Anthrax. 2nd ed., revised and supplemented. Moscow: Kolos; 1996. 335 p. (in Russ.)

6. Listishenko A. A. New approaches to anthrax in the Far North. *Issues of Regulatory Regulation in Veterinary Medicine*. 2014; (2): 87–90. https://elibrary.ru/sfkzjr (in Russ.)

7. Popova A. Yu., Demina Yu. V., Ezhlova E. B., Kulichenko A. N., Ryazanova A. G., Maleev V. V., et al. Outbreak of anthrax in the Yamalo-Nenets autonomous district in 2016, epidemiological peculiarities. *Problems of Particularly Dangerous Infections*. 2016; (4): 42–46. https://doi.org/10.21055/0370-1069-2016-4-42-46 (in Russ.)

8. Selyaninov Yu. O., Egorova I. Yu., Kolbasov D. V., Listishenko A. A. Anthrax in Yamal: re-emergence causes and diagnostic issues. *Veterinariya*. 2016; (10): 3–7. https://elibrary.ru/wzixfl (in Russ.)

9. Liskova E. A., Egorova I. Y., Selyaninov Y. O., Razheva I. V., Gladkova N. A., Toropova N. N., et al. Reindeer anthrax in the Russian Arctic, 2016: Climatic determinants of the outbreak and vaccination effectiveness. *Frontiers in Veterinary Science*. 2021; 8:668420. https://doi.org/10.3389/fvets.2021.668420

10. Ezhova E., Orlov D., Suhonen E., Kaverin D., Mahura A., Gennadinik V., et al. Climatic factors influencing the anthrax outbreak of 2016 in Siberia, Russia. *EcoHealth*. 2021; 18 (2): 217–228. https://doi.org/10.1007/s10393-021-01549-5

11. Kazanovsky E. S., Karabanov V. P., Klebenson K. A. Treatment and prophylactic effectiveness of composition ganamectin and vaccines of st. 55 against oedemagenosis and anthrax of reindeers. *Perm Agrarian Journal*. 2014; (2): 60–65. https://elibrary.ru/sfbdgv (in Russ.)

12. Zabrodin V. A., Gordienko L. N., Karepin E. V., Tolstikov V. G., Chabanov Yu. P., Kulikova E. V. Khishchniki tundry i lesotundry – rezervuar vozbuditelya brutselleza severnykh olenei = Predators of tundra and forest tundra – reservoirs of brucellosis pathogen in reindeer. *Bolezni dikikh zhivotnykh: sbornik trudov konferentsii (g. Pokrov, 28–30 sentyabrya 2004 g.) = Diseases of Wild Animals: Proceedings of the Conference (Pokrov, 28–30 September, 2004)*. Pokrov: VNIIVViM; 2004; 95–99. (in Russ.)

13. Zaberezhny A. D., Iskandarov M. I., Fedorov A. I., Borodulina P. I., Iskandarova S. S., Layshev K. A., et al. Catalogue of Brucella strain genotypes, stored in the All-Russian Collection of pathogenic and vaccine strains of the microorganisms causing animal infectious diseases FGBNU FNTs VIEV RAN: study guide. Novosibirsk: OOO "SibAK"; 2020. 76 p. https://doi.org/10.13140/RG.2.2.13668.81283 (in Russ.)

14. Vinokurov N. V., Zakharova O. I. Brucellosis of reindeer: guidance manual. Saint Petersburg: Naukoemkie tekhnologii; 2022. 129 p. https://elibrary.ru/edoyyu (in Russ.)

15. Vinokurov N. V., Iskandarov M. I., Layshev K. A., Sleptsov E. S., Grigoriev I. I., Tatarinova Z. G. Epizootological and epidemiological role of brucellosis of different animal species in the Russian Federation. *Veterinaria i kormlenie*. 2020; (6): 13–15. https://doi.org/10.30917/ATT-VK-1814-9588-2020-6-3 (in Russ.)

16. Iskandarov M. I., Nifontov K. R., Tomashevskaya E. P. Epidemiological danger some species of brucella and taxonomic position of reindeer. *Hippology and Veterinary Medicine*. 2021; (2): 113–117. https://doi.org/10.13140/RG.2.2.13714.99525 (in Russ.)

17. Laishev K., Sleptsov E., Fogel L., Kisil A., Veretennikov V. Concept development to optimize the reindeer brucellosis prevention. *Advances in Animal and Veterinary Sciences*. 2020; 8 (2): 18–23. https://doi.org/10.17582/journal.aavs/2020/8.s2.18.23

18. Ekkert N. I. Poval'nye bolezni severnykh olenei: predvaritel'nyi otchet Veterinarnomu upravleniyu = Mass diseases of reindeer: preliminary report to the Veterinary Authority. *Arkhiv veterinarnykh nauk*. 1898; Book 1: 1–31. (in Russ.)

19. Pavlovsky E. N. Ovodovaya bolezn' severnykh olenei = Warble fly disease of reindeer. *Vestnik obshchestvennoi veterinarii*. 1909; 6: 288–291. (in Russ.)

20. Golosov I. M., Maslukhin B. V. Footrot in reindeer. Norilsk; 1969. 148 p. (in Russ.)

21. Machakhtyrov I. G. Epidemiology and vaccination against footrot in reindeer in the Republic of Sakha (Yakutia): Author's abstract of thesis for degree of Cand. Sci. (Veterinary Medicine). Yakutsk; 2000. 22 p. (in Russ.)

22. Ilina L. A., Filippova V. A., Brazhnik E. A., Dubrovin A. V., Yildirim E. A., Dunyashev T. P., et al. The comparative analysis of the ruminal bacterial population in reindeer (*Rangifer tarandus* L.) from the Russian Arctic zone: regional and seasonal effects. *Animals*. 2021; 11 (3): 911. https://doi.org/10.3390/ani11030911

23. Laishev K. A., Ilyina L. A., Yuzhakov A. A. Features of the rumen microbiome in reindeer with necrobacteriosis. *International Bulletin of Veterinary Medicine*. 2023; (2): 18–24. https://doi.org/10.52419/issn2072-2419.2023.2.18 (in Russ.)

24. Samandas A. M., Layshev K. A., Gulyukin M. I., Gulyukin A. M., Iskandarov M. I., Sleptsov E. S., et al. Optimization of reindeer protection from gnat, warble flies and footrot in the far North: monograph. Novosibirsk: ANS "SibAK"; 2019. 190 p. https://doi.org/10.13140/RG.2.2.26417.43360 (in Russ.)

25. Ilina L. A., Layshev K. A., Laptev G. Yu., Philippova V. A., Yildirim E. A., Dunyashev T. P., et al. Microbiome of reindeer rumen *Rangifer tarandus* in the Russian Arctic regions. Saint Petersburg: Biotroph Ltd.; 2020. 272 p. https://elibrary.ru/bzidui (in Russ.)

26. Makarov V. V., Gulyukin A. M., Gulyukin M. I. Rabies: Natural History at Centuries Boundary. Moscow: ZooVetKniga; 2015. 121 p. (in Russ.)

27. Romanenko T. M., Anufriev V. V., Vylko Yu. P., Layshev K. A., Ivkina M. V. About epizootic situation on rabies the reindeer herding Nenets Autonomous Area. *Bulletin of the Northern Trans-Ural State Agricultural University*. 2016; (1): 91–98. https://elibrary.ru/vzrzsx (in Russ.)

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The problem of norovirus infection in animals (literature review)

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ABSTRACT

Livestock industry efficiency strongly depends on the livability of young animals, mainly during the early postnatal period. Infectious gastroenteritis of newborns manifested as diarrhea occupies the leading place among the diseases of young animals and brings the production and economic losses. The cause of numerous gastrointestinal disorders are physiological, hygienic, infectious and other factors. This pathology is reported in 50–80% of newborn calves, while 15–55% of diseased animals die. The investigations of the etiology of numerous diarrhea cases revealed rota-, corona-, parvo-, enteroviruses and bovine viral diarrhea virus in fecal samples from calves. Inactivated vaccines have been developed in the Russian Federation to prevent viral diarrhea in cattle. Despite their high antigenicity and field effectiveness, numerous cases of diarrhea in newborn calves have been reported in a number of large livestock farms. In fecal samples collected from diseased individuals, noroviruses along with the above-mentioned viruses were detected by electron microscopy. The noroviruses were detected in fecal samples from humans, cattle, pigs, sheep, dogs, cats, mice, as well as in pork and milk samples. The norovirus genome is prone to mutations, resulting in antigenic shifts and recombination, as well as the emergence and rapid spread of new epidemic and epizootic variants. Epidemiological features of norovirus infection include: prolonged shedding of the virus by the diseased animals and carriers, various transmission routes (fecal-oral, contact) and high contagiousness. In late 20th and early 21st century a large number of dairy and meat cattle were imported to the Russian Federation from various countries, including norovirus-infected countries. All this suggests the need to take noroviruses and other viruses (neboviruses, toroviruses, astroviruses, kobuviruses) into account when investigating the etiology of numerous diarrhea cases in newborn calves and necessitates the development of norovirus diagnostic tools and methods, as well as control measures.

Keywords: review, noroviruses, *Caliciviridae,* diarrhea, calves, pigs, genotypes, genogroups, zoonosis, fecal-oral transmission

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Проблема норовирусной инфекции животных (обзор литературы)

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РЕЗЮМЕ

Основой повышения эффективности животноводства является сохранность молодняка, главным образом в ранний постнатальный период. Ведущее место среди болезней молодняка занимают инфекционные гастроэнтериты новорожденных животных, которые проявляются диареей и приводят к производственным и экономическим потерям. Причиной массовых нарушений функции органов пищеварения являются физиологические, санитарно-гигиенические, инфекционные и другие факторы. Данная патология регистрируется у 50–80% новорожденных телят, во многих случаях отмечается гибель от 15 до 55% больных животных. При установлении этиологии массовых диарей в пробах фекалий телят выявляли рота-, корона-, парво-, энтеровирусы и возбудители вирусной диареи – болезни слизистых. Для профилактики вирусных диарей крупного рогатого скота в Российской Федерации были разработаны инактивированные вакцины. Несмотря на их высокую антигенную активность и полевую эффективность, в ряде крупных животноводческих

хозяйств были зарегистрированы случаи массовых диарей новорожденных телят. В пробах фекалий, отобранных от отдельных больных животных, наряду с возбудителями указанных инфекций методом электронной микроскопии выявлялись норовирусы. Возбудитель норовирусной инфекции был обнаружен в пробах фекалий человека, крупного рогатого скота, свиней, овец, собак, кошек, мышей, а также в свинине и молоке. Геном норовируса подвержен мутациям, что приводит к антигенному сдвигу и рекомбинациям, а также возникновению и быстрому распространению новых эпидемических и эпизоотических вариантов возбудителя. Эпизоотологическими особенностями норовирусной инфекции являются: длительное выделение возбудителя из организма больных животных и животных-вирусоносителей, реализация различных путей передачи (фекально-орального, контактного) и высокая контагиозность. В конце XX и в начале XXI века в Российскую Федерацию из разных стран, в том числе и из неблагополучных по норовирусной инфекции, было завезено большое количество крупного рогатого скота молочных и мясных пород. Все это свидетельствует о необходимости учета норовирусов и других патогенов (небовирусов, торовирусов, астровирусов, кобувирусов) при выяснении этиологии массовых случаев диарей новорожденных телят, а также разработки средств и методов диагностики и мер борьбы с норовирусной инфекцией животных.

Ключевые слова: обзор, норовирусы, *Caliciviridae*, диарея, телята, свиньи, генотипы, геногруппы, зооноз, фекально-оральный путь заражения

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Historically, new pathogens of viral gastrointestinal infections in cattle were described based on electron microscopy results of faecal samples from newborn diarrheic calves. Subsequently, other methods, including molecular biology, began to be used for this purpose. Rotaviruses, coronaviruses, caliciviruses, toroviruses, astroviruses, kobuviruses, neboviruses and pestiviruses (causing bovine mucosal complex) were found in fecal samples from diarrheic calves using electron microscopy, molecular biology and fecal immunochemical test) [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13]. A study of 269 fecal samples from sheep, goats, cattle, pigs and rabbits taken from livestock farms in Hungary revealed a new picornavirus, which was classified as *Bopivirus* genus [14].

Caliciviruses infecting a wide range of vertebrates, as well as humans, were separated from the *Picornaviridae* family in 1979 [12]. The *Caliciviridae* family unites a group of RNA viruses with similar morphology and different antigenic properties [15]. Caliciviruses are stable and highly resistant to physical and chemical exposures (factors) of the environment; remain infectious at pH 2.7 for 3 hours at room temperature. Viruses are resistant to ether, chloroform, guanidine, sodium deoxycholate, as well as to pH 4–5, and are active for 30 minutes when heated to 60 °C [3, 15, 16, 17]. Calicivirus virions are small non-enveloped particles 27–40 nm in diameter with icosahedral symmetry (T = 3). A characteristic feature of calicivirus capsid architecture is 32 cup-shaped depressions at each of the icosahedral five-fold and three-fold axes (*calyx* is derived from the Latin which means cup). The molecular weight of the virion is 15 MDa, the sedimentation constant is 170–183 S, the buoyant density in CsCl gradient is 1.36–1.41 g/cm^3. The capsid is comprised of 180 copies of the major capsid protein VP1 and 1–2 copies of the minor structural protein VP2, around the VPg-linked genome. VP1 dimers form 90 arch-shaped capsomers that form visible 40 Å deep and 90 Å wide depressions (cups). The genome of caliciviruses is positive-sense, single-stranded RNA with a molecular weight of 2.6–2.8 MDa and 7,500–7,700 bp in size. The infectivity of calicivirus RNA is caused by the VPg peptide covalently linked to genomic RNA [2, 3, 16, 17, 18, 19, 20]. The classification of caliciviruses was approved by the decision of the International Committee on Taxonomy of Viruses in 2002. This classification was based on the results of nucleotide sequence phylogenetic analysis [20, 21, 22]. Currently, the *Caliciviridae* family comprises pathogens belonging to eleven genera, among them noroviruses, neboviruses, sapoviruses, vesiviruses, lagoviruses, etc.[1]

In 1972, a new virus was discovered by immune electron microscopy in an infectious stool filtrate derived from an outbreak of human gastroenteritis in Norwalk, Ohio. The virus was named *Norwalk virus* [16, 17, 23], and the disease was named a norovirus infection. The results of numerous studies conducted in many countries indicate that all identified noroviruses have closely related genome structures, but are genetically and antigenically highly diverse and infect a wide range of mammalian host species including humans. This virus was detected in biological samples from cattle [24, 25, 26], pigs [12, 27, 28], sheep [29], cats [30, 31], dogs [32, 33], and mice [34].

Based on the phylogenetic analysis of the genome nucleotide sequences, noroviruses were classified into 7 genogroups [16, 18, 35]. Subsequently, separate clusters (genotypes) and genetic variants were recognized in each genogroup [11, 16, 18, 20, 35, 36, 37, 38, 39, 40, 41, 42, 43]. Noroviruses are characterized by rapid genetic variability [18]. The results of the VP1 phylogenetic analysis suggest that new norovirus strains emerge every 2–3 years and there is a risk of highly virulent strain occurrence. About 5% of *Norwalk virus* population evolve into new genetic variants every year [16, 44]. Recombinations of caliciviruses are frequently reported, being the reason of antigenically altered viral strain emergence [16, 39, 45, 46]. The *Norwalk virus* genome is prone to mutations, resulting in antigenic shifts and recombinations, as well as the

[1] Current ICTV Taxonomy Release. https://ictv.global/taxonomy

emergence and rapid spread of new epidemic and epizootic variants [17, 18, 24, 41, 45]. The mutation processes involve the genome regions responsible for the virus binding to host receptors on the intestinal mucosal epithelium [16, 41].

Epidemiological features of norovirus infection include: prolonged shedding of the virus by the diseased animals and carriers, various transmission routes (fecal-oral, contact) and high contagiousness [16, 18]. Norovirus-contaminated feed and water can serve as transmission factors. The virus reservoir and source are infected (diseased and convalescent) animals. Bovine norovirus or human norovirus are responsible for the infection in cattle [24, 28]. One gram of feces from a diseased animal contains 10^8 viral particles or norovirus RNA copies [2, 8, 16, 17, 18, 38]. It has been proven that the ingestion of 10 norovirus virions is sufficient for the development of clinical manifestations [8, 16, 17, 18]. The norovirus infection incubation period in newborn calves infected with the virus isolated from cattle is 14–48 hours, the duration of the disease is from 2 to 30 days. Following the recovery, the virus is still shed for 5–50 days in the amount of 10^4 copies of viral RNA per 1 g of feces. Calves infected with human norovirus start demonstrating clinical signs 2–6 days post infection [47].

Virions replicate and assemble in the cytoplasm, and viral particles are released when the cell is destroyed. The replication cycles of the caliciviruses are similar as far as they have been explored: viruses interact with a multitude of cell surface attachment factors (glycans) and co-receptors (proteins) for adsorption and penetration, use cellular membranes for the formation of replication complexes [48].

Noroviruses propagate in the epithelium cells of small intestinal villi, as well as the immune system cells (macrophages, dendritic cells, T and B-lymphocytes) [18, 38, 49, 50, 51]. At the same time, a broadening and blunting of the intestinal villi, epithelial cell peeling, crypt epithelial hyperplasia, cytoplasmic vacuolization, infiltration of the affected cells into the *lamina propria* are observed. The lesions are more severe in the small intestine (duodenum, jejunum and ileum), where mucosal inflammation involving atrophy of intestinal villi and hypertrophy of intestinal glands are detected. Decreased cell enzymatic activity and development of secondary disaccharide deficiency are observed. In the setting of this infection gastric motility disorder frequently occurs. Increased intestinal epithelial apoptosis, epithelial barrier malfunction, and development of diarrhea due to loss of ions and water from subepithelial capillaries into the lumen are observed [16, 18]. Moreover, villous necrosis and villous atrophy are reported [38, 45, 50, 51]. Norovirus was detected in the epithelial cells of the duodenum, jejunum and ileum, Peyer's patches and large intestinal mesenteric lymph nodes [38].

Table
Genetic characteristics of noroviruses isolated from fecal samples

Hosts	Genogroups
Human	GI, GII, GIV, GVI, GVII
Ruminants (cattle, sheep)	GIII, GV
Pigs	GII
Mice	GV
Dogs	GIV, GVII
Cats	GIV

Macroscopic lesions and clinical signs caused by norovirus infection are similar to those caused by rotavirus and coronavirus infections, which complicates clinical and post-mortem diagnosis [1, 3, 4, 8, 19, 52]. Noroviruses are detected in fecal samples from cattle of different ages. The greatest economic losses are caused by norovirus infection in calves, who manifest diarrhea, depression, fever, and digestive disorders. Diarrhea is observed on days 3–7 post infection and can persist for a month. Diarrhoea is more severe in 3-week old calves than in neonatal animals [24]. In addition to norovirus, rota-, corona-, neboviruses, bovine diarrhea virus [53], and other microorganisms [10, 11] were frequently isolated from fecal samples collected from diarrheic calves. When investigating the reasons of gastrointestinal disorders in newborn calves in England, Belgium, Hungary, Germany, Italy, the Netherlands, France, Slovenia, Norway, Sweden, China, South Korea, India, Iran, Turkey, Egypt, Tunisia, the USA, Australia and New Zealand, noroviruses were detected in faecal samples. The results of numerous tests suggested that norovirus infection is a highly contagious zoonotic disease with the fecal-oral route of transmission [11, 20, 28, 29, 36, 38, 39, 40, 41, 44, 49, 53, 54, 55, 56].

The table provides data on the detection of different norovirus genogroups in different hosts. Each norovirus genogroup comprises several genetic clusters (genotypes) depending on the similarity of genetic characteristics [45, 57].

The results of the VP1 phylogenetic analyses suggest a high frequency of norovirus recombination. Noroviruses of genogroup II (GII) isolated from fecal samples of diseased humans and pigs are characterized by a high level of variability [16, 12, 28, 58]. The study of genogroup GII norovirus RNA isolated from pig faecal samples in Japan, the USA and several European countries, revealed that porcine/human recombinants can emerge in subclinically infected adult animals, and pigs may be reservoirs of new human noroviruses [42, 52, 59].

Noroviruses have been shown to undergo extensive genetic recombination. Co-infection of calves with bovine and human strains of norovirus can produce a recombinant virus with altered virulence properties [46, 60]. Noroviruses of genogroup GIII (bovine) and genotype GII.4 (human) were simultaneously identified in fecal samples from diarrheic calves in Canada [12].

There is a high probability that recombinant norovirus strains can emerge which potentially can transmit to human population.

The results of the experimental infection of gnotobiotic calves and piglets with human norovirus confirmed virus replication and seroconversion in infected animals [45, 47]. Spontaneous infections of piglets with norovirus have been reported. In this case, diarrhea occurred 2–6 days after the experimental infection. The data from these studies led to the assumption that cattle and pigs may serve as a reservoir of human norovirus, due to viral mutations in the animal organism and emergence of strains with new properties. Long-term contacts between humans and viruses, which previously infected only animals, can lead to mutations and replication in the intestinal epithelium of the human [9, 11, 12, 38, 42, 46, 47, 52, 58, 59, 61]. It is

believed that humans can be infected with bovine and porcine noroviruses through contaminated animal meat and milk [46]. The detection of human noroviruses in animals, as well as the simultaneous presence of human and animal noroviruses in bivalves, suggests a risk of the human norovirus transmission [62].

The results of serological tests showed antibodies to human norovirus in porcine sera in 36–71% of cases [27]. In the Netherlands, antibodies (IgG) to bovine norovirus were detected in 28 and 20% of serum samples, collected from 210 veterinarians and 630 animal owners, respectively [49]. In Sweden, 26.7% of blood donors were antibody-positive for bovine norovirus (GIII.2) [61]. It has been established that human norovirus has a clear tropism to the canine intestinal epithelial cells [18].

These data are based on the assumption that zoonotic transmission is typical for norovirus [20, 45, 46, 59, 61, 63].

CONCLUSION

These data suggest a high prevalence of highly contagious norovirus infection in the world, which is of social and economic importance. Noroviruses are the most common cause of epidemic gastroenteritis, responsible for at least 50% of all gastroenteritis outbreaks in humans worldwide, and a major cause of foodborne illness. Norovirus infection outbreaks among children have also been reported in the Russian Federation. Norovirus is extremely contagious, with an estimated infectious dose as low as 10–1,000 viral particles. Many researchers have revealed that transmission might occur directly through the fecal-oral route and there is a potential for zoonotic transmission. Noroviruses have been found in fecal samples from humans, cattle, pigs, sheep, dogs, cats, as well as in pork and milk. Epidemiological features of the norovirus infection include long-term viral shedding with feces in high concentrations. Transmission of noroviruses occurs in three general routes typical of acute gastrointestinal infections: waterborne, foodborne, and contacts. At the beginning of the 21st century, a large number of cattle were imported to Russia from norovirus-infected countries. This suggests the need for monitoring tests, the development of diagnostic agents and methods, and measures to control norovirus and other emergent infections.

REFERENCES

1. Current infectious diseases of cattle: A manual. Ed. by T. I. Aliper. Moscow: Sel'skokhozyaistvennye tekhnologii; 2021. 832 p. (in Russ.)

2. Shevchenko A. A., Chernykh O. Yu., Mishchenko V. A., Mishchenko A. V., Shevkoplyas V. N. Astrovirus infection of cattle. *Proceedings of the Kuban State Agrarian University*. 2015; 57: 156–160. https://www.elibrary.ru/whwydv (in Russ.)

3. Sergeev V. A., Nepoklonov E. A., Aliper T. I. Viruses and viral vaccines. Moscow: Biblionika; 2007. 524 p. (in Russ.)

4. Gaffarov H. Z., Ivanov A. V., Nepoklonov E. A., Ravilov A. Z. Mono- and mixed infectious diarrhea of neonatal calves and piglets. Kazan: Fen; 2002. 592 p. (in Russ.)

5. Animal infectious pathology: in 2 vlms. Vol. 1. Ed. by A. Ya. Samuylenko, B. V. Soloviev, E. A. Nepoklonov, E. S. Voronin. Moscow: Akademkniga; 2006. 910 p. (in Russ.)

6. Gorelov A. V., Ploskireva A. A., Doroshina E. A., Podkolzin A. T., Tkhakushinova N. Kh. Norovirus infection at the modern stage: clinical manifestations and therapeutic approaches. *Infectious Diseases*. 2011; 9 (2): 100–105. https://www.elibrary.ru/obfxht (in Russ.)

7. Mishchenko V. A., Mishchenko A. V., Yashin R. V., Chernykh O. Yu., Lysenko A. A., Krivonos R. A. Features of cobuvirus infection of farm animals. *Veterinaria Kubani*. 2021; 5: 3–6. https://doi.org/10.33861/2071-8020-2021-5-3-6 (in Russ.)

8. Guidance on virology. Human and animal viruses and viral infections. Ed. by D. K. Lvov. Moscow: Meditsinskoe informatsionnoe agentstvo; 2013. 1200 p. (in Russ.)

9. Bridger J. C., Hall G. A., Brown J. F. Characterization of a calici-like virus (Newbury agent) found in association with astrovirus in bovine diarrhea. *Infection and Immunity*. 1984; 43 (1): 133–138. https://doi.org/10.1128/iai.43.1.133-138.1984

10. Castells M., Colina R. Viral enteritis in cattle: to well known viruses and beyond. *Microbiology Research*. 2021; 12 (3): 663–682. https://doi.org/10.3390/microbiolres12030048

11. Di Martino B., Di Profio F., Di Felice E., Melegari I., Ceci C., Mauroy A., et al. Genetic heterogeneity of bovine noroviruses in Italy. *Archives of Virology*. 2014; 159 (10): 2717–2722. https://doi.org/10.1007/s00705-014-2109-0

12. Mattison K., Shukla A., Cook A., Pollari F., Friendship R., Kelton D., et al. Human noroviruses in swine and cattle. *Emerging Infectious Diseases*. 2007; 13 (8): 1184–1188. https://doi.org/10.3201/eid1308.070005

13. Woode G. N., Bridger J. C. Isolation of small viruses resembling astroviruses and caliciviruses from acute enteritis of calves. *Journal of Medical Microbiology*. 1978; 11 (4): 441–452. https://doi.org/10.1099/00222615-11-4-441

14. László Z., Pankovics P., Reuter G., Ságola A., Bálint Á., Albert M., Boros Á. Multiple types of novel enteric bopiviruses (*Picornaviridae*) with the possibility of interspecies transmission identified from cloven-hoofed domestic livestock (ovine, caprine and bovine) in Hungary. *Viruses*. 2021; 13 (1):66. https://doi.org/10.3390/v13010066

15. Green K. Y., Ando T., Balayan M. S., Berke T., Clarke I. N., Estes M. K., et al. Taxonomy of the caliciviruses. *The Journal of Infectious Diseases*. 2000; 181 (Suppl. 2): S322–S330. https://doi.org/10.1086/315591

16. Noroviral infection: etiology, epidemiology, diagnosis: analytical review. Nizhny Novgorod: Academician I. N. Blokhina Nizhny Novgorod Scientific Research Institute of Epidemiology and Microbiology; 2009. 64 p. (in Russ.)

17. Ando T., Noel J. S., Fankhauser R. L. Genetic classification of "Norwalk-like viruses". *The Journal of Infectious Diseases*. 2000; 181 (Suppl. 2): S336–S348. https://doi.org/10.1086/315589

18. Khokhlova N. I., Kapustin D. V., Krasnova E. I., Izvekova I. Ya. Norovirus infection (systematic review). *Journal Infectology*. 2018; 10 (1): 5–14. https://doi.org/10.22625/2072-6732-2018-10-1-5-14 (in Russ.)

19. MU 3.1.1.2969-11 Epidemiological surveillance, laboratory diagnosis and prevention of norovirus infection: guidelines. Moscow: Federal Hygienic and Epidemiological Center of Rospotrebnadzor; 2012. 36 p. (in Russ.)

20. Oliver S. L., Dastjerdi A. M., Wong S., El-Attar L. M. R., Gallimore C., Brown D. W. G., et al. Molecular characterization of bovine enteric caliciviruses: A distinct third genogroup of noroviruses (Norwalk-like viruses) unlikely to be of risk to humans. *Journal of Virology*. 2003; 77 (4): 2789–2798. https://doi.org/10.1128/jvi.77.4.2789-2798.2003

21. Gomez D. E., Weese J. S. Viral enteritis in calves. *The Canadian Veterinary Journal*. 2017; 58 (12): 1267–1274. https://pubmed.ncbi.nlm.nih.gov/29203935

22. Vinje J., Estes M. K., Esteves P., Green K. Y., Katayama K., Knowles N. J., et al. ICTV virus taxonomy profile: *Caliciviridae*. *Journal of General Virology*. 2019; 100 (11): 1469–1470. https://doi.org/10.1099/jgv.0.001332

23. Kapikian A. Z. The discovery of the 27-nm Norwalk virus: an historic perspective. *The Journal of Infectious Diseases*. 2000; 181 (Suppl. 2): S295–S302. https://doi.org/10.1086/315584

24. Di Felice E., Mauroy A., Pozzo F. D., Thiry D., Ceci C., Di Martino B., et al. Bovine noroviruses: A missing component of calf diarrhoea diagnosis. *The Veterinary Journal*. 2016; 207: 53–62. https://doi.org/10.1016/j.tvjl.2015.10.026

25. Cui Y., Chen X., Yue H., Tang C. First detection and genomic characterization of bovine norovirus from yak. *Pathogens*. 2022; 11 (2):192. https://doi.org/10.3390/pathogens11020192

26. Van der Poel W. H. M., van der Heide R., Verschoor F., Gelderblom H., Vinjé J., Koopmans M. P. G. Epidemiology of Norwalk-like virus infections in cattle in the Netherlands. *Veterinary Microbiology*. 2003; 92 (4): 297–309. https://doi.org/10.1016/s0378-1135(02)00421-2

27. Farkas T., Nakajima S., Sugieda M., Deng X., Zhong W., Jiang X. Seroprevalence of noroviruses in swine. *Journal of Clinical Microbiology*. 2005; 43 (2): 657–661. https://doi.org/10.1128/JCM.43.2.657-661.2005

28. Mijovski J. Z., Poljšak-Prijatelj M., Steyer A., Barlič-Maganja D., Koren S. Detection and molecular characterisation of noroviruses and sapoviruses in asymptomatic swine and cattle in Slovenian farms. *Infection, Genetics and Evolution*. 2010; 10 (3): 413–420. https://doi.org/10.1016/j.meegid.2009.11.010

29. Wolf S., Williamson W., Hewitt J., Lin S., Rivera-Aban M., Ball A., et al. Molecular detection of norovirus in sheep and pigs in New Zealand farms. *Veterinary Microbiology*. 2009; 133 (1–2): 184–189. https://doi.org/10.1016/j.vetmic.2008.06.019

30. Takano T., Kusuhara H., Kuroishi A., Takashina M., Doki T., Nishinaka T., Hohdatsu T. Molecular characterization and pathogenicity of a genogroup GVI feline norovirus. *Veterinary Microbiology*. 2015; 178 (3–4): 201–207. https://doi.org/10.1016/j.vetmic.2015.05.018

31. Di Martino B., Di Profio F., Melegari I., Sarchese V., Cafiero M. A., Robetto S., et al. A novel feline norovirus in diarrheic cats. *Infection, Genetics and Evolution*. 2016; 38: 132–137. https://doi.org/10.1016/j.meegid.2015.12.019

32. Ma H., Yue H., Luo Y., Li S., Tang C. First detection of canine norovirus in dogs and a complete GVI.2 genome in mainland China. *Infection, Genetics and Evolution*. 2021; 92:104879. https://doi.org/10.1016/j.meegid.2021.104879

33. Lyoo K. S., Jung M. C., Yoon S. W., Kim H. K., Jeong D. G. Identification of canine norovirus in dogs in South Korea. *BMC Veterinary Research*. 2018; 14:413. https://doi.org/10.1186/s12917-018-1723-6

34. Kennedy E. A., Aggarwal S., Dhar A., Karst S. M., Wilen C. B., Baldridge M. T. Age-associated features of norovirus infection analysed in mice. *Nature Microbiology*. 2023; 8 (6): 1095–1107. https://doi.org/10.1038/s41564-023-01383-1

35. Scipioni A., Mauroy A., Vinjé J., Thiry E. Animal noroviruses. *The Veterinary Journal*. 2008; 178 (1): 32–45. https://doi.org/10.1016/j.tvjl.2007.11.012

36. Deng Y., Batten C. A., Liu B. L., Lambden P. R., Elschner M., Günther H., et al. Studies of epidemiology and seroprevalence of bovine noroviruses in Germany. *Journal of Clinical Microbiology*. 2003; 41 (6): 2300–2305. https://doi.org/10.1128/JCM.41.6.2300-2305.2003

37. Ike A. C., Roth B. N., Böhm R., Pfitzner A. J., Marschang R. E. Identification of bovine enteric Caliciviruses (BEC) from cattle in Baden-Württemberg. *Deutsche Tierärztliche Wochenschrift*. 2007; 114 (1): 12–15. https://pubmed.ncbi.nlm.nih.gov/17252930

38. Otto P. H., Clarke I. N., Lambden P. R., Salim O., Reetz J., Liebler-Tenorio E. M. Infection of calves with bovine norovirus GIII.1 strain Jena virus: an experimental model to study the pathogenesis of norovirus infection. *Journal of Virology*. 2011; 85 (22): 12013–12021. https://doi.org/10.1128/JVI.05342-11

39. Parra G. I. Emergence of norovirus strains: a tale of two genes. *Virus Evolution*. 2019; 5 (2):ves048. https://doi.org/10.1093/ve/vez048

40. Pourasgari F., Kaplon J., Sanchooli A., Fremy C., Karimi-Naghlani S., Otarod V., et al. Molecular prevalence of bovine noroviruses and neboviruses in newborn calves in Iran. *Archives of Virology*. 2018; 163 (5): 1271–1277. https://doi.org/10.1007/s00705-018-3716-y

41. Reuter G., Pankovics P., Egyed D. Detection of genotype 1 and 2 bovine noroviruses in Hungary. *Veterinary Record*. 2009; 165 (18): 537–538. https://doi.org/10.1136/vr.165.18.537

42. Vinjé J. Advances in laboratory methods for detection and typing of norovirus. *Journal of Clinical Microbiology*. 2015; 53 (2): 373–381. https://doi.org/10.1128/JCM.01535-14

43. Zheng D. P., Ando T., Fankhauser R. L., Beard R. S., Glass R. I., Monroe S. S. Norovirus classification and proposed strain nomenclature. *Virology*. 2006; 346 (2): 312–323. https://doi.org/10.1016/j.virol.2005.11.015

44. Bank-Wolf B. R., König M., Thiel H. J. Zoonotic aspects of infections with noroviruses and sapoviruses. *Veterinary Microbiology*. 2010; 140 (3–4): 204–212. https://doi.org/10.1016/j.vetmic.2009.08.021

45. Cheetham S., Souza M., Meulia T., Grimes S., Han M. G., Saif L. J. Pathogenesis of a genogroup II human norovirus in gnotobiotic pigs. *Journal of Virology*. 2006; 80 (21): 10372–10381. https://doi.org/10.1128/JVI.00809-06

46. Oliver S. L., Batten C. A., Deng Y., Elschner M., Otto P., Charpilienne A., et al. Genotype 1 and genotype 2 bovine noroviruses are antigenically distinct but share a cross-reactive epitope with human noroviruses. *Journal of Clinical Microbiology*. 2006; 44 (3): 992–998. https://doi.org/10.1128/JCM.44.3.992-998.2006

47. Souza M., Azevedo M. S. P., Jung K., Cheetham S., Saif L. J. Pathogenesis and immune responses in gnotobiotic calves after infection with the genogroup II.4-HS66 strain of human norovirus. *Journal of Virology*. 2008; 82 (4): 1777–1786. https://journals.asm.org/doi/abs/10.1128/jvi.01347-07

48. Desselberger U. *Caliciviridae* other than noroviruses. *Viruses*. 2019; 11 (3):286. https://doi.org/10.3390/v11030286

49. Widdowson M. A., Rockx B., Schepp R., van der Poel W. H., Vinje J., van Duynhoven Y. T., Koopmans M. P. Detection of serum antibodies to bovine norovirus in veterinarians and the general population in the Netherlands. *Journal of Medical Virology*. 2005; 76 (1): 119–128. https://doi.org/10.1002/jmv.20333

50. Wobus C. E., Karst S. M., Thackray L. B., Chang K. O., Sosnovtsev S. V., Belliot G., et al. Replication of norovirus in cell culture reveals a tropism for dendritic cells and macrophages. *PLoS Biology.* 2004; 2 (12):e432. https://doi.org/10.1371/journal.pbio.0020432

51. Wobus C. E. The dual tropism of noroviruses. *Journal of Virology*. 2018; 92 (16):e01010-17. https://doi.org/10.1128/JVI.01010-17

52. Villabruna N., Koopmans M. P. G., de Graaf M. Animals as reservoir for human norovirus. *Viruses*. 2019; 11 (5):478. https://doi.org/10.3390/v11050478

53. Tråvén M., Axén C., Svensson A., Björkman C., Emanuelson U. Prevalence of bovine norovirus and nebovirus and risk factors of infection in Swedish dairy herds. *Dairy*. 2022; 3 (1): 137–147. https://doi.org/10.3390/dairy3010011

54. Guo Z., He Q., Yue H., Zhang B., Tang C. First detection of Nebovirus and Norovirus from cattle in China. *Archives of Virology.* 2018; 163 (2): 475–478. https://doi.org/10.1007/s00705-017-3616-6

55. Symes S. J., Allen J. L., Mansell P. D., Woodward K. L., Bailey K. E., Gilkerson J. R., Browning G. F. First detection of bovine noroviruses and detection of bovine coronavirus in Australian dairy cattle. *Australian Veterinary Journal*. 2018; 96 (6): 203–208. https://doi.org/10.1111/avj.12695

56. Yilmaz H., Turan N., Altan E., Bostan K., Yilmaz A., Helps C. R., Cho K. O. First report on the phylogeny of bovine norovirus in Turkey. *Archives of Virology*. 2011; 156 (1): 143–147. https://doi.org/10.1007/s00705-010-0833-7

57. Turan T., Işıdan H., Atasoy M. O., Irehan B. Detection and molecular analysis of bovine enteric norovirus and nebovirus in Turkey. *Journal of Veterinary Research*. 2018; 62 (2): 129–135. https://doi.org/10.2478/jvetres-2018-0021

58. Nguyen G. T., Phan K., Teng I., Pu J., Watanabe T. A systematic review and meta-analysis of the prevalence of norovirus in cases of gastroenteritis in developing countries. *Medicine.* 2017; 96 (40):e8139. https://doi.org/10.1097/MD.0000000000008139

59. Wang Q. H., Han M. G., Cheetham S., Souza M., Funk J. A., Saif L. J. Porcine noroviruses related to human noroviruses. *Emerging Infectious Diseases*. 2005; 11 (12): 1874–1881. https://doi.org/10.3201/eid1112.050485

60. Mauroy A., Scipioni A., Mathijs E., Ziant D., Daube G., Thiry E. Genetic and evolutionary perspectives on genogroup III, genotype 2 bovine noroviruses. *Archives of Virology*. 2014; 159 (1): 39–49. https://doi.org/10.1007/s00705-013-1791-7

61. Vildevall M., Grahn A., Oliver S. L., Bridger J. C., Charpilienne A., Poncet D., et al. Human antibody responses to bovine (Newbury-2) norovirus (GIII.2) and association to histo-blood group antigens. *Journal of Medical Virology*. 2010; 82 (7): 1241–1246. https://doi.org/10.1002/jmv.21776

62. Savini F., Giacometti F., Tomasello F., Pollesel M., Piva S., Serraino A., De Cesare A. Assessment of the impact on human health of the presence of norovirus in bivalve molluscs: what data do we miss? *Foods*. 2021; 10 (10):2444. https://doi.org/10.3390/foods10102444

63. Mauroy A., Scipioni A., Mathijs E., Saegerman C., Mast J., Bridger J. C., et al. Epidemiological study of bovine norovirus infection by RT-PCR and a VLP-based antibody ELISA. *Veterinary Microbiology*. 2009; 137 (3–4): 243–251. https://doi.org/10.1016/j.vetmic.2009.01.031

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Infectious hematopoietic necrosis (review)

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ABSTRACT

Aquaculture in the Russian Federation is an integral part of the agricultural industry of the state economy. Countries with high rates of aquaculture growth (Norway, USA, China, Japan, Canada, etc.) and increasing efficiency of fish farming are the cradles of infectious diseases, which, in case of improper control, invade the territory of other countries and spread to new areas, bearing the risks for the domestic industry too. In recent years, infectious hematopoietic necrosis (IHN) has caused significant damage to fish farms. In 2020, Estonia suffered heavy losses; more than 65 tons of rainbow trout died and were destroyed during the IHN outbreak with a mortality rate of 71%. This was the first IHN case in this country. The aggravation of the epidemic situation at Estonian fish farms poses a threat to the northwestern regions of the Russian Federation, where aquaculture is practiced (the Leningrad Oblast and the Republic of Karelia). In 2022, IHN outbreaks were reported in France, Italy, Finland, Germany, Denmark and Macedonia. IHN-caused deaths were reported at the river trout farm in Georgia in 2023 for the first time. The domestic aquaculture depends on the import of eggs and seed material from Norway, Denmark, Finland and other countries, therefore a regular disease monitoring is urgently needed. The paper provides a brief description of the IHN causative agent, describes its epidemiology, pathogenesis, clinical signs, post-mortem lesions, diagnostic tests, infection control and prevention measures. We have reviewed 88 literature sources to summarize the information.

Keywords: review, infectious hematopoietic necrosis virus, fish diseases, disease situation

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Инфекционный некроз гемопоэтической ткани лососевых рыб (обзор)

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РЕЗЮМЕ

Производство аквакультуры на территории Российской Федерации является неотъемлемой частью сельскохозяйственного сектора экономики страны. Страны с высоким уровнем и темпами развития аквакультуры (Норвегия, США, Китай, Япония, Канада и др.) и растущей эффективностью производства рыб являются центрами возникновения и распространения инфекционных заболеваний, которые при ненадлежащем контроле проникают на территорию других государств и распространяются в новых ареалах, угрожая в том числе и отечественной отрасли. В последние годы значительный ущерб рыбоводным хозяйствам наносит инфекционный некроз гемопоэтической ткани лососевых рыб. В 2020 г. большие потери понесла Эстония, где во время вспышки данного инфекционного заболевания погибло и было уничтожено более 65 тонн радужной форели, показатель смертности при этом составил 71%. Это был первый случай инфекционного некроза гемопоэтической ткани в этой стране. Обострение эпизоотической ситуации на рыбоводческих предприятиях Эстонии представляет угрозу северо-западным регионам Российской Федерации с развитой аквакультурой (в Ленинградской области и Республике Карелии). В 2022 г. вспышки инфекционного некроза гемопоэтической ткани отмечали во Франции, Италии, Финляндии, Германии, Дании и Македонии. А в 2023 г. впервые в Грузии отмечена гибель рыб от данного заболевания на речной форелевой ферме. Отечественное производство продукции аквакультуры зависит от импорта икры и посадочного материала из Норвегии, Дании, Финляндии и других стран, поэтому возникает необходимость в регулярном эпизоотологическом мониторинге. В статье дана краткая характеристика возбудителя инфекционного некроза гемопоэтической ткани, описаны эпизоотология, патогенез, клинические признаки, патолого-анатомические изменения, методы диагностики, профилактики и меры борьбы с инфекцией. Обзор составлен на основе анализа 88 источников.

Ключевые слова: обзор, вирус инфекционного некроза гемопоэтической ткани, болезни рыб, эпизоотическая ситуация

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INTRODUCTION

Infectious hematopoietic necrosis (IHN) is a highly contagious viral disease of salmonid species, occurring in freshwater and marine fish and characterized by high mortality, decreased fish production levels and deformities that occur in the survivors. The disease may be referred to by a number of other names such as sockeye salmon viral disease, Columbia River sockeye disease, Oregon sockeye disease and Sacramento River Chinook disease. However, currently the generally accepted name of the disease is infectious hematopoietic necrosis. IHN is included into the list of dangerous and economically significant diseases, notifiable to the World Organization for Animal Health (WOAH) [1]. A wide range of salmonid species, both farmed and wild, are susceptible to the disease. Juveniles up to 2–6 months of age are most susceptible. The disease of the majority of the juveniles causes significant damage and losses, thus posing threat of complete ruin of the farmer. The disease is characterized by a high mortality rate (90–100%), loss of productivity and fish production efficiency and impaired fish quality and commodity size. Both freshwater and marine aquacultured fish manifest the disease. The disease outbreaks in the countries, where aquaculture is well-developed, cause significant economic damage [2, 3, 4].

PATHOGEN CHARACTERISTICS

The IHN causative agent is an RNA-containing virus of the *Rhabdoviridae* family from the *Novirhabdovirus* genus, which was isolated into a separate taxon by the International Committee on Taxonomy of Viruses in 2014[1]. The novirhabdoviruses were classified as a separate taxon due to the presence of the NV gene, which is the major difference from vesiculoviruses [5]. The virion is a bullet-shaped spiral nucleocapsid, approximately 110 nm long and 70 nm in diameter (Fig. 1) [6, 7]. There is only one serotype of the virus. Both low-virulent and high-virulent viruses are reported among field isolates. IHNV is isolated and cultured in continuous cell lines EPC, AS, BF-2, CHSE-214, FHM, ICO, RTH-149, RTG-2 and STE-137 [8, 9, 10, 11, 12, 13].

The IHNV genome is a non-segmented, negative-sense, single-stranded RNA genome of approximately 11,000 nucleotides. The viral genome codes six proteins in the following order: a nucleoprotein (N), a phosphoprotein (P), a matrix protein (M), a glycoprotein (G), a non-virion protein (NV), and a polymerase (L) [14, 15, 16, 17, 18, 19].

The inner helical ribonucleocapsid core consists of the ribonuclease genome and N, M and L proteins. The matrix protein (M) attaches to both the G protein on the internal side of the membrane and to the ribonucleocapsid. The outer membrane consists of a lipid bilayer membrane and the glycoprotein (G) that projects externally and forms noncovalently bound homotrimer spikes [19, 20, 21, 22].

The N protein of IHNV contains 413 amino acids and has a molecular mass of 40.5–44.0 kDa. This is the earliest expressed and most abundant protein produced by the virus during an IHNV infection. The P protein, previously called the M1 protein, contains 231 amino acids and has a molecular mass of 25.6 kDa. The M protein, previously called the M2 protein, is a highly basic protein. It contains a number of basic amino acids at the N-terminal end that are conserved among the homologous matrix proteins of other fish rhabdoviruses. The G protein with a molecular mass of 67–70 kDa contains 508 amino acid residues and forms the spike-like projections on the surface of the mature virion. This protein binds to cell receptors and is responsible for the attachment of the virus to the membrane of the host cell, cell fusion, syncytia formation and typical cytopathic effect. The G protein is also the target of neutralizing antibodies [23]. The L encodes a protein of 1986 amino acids with a predicted molecular weight of approximately 225 kDa and shows similarity to the RNA-dependent RNA polymerase genes of other rhabdoviruses. The NV gene was discovered first in IHNV between the G and L genes

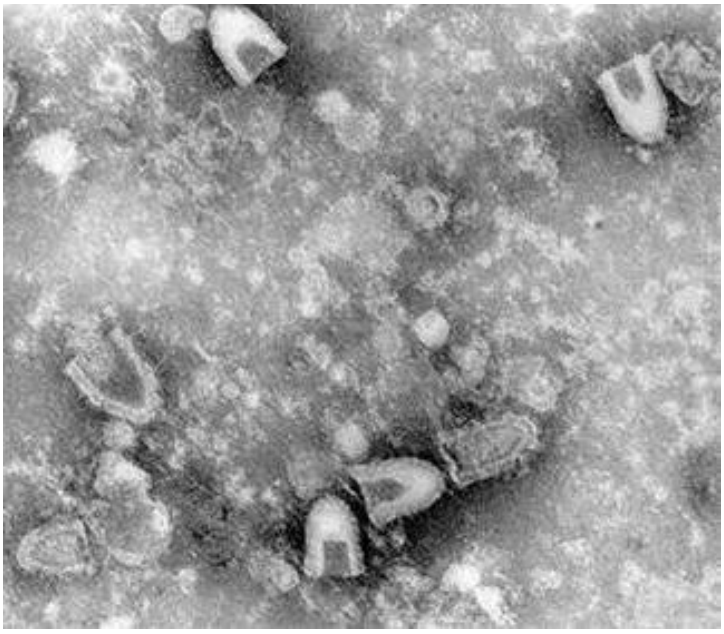

Fig. 1. IHNV viewed under an electron microscope [7]

[1] International Committee on Taxonomy of Viruses (ICTV). https://ictv.global/taxonomy

and subsequently in other aquatic rhabdoviruses such as viral hemorrhagic septicemia virus, Hirame novirhabdovirus, snakehead rhabdovirus, and various eel viruses [24, 25]. The NV gene encodes a nonstructural protein, which can be identified within infected cells but not in purified virions. This protein is required for efficient replication of IHNV *in vivo* [25].

G gene sequencing of North American IHNV isolates has revealed 3 major genogroups, designated U, M and L [26, 27, 28]. Representatives of these genogroups circulate in certain geographically isolated populations of wild salmonids. The U genotype group isolates are most spread in Alaska and British Columbia, and watersheds of coastal Washington and the Columbia River basin in Washington, Oregon and Idaho states. The M group contains isolates from Idaho, the Columbia and Snake River basins, and a virus from the Washington coast. The L genotype contains most of the viruses from California and the southern Oregon coast. Molecular genetic methods confirmed that IHNV European and Asian isolates are of North American origin [29, 30, 31]. Tests have shown that different viral genogroups are species-specific. For example, IHNV genogroup U isolates have been shown to have higher virulence in sockeye salmon, whereas genogroup M viral isolates cause a significantly lower mortality in sockeye salmon. However, genogroup M viruses are highly pathogenic for rainbow trout, though low mortality rate is reported in case of infection with genogroup U viruses [32]. Genogroup L viruses are most virulent in chinook salmon [33].

EPIDEMIOLOGY

Resistance to physicochemical factors and disinfection. IHNV survives in fresh water at 15 °C for 1 month, especially if organic material is present. IHNV is heat, acid and ether labile; readily inactivated by common disinfectants and drying. The virus is not resistant to high temperatures and is almost completely inactivated in 15 minutes at 45 °C, and completely destroyed at 60 °C [34].

Susceptible host species. Fry is the most highly susceptible age group. Fish become increasingly resistant to infection with age until spawning, when they once again become highly susceptible.

There is a high degree of variation in susceptibility to infection with different IHNV strains; the same viral strain can cause infection of different intensity in different fish species.

A wide range of salmonids are susceptible to the virus, including Arctic char (*Salvelinus alpinus*), Atlantic salmon (*Salmo salar*), brook trout (*Salvelinus fontinalis*), whitespotted char (*Salvelinus leucomaenis*), brown trout (*Salmo trutta*), chinook salmon (*Oncorhynchus tshawytscha*), chum salmon (*Oncorhynchus keta*), coho salmon (*Oncorhynchus kisutch*), cutthroat trout (*Oncorhynchus clarkii*), lake trout (*Salvelinus namaycush*), masu salmon (*Oncorhynchus masou*), marbled trout (*Salmo marmoratus*), rainbow trout (*Oncorhynchus mykiss*), mountain whitefish (*Prosopium williamsoni*) and sockeye salmon (*Oncorhynchus nerka*). The most susceptible to the disease are rainbow trout, chinook salmon, sockeye salmon and chum salmon. Sockeye salmon juveniles are highly susceptible IHNV [1, 11, 35, 36].

It is believed that white sturgeon (*Acipenser transmontanus*), European eel (*Anguilla anguilla*), tube-snout (*Aulorhynchus flavidus*), Pacific herring (*Clupea pallasii*), Shiner perch (*Cymatogaster aggregate*), turbot (*Scophthalmus maximus*), burbot (*Lota lota*), Arctic grayling (*Thymallus arcticus*), American yellow perch (*Perca flavescens*) and all varieties and species of common carp (*Cyprinus carpio*) [2], are also susceptible to the disease, but there is not enough evidence to confirm this fact. Despite the fact that these species are less susceptible to IHNV, they can serve as a natural reservoir of infection [37, 38, 39].

Geographical distribution. IHNV was first detected in fish farms on the North American west coast in the 1940s [9]. Historically, the geographical range of this pathogen was limited to the western (Pacific) part of North America in the territories of the USA and Canada, where IHN is endemic among populations of wild salmonids [7, 10, 34].

However, the disease was introduced to Europe and Asia with exported infected fish and eggs in the late 1980s. Currently, the disease is spread all over the world, including Japan, South Korea, Chile, China, Taiwan, Turkey and many European Union countries [14, 40, 41, 42, 43, 44].

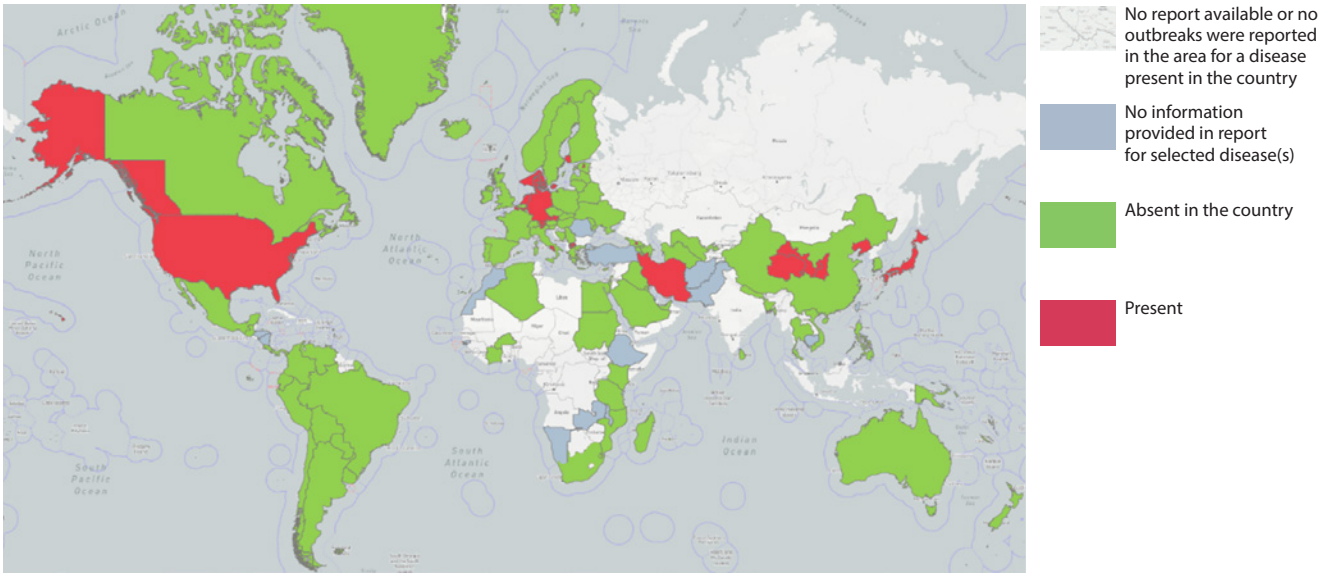

Fig. 2. The IHN spread in the world in 2021–2023 (WOAH data) [46]

Fig. 3. Salmon louse (Lepeophtheirus salmonis) on Atlantic salmon (photo made by the staff of the Reference Laboratory for Aquaculture Diseases, FGBI "ARRIAH")

In Russia, the IHNV virus was isolated in the Krasnodar Krai and the Republic of Karelia [45].

From 2021 to 2023 IHNV outbreaks were reported in Estonia, Denmark, Finland, Germany, France and Italy (Fig. 2).

In 2023, IHN-induced deaths of fish were reported at a river trout farm near Gori in Georgia. By July 12, 2023, 1.1 thousand fish died and 1.5 thousand fish were emergently killed out of 40 thousand on the farm.

Transmission mechanism. The source of infection is diseased fish, virus carriers and freshly dead fish. IHNV enters the body through the gill, damaged skin, fins and oral/gastrointestinal tract. The transmission of IHNV between fish is primarily horizontal and high levels of virus are shed from infected juvenile fish. During spawning fish become highly susceptible to the infection and may shed large amounts of virus in sexual products. Cases of vertical or egg-associated transmission have been recorded. Although egg-associated transmission is significantly reduced by the now common practice of surface disinfection of eggs with an iodophor solution [47]. The virus is transmitted from fish to fish by direct contact, through water, silt, and fish handling equipment. An oral route of transmission is possible through cannibalism and feeding on infected fish. Unauthorized movement of eggs and fish from infected farms also contributes to the viral spread [9, 48]. Once IHNV is introduced into a farmed stock, the disease may become established among susceptible species of wild fish in the watershed. The length that individual fish are infected with IHNV varies with temperature. Survivors of infection with IHNV demonstrate a strong protective immunity with the synthesis of circulating antibodies to the virus [49]. Reservoirs of IHNV are clinically infected fish and covert carriers among cultured or wild fish but a true, life-long IHNV carrier state appears to be a rare event. Virus is shed via urine, sexual fluids and from external mucus (more seldom with feces), through gills, skin and fins [9, 37, 38, 50].

Vectors. Invertebrate vectors have been proposed to play a role in IHNV transmission. Blood-sucking parasites (leeches, copepodes) as well as some piscivorous birds are potential vectors for IHNV [50].

Study by E. Jakob et al. [51] showed that the salmon louse (*Lepeophtheirus salmonis*) (Fig. 3) is capable of IHNV transmitting under laboratory conditions. Although salmon lice are often considered not to transfer between hosts, such transfers have been observed under farmed and laboratory conditions, particularly when the host fish were kept at high densities [52]. Lice that were exposed to IHNV in water or had parasitized experimentally infected Atlantic salmon were put in different tanks containing naive Atlantic salmon. Mortalities of 70.6 and 66.6% respectively were observed in the two tanks of fish respectively in 7–9 days. IHNV was recovered from the majority of exposed fish. The authors concluded that under the experimental conditions the lice are mechanical vectors [51].

IHNV was isolated from adult Mayflies (*Callibaetis* sp.) collected from streams and an abandoned fish hatchery on a number of occasions [53].

A wide range of farmed fish from freshwater and the northern European marine environment, and to a much lesser degree farmed marine Mediterranean fish, are considered possible vectors of IHNV. Furthermore, there is evidence for the potential of IHNV transmission via invertebrates and piscivorous birds, and other animals may play a role.

Cyprinidae and other freshwater fish, marine fish and freshwater crustaceans are judged to be potential vectors of IHNV [54].

Mortality and morbidity. Depending on the species of fish, farming conditions, temperature, and, to some extent, the virus strain, outbreaks of infection with IHNV may range from explosive to chronic. Losses in acute outbreaks will exceed several per cent of the population per day and cumulative mortality may reach 90–95% or more [50]. In chronic cases, clinical signs are less pronounced, losses are protracted and fish in various stages of disease can be observed in the pond.

Larvae may die immediately after hatching and mortality rate may be up to 80–90%. Adults are more resistant and mortality rate among yearlings is most often 20–30%. Infection with IHNV can produce mortality in water temperatures from 3 to 18 °C. In Alaska, the disease can cause up to 100% mortality in sockeye salmon at water temperatures as low as 1–2 °C [55].

Disease factors. Older fish are typically more resistant to clinical disease. But among individuals, there is a high

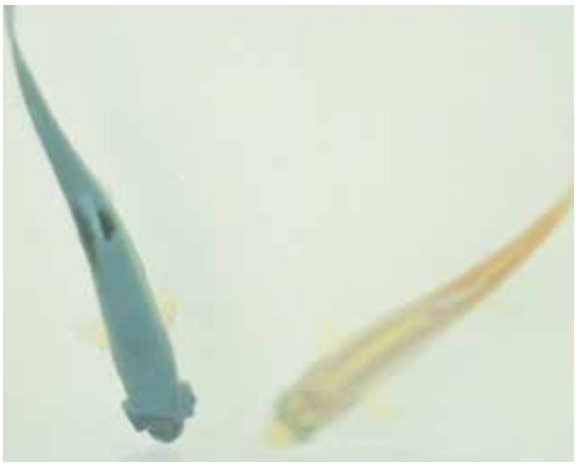

Fig. 4. Rainbow trout fry. IHNV infected fish (left) shows darker coloring [61]

Fig. 5. Cephalic bumps on sockeye salmon fry, characteristic of IHN disease [55]

degree of variation in susceptibility to infection with IHNV. Good fish health condition seems to decrease susceptibility to overt infection with IHNV, while co-infections with bacterial diseases (e.g. bacterial coldwater disease), handling and other stressors can cause subclinical infections to become overt.

The most important environmental factor affecting the progress of infection with IHNV is water temperature. In natural conditions, the disease occurs at water temperatures of 3 to 15 °C and morbidity decreases with the water temperature increase. IHNV epizootics usually occur during spring season (end of winter – beginning of summer) and less often during autumn season (end of summer and autumn), but if the temperature is suitable, the outbreaks can be observed at any time of the year. The disease is most acute at 10–12 °C. Up to 80–100% of juveniles may die [11]. In 100–500 g fish, the disease, as a rule, proceeds in a chronic form and mortality rate does not exceed 10–25%. The younger the fish is, the more it is susceptible to the virus even at higher temperatures. This is associated with the immature immunity in fry.

IHN outbreak may not occur even if the virus circulates in the fish population. The disease in fish is provoked by stressful conditions caused by handling and rearing violations (transportation, sorting, temperature fluctuations, low oxygen levels, sudden pH changes, metabolite accumulation in water, etc.) [9, 56].

PATHOGENESIS

The incubation period in case of natural infection in fingerlings at a water temperature of 10–15 °C is about 7–12 days [57].

Virus entry is thought to occur through the gills, skin, fins and anterior gastrointestinal track. Harmache A. et al. proved that that the fin bases are the major portal of entry for IHNV in 2006 [58]. IHNV exhibits a specific tissue tropism for connective tissue while splenic and renal hematopoietic tissues are the first and most severely affected areas. These organs are the sites in which virus is most abundant during the course of overt infection [34, 50].

Virus multiplication in endothelial cells of blood capillaries, hematopoietic tissues, and cells of the kidney underlies the clinical signs. Impairment of osmotic balance makes plasma release from blood cells into the interstitial space and body cavity, and occurs within a clinical context of oedema and haemorrhage [59]. The disease can progress to a lethal necrosis of the hematopoietic tissues of the kidney and spleen, a generalized viraemia with associated necrosis in all tissues. Death is due to renal failure caused by electrolyte imbalance [60].

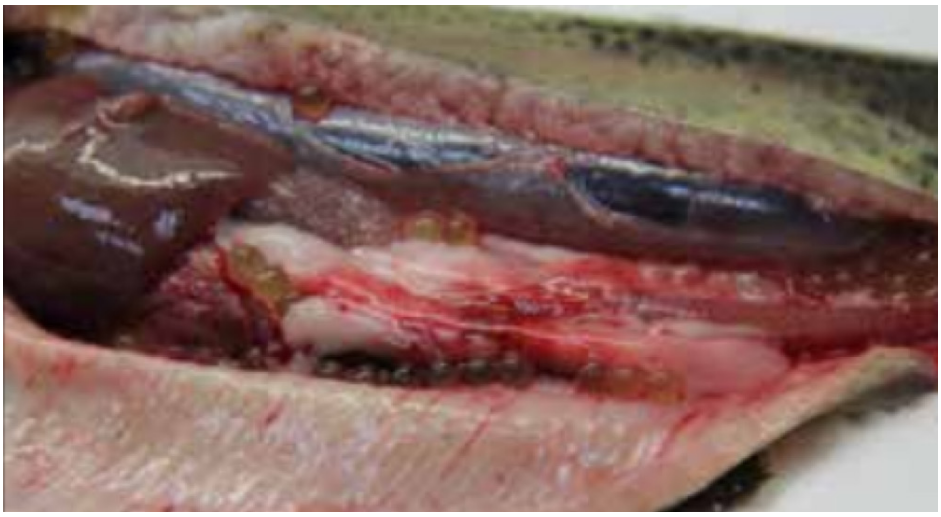

Fig. 6. Haemorrhages on the swim bladder, intestine and fat tissue of IHNV infected fish [63]

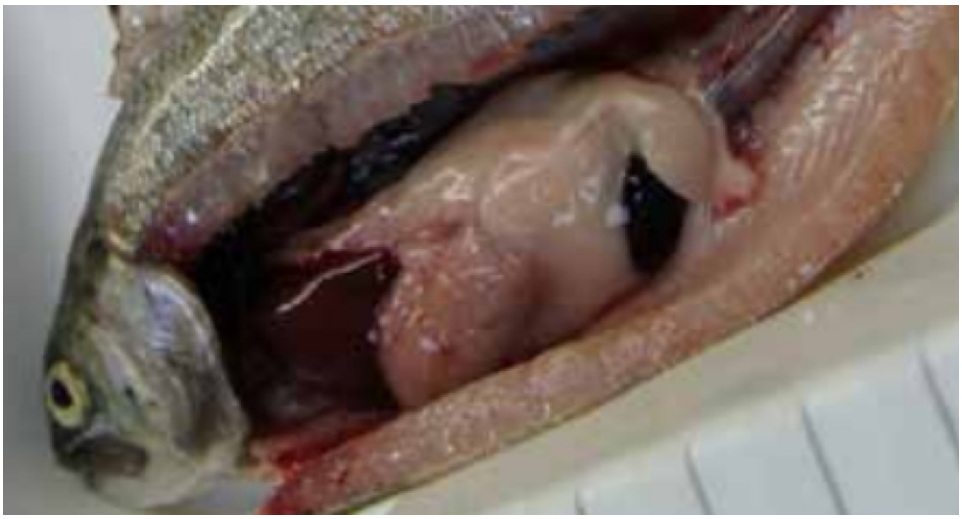

Fig. 7. Spleenomegaly in IHNV infected fish [63]

PATHOLOGY

The disease is characterized by sepsis, severe affection of hematopoetic organs, hemorrhages in organs and tissues.

First clinical signs of classical (acute) IHNV infection in 0.2–8.0 g fry are: anorexia and depression, lethargy. Other symptoms include darkening of the skin (Fig. 4), laying on the bottom or hanging around the water surface and swimming along the sides of the tank to avoid the currents.

In acute disease there is a sudden increase in fish mortality, but the fish may not show clinical signs and may die without apparent cause [62]. During outbreaks, fish are typically lethargic with bouts of frenzied, abnormal activity (spiral swimming, whirling and flashing), dark coloration, exophthalmia, pale gills, petechial hemorrhages around the eyes and fins, less often on the abdomen and behind the head, ascites (distended abdomen). Some affected fish show trailing faecal casts (of grey color, sometimes with blood). The larvae demonstrate multiple hemorrhages in the yolk sac and characteristic cephalic bumps on the head (Fig. 5). The fry exhibit hemorrhages at the base of the fins and on the mucous membranes, as well as in the yolk sac.

A post-mortem examination reveals the accumulation of a watery, yellowish (sometimes bloody) fluid and there may be petechial haemorrhages in the visceral mesenteries, adipose tissue, musculature, peritoneum, intestine and swim-bladder (Fig. 6). Necrotic changes and hemorrhages are observed in the kidneys and liver. The spleen is pale. The liver, kidneys and spleen are enlarged (Fig. 7). Fish may have empty stomachs, intestines filled with yellowish bloody mucus [59, 62].

In some fish over 8 g, usually at the final stage of the epizootic, a nervous form of the disease develops, manifested by behavior changes (periods of hyperactivity and depression). Usually there are no clinical signs, with the exception of a darker coloration, in such fish. This IHN form is due to damaged central nervous system, that's why the virus in such fish can only be detected in the brain. It is assumed that the virus concentrates in the central nervous system, where immune surveillance is less effective, replicates to about 10^6 PFU/g and destroys brain tissue, resulting in spinal deformities – scoliosis (Fig. 8) of 1–5% of survivors.

The third form of IHN is epitheliotropic, or gill targeting, is observed in older fish of about 50–100 g of weight. Large fish can become infected with the IHNV, but the infection does not become systemic due to the age of the fish or any other factor. However, the agent replicates very effectively in the epithelial cells of the fins, skin and gills and can cause serious breathing problems due to anemia, often hemorrhages in the gills (Fig. 9). Mortality is sporadic, but due to the fact that larger fish are affected, losses (in the total weight of the product) can be high. This ultimately reduces the economic efficiency of the fish farm (decreased weight gain and increased feed conversion) [64, 65].

Diseased fish usually demonstrate some of the above mentioned signs. Only a few affected fish can exhibit all characteristic clinical signs and gross internal lesions during the epizootic. None of the signs described are considered to be pathognomonic for the disease. The blood of affected fry shows reduced haematocrit, leukopenia, degeneration of leucocytes and thrombocytes, and large amounts of cellular debris. As with other haemorrhagic viraemias of fish, blood chemistry is altered in severe cases.

Histopathological findings reveal degenerative necrosis in hematopoietic tissues, kidney, spleen, liver, pancreas, and digestive tract. Necrosis of eosinophilic granular cells in the intestinal wall is pathognomonic of IHNV infection [50].

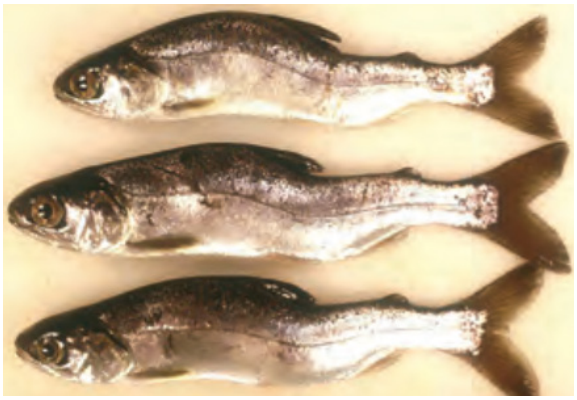

Fig. 8. Scoliosis in sockeye salmon smolts surviving IHNV infection [55]

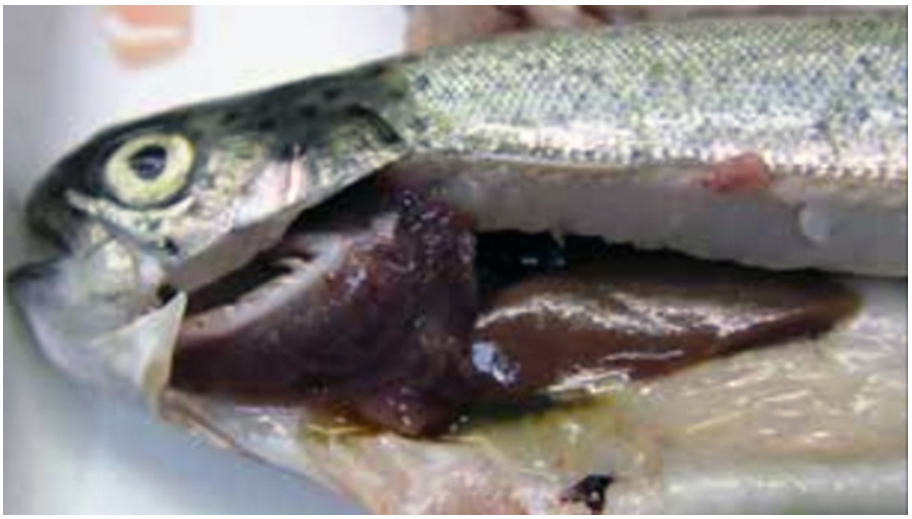

Fig. 9. Anemia and hemorrhages in the gills of IHNV-infected fish [63]

DIAGNOSIS

A preliminary diagnosis should be based on the epidemiological data, clinical appearance of the disease and post mortem findings. The final diagnosis should be based on the results of the virological examination, including isolation and serological identification of the virus, and, if necessary, a bioassay [9, 13, 39, 62].

The optimal tissue material to be examined is spleen, anterior kidney, and heart or encephalon. In some cases, ovarian fluid and milt must be examined.

The "Gold Standard" for detection of IHNV is the isolation of the virus in cell culture followed by its immunological or molecular identification.

Various continuous fish cell lines are used to isolate the virus: EPC, AS, BF-2, CHSE-214, FHM, ICO, RTH-149, RTG-2 and STE-137 [8, 9, 10, 11, 12, 13]. The cytopathic effect in cell culture can be observed 48–72 hours post inoculation (Fig. 10).

Serological identification of the IHNV is performed using enzyme-linked immunosorbent assay (ELISA), indirect fluorescent antibody (IFA) test and neutralization test (NT). The ELISA advantages are high diagnostic sensitivity and specificity, it is less laborious and time-consuming [2, 12, 13, 59, 67, 68, 69, 70, 71]. Molecular genetic diagnostic tests are most rapid and sensitive among all IHNV detection methods. These include reverse transcription polymerase chain reaction (RT-PCR) and real-time RT-PCR using primers targeting G and N genes to detect viral RNA [13, 72, 73, 74, 75, 76, 77, 78, 79].

PREVENTION AND CONTROL MEASURES

Since there is no treatment against IHN to date and there are no commercially available vaccines on the market of the Russian Federation, the main strategy for the disease control is to ensure biosafety and to culture genetically resistant fish.

The IHN prevention shall rely on the avoidance of the disease introduction and spread in free farms, through the implementation of strict control policies and sound hygiene practices, compliance with fish farming standards to exclude or minimize the risk of IHNV introduction into fish farms [9, 37, 38, 39].

Eggs and fish seed materials shall be supplied from farms free from infectious diseases, including infectious

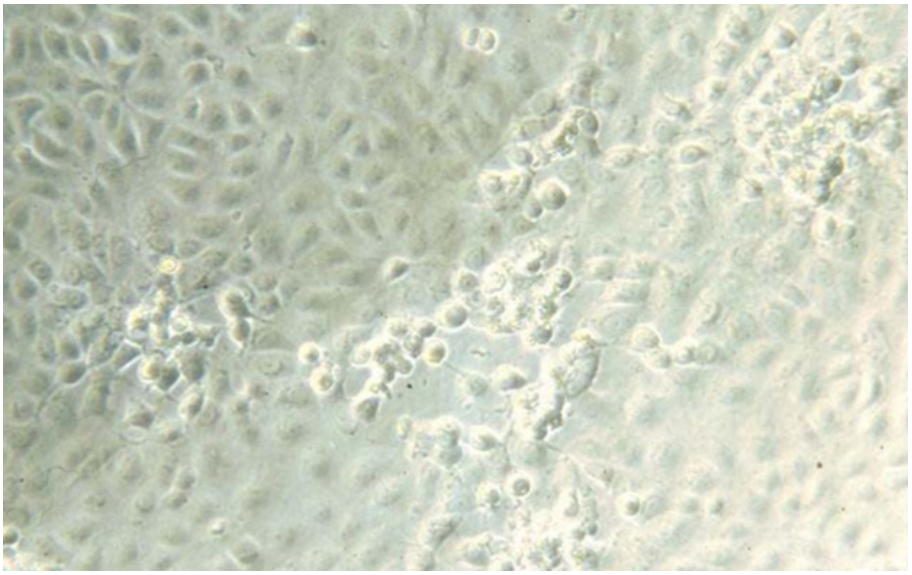

Fig.10. Cytopathic effect (CPE) on CHSE-214 cell line 72 hours post-inoculation [66]

hematopoetic necrosis. Optimal ecological and hygienic conditions shall be established in fry rearing tanks. Feeds shall contain only high-quality virus-free raw materials [37].

The seed material and eggs of a new batch shall be isolated and kept in separate ponds or tanks. Disinfection of eggs is a common practice to effectively mitigate egg-associated transmission of IHNV in aquaculture practice. The method is widely practiced in areas where the virus is endemic [80, 81, 82, 83].

In case of IHN outbreak, the farm shall be recognized disease-infected and subjected to quarantine restrictions (according to the order of the Russian Federation Ministry of Agriculture No. 173 of September 29, 2005 on approval of the list of quarantinable and highly dangerous fish diseases). All diseased fish shall be destroyed. Fish tanks and water supply channels shall be disinfected with chlorine or lime. The handling tools shall be treated with formalin, and the low-value tools shall be destroyed. If no IHN clinical signs are observed in fish during the year, and the results of virological examinations are negative, the quarantine can be lifted [37, 62].

Another control strategy is the farming of virus-resistant populations. Within endemic areas, the use of less susceptible species (chinook salmon, brook trout, cutthroat trout, brown trout, etc.) has been used to reduce the impact of infection with IHNV in aquaculture.

Experimental trials of triploid or inter-species hybrids have shown promise and the genetic basis of resistance to IHNV has been an active area of recent research [84, 85, 86].

Experimental vaccines to protect salmonids against infection with IHNV have been the subject of research for more than 50 years. Research on genetically engineered (recombinant) vaccines against IHN is undertaken in the USA, Germany and Canada [87, 88].

CONCLUSION

Infectious hematopoietic necrosis is a highly contagious disease, classified by the WOAH as a dangerous and economically significant notifiable diseases. This disease can infect a wide range of salmonids and is characterized by high mortality rates (up to 100%) and impaired fish quality and commodity size.

The situation analysis demonstrates that IHNV is the cause of frequent outbreaks in countries where aquaculture is practiced, inflicting significant economic losses. The global epizootic situation remains complicated, especially in the countries bordering the Russian Federation. Prevention is the only way to control the disease.

REFERENCES

1. Infection with infectious haematopoietic necrosis virus. Chapter 10.6. *In: WOAH. Aquatic Animal Health Code.* https://www.woah.org/fileadmin/Home/eng/Health_standards/aahc/current/chapitre_ihn.pdf

2. Infection with infectious haematopoietic necrosis virus. Chapter 2.3.5. *In: WOAH. Manual of Diagnostic Tests for Aquatic Animals. https://www.woah.org/fileadmin/Home/eng/Health_standards/aahm/current/2.3.05_IHN.pdf*

3. Rudakova S. L. Nekroz gemopoeticheskoi tkani u proizvoditelei nerki i predpolagaemye istochniki infektsii = Infectious hematopoetic necrosis in sockeye salmon broodstock and potential infection sources. *Problems of Fishiries.* 2003; 4 (1): 93–102. https://www.elibrary.ru/hneowo (in Russ.)

4. Shchelkunov I. S. Epizootic situation of viral diseases of breeding fish. *Veterinariya.* 2006; 4: 22–25. https://www.elibrary.ru/htbdbn (in Russ.)

5. Kurath G., Higman K. H., Björklund H. V. Distribution and variation of NV genes in fish rhabdoviruses. *Journal of General Virology*. 1997; 78 (1): 113–117. https://doi.org/10.1099/0022-1317-78-1-113

6. Family – Rhabdoviridae. *In: Virus Taxonomy. Eds. A. M. Q. King, M. J. Adams, E. B. Carstens, E. J. Lefkowitz.* Amsterdam: Elsevier; 2012; 686–713. https://doi.org/10.1016/B978-0-12-384684-6.00057-4

7. Dixon P., Paley R., Alegria-Moran R., Oidtmann B. Epidemiological characteristics of infectious hematopoietic necrosis virus (IHNV): a review. *Veterinary Research*. 2016; 47:63. https://doi.org/10.1186/s13567-016-0341-1

8. Akinshina G. T., Belokon V. S., Bilko N. M., Gulyukin M. I., Galnbek T. V., Dagdanova A. V., et al. Animal cell in culture (methods and implementation in biotechnology). 2nd ed., supplemented. Moscow: Sputnik+; 2009. 652 p. (in Russ.)

9. Bogdanova E. A. Diseases of Aquacultured Salmonids and Whitefish. Saint Petersburg: GosNIORKH; 1994; 14–17. (in Russ.)

10. Miller T. A., Rapp J., Wastlhuber U., Hoffmann R. W., Enzmann P. J. Rapid and sensitive reverse transcriptase-polymerase chain reaction based detection and differential diagnosis of fish pathogenic rhabdoviruses in organ samples and cultured cells. *Diseases of Aquatic Organisms*. 1998; 34 (1): 13–20. https://doi.org/10.3354/dao034013

11. Rudakova S. L. Descriptive model of the infectious hematopoietic necrosis virus distribution in a sockeye population. *Izvestiya TINRO.* 2008; 152: 173–185. https://www.elibrary.ru/jvuidn (in Russ.)

12. Doronin M. I., Pylnov V. A., Nasarov N. A., Rybakov S. S. Latex-agglutination tests for detection of salmon infectious hematopoietic necrosis virus antigen. *Veterinariya*. 2014; 9: 56–61. https://www.elibrary.ru/slplcr (in Russ.)

13. Hostnik P., Barlic-Maganja D., Strancar M., Jencic V., Toplak I., Grom J. Influence of storage temperature on infectious hematopoietic necrosis virus detection by cell culture isolation and RT-PCR methods. *Diseases of Aquatic Organisms*. 2002; 52 (3): 179–184. https://doi.org/10.3354/dao052179

14. Nishizawa T., Savaş H., Işıdan H., Üstündağ C., Iwamoto H., Yoshimizu M. Genotyping and pathogenicity of viral hemorrhagic septicemia virus from free-living turbot (*Psetta maxima*) in a Turkish coastal area of the Black Sea. *Applied and Environmental Microbiology.* 2006; 72 (4): 2373–2378. https://doi.org/10.1128/AEM.72.4.2373-2378.2006

15. Bearzotti M., Delmas B., Lamoureux A., Loustau A.-M., Chilmonczyk S., Bremont M. Fish rhabdovirus cell entry is mediated by fibronectin. *Journal of Virology*. 1999; 73 (9): 7703–7709. https://doi.org/10.1128/JVI.73.9.7703-7709.1999

16. Einer-Jensen K., Ahrens P., Forsberg R., Lorenzen N. Evolution of the fish rhabdovirus viral haemorrhagic septicaemia virus. *Journal of General Virology.* 2004; 85 (5): 1167–1179. https://doi.org/10.1099/vir.0.79820-0

17. Gaudin Y., de Kinkelin P., Benmansour A. Mutations in the glycoprotein of viral haemorrhagic septicaemia virus that affect virulence for fish and the pH threshold for membrane fusion. *Journal of General Virology.* 1999;

80 (5): 1221–1229. https://doi.org/10.1099/0022-1317-80-5-1221

18. Nishizawa T., Iida H., Takano R., Isshiki T., Nakajima K., Muroga K. Genetic relatedness among Japanese, American and European isolates of viral hemorrhagic septicemia virus (VHSV) based on partial G and P genes. *Diseases of Aquatic Organisms*. 2002; 48 (2): 143–148. https://doi.org/10.3354/dao048143

19. Schütze H., Enzmann P. J., Kuchling R., Mundt E., Niemann H., Mettenleiter T. C. Complete genomic sequence of the fish rhabdovirus infectious haematopoietic necrosis virus. *Journal of General Virology*. 1995; 76 (10): 2519–2527. https://doi.org/10.1099/0022-1317-76-10-2519

20. Björklund H. V., Higman K. H., Kurath G. The glycoprotein genes and gene junctions of the fish rhabdoviruses spring viremia of carp virus and hirame rhabdovirus: analysis of relationships with other rhabdoviruses. *Virus Research*. 1996; 42 (1–2): 65–80. https://doi.org/10.1016/0168-1702(96)01300-7

21. Hoffmann B., Schütze H., Mettenleiter T. C. Determination of the complete genomic sequence and analysis of the gene products of the virus of spring viremia of carp, a fish rhabdovirus. *Virus Research*. 2002; 84 (1–2): 89–100. https://doi.org/10.1016/s0168-1702(01)00441-5

22. Morzunov S. P., Winton J. R., Nichol S. T. The complete genome structure and phylogenetic relationship of infectious hematopoietic necrosis virus. *Virus Research*. 1995; 38 (2–3): 175–192. https://doi.org/10.1016/0168-1702(95)00056-v

23. Lorenzen N., Lapatra S. E. Immunity to rhabdoviruses in rainbow trout: the antibody response. *Fish and Shellfish Immunology*. 1999; 9 (4): 345–360. https://doi.org/10.1006/fsim.1999.0194

24. Hoffmann B., Beer M., Schütze H., Mettenleiter T. C. Fish rhabdoviruses: Molecular epidemiology and evolution. *In: The World of Rhabdoviruses. Current Topics in Microbiology and Immunology*. Berlin, Heidelberg: Springer; 2005; 292: 81–117. https://doi.org/10.1007/3-540-27485-5_5

25. Kurath G., Ahern K., Pearson G. D., Leong J. C. Molecular cloning of the six mRNA species of infectious hematopoietic necrosis virus, a fish rhabdovirus, and gene order determination by R-loop mapping. *Journal of Virology*. 1985; 53 (2): 469–476. https://doi.org/10.1128/JVI.53.2.469-476.1985

26. Garver K. A., Troyer R. M., Kurath G. Two distinct phylogenetic clades of infectious hematopoietic necrosis virus overlap within the Columbia River basin. *Diseases of Aquatic Organisms*. 2003; 55 (3): 187–203. https://doi.org/10.3354/dao055187

27. Troyer R. M., LaPatra S. E., Kurath G. Genetic analyses reveal unusually high diversity of infectious haematopoietic necrosis virus in rainbow trout aquaculture. *Journal of General Virology*. 2000; 81 (12): 2823–2832. https://doi.org/10.1099/0022-1317-81-12-2823

28. Kurath G., Garver K. A., Troyer R. M., Emmenegger E. J., Einer-Jensen K., Anderson E. D. Phylogeography of infectious haematopoietic necrosis virus in North America. *Journal of General Virology*. 2003; 84 (4): 803–814. https://doi.org/10.1099/vir.0.18771-0

29. Enzmann P. J., Kurath G., Fichtner D., Bergmann S. M. Infectious hematopoietic necrosis virus: monophyletic origin of European isolates from North American genogroup M. *Diseases of Aquatic Organisms*. 2005; 66 (3): 187–195. https://doi.org/10.3354/dao066187

30. Yu Z.-H., Deng M.-L., Geng Y., Zhou Y., Wang K.-Y., Chen D.-F., et al. An outbreak of infectious haematopoietic necrosis virus (IHNV) infection in cultured rainbow trout (*Oncorhynchus mykiss*) in Southwest China. *Aquaculture Research*. 2016; 47 (7): 2355–2362. https://doi.org/10.1111/are.12680

31. Nishizawa T., Kinoshita S., Kim W.-S., Higashi S., Yoshimizu M. Nucleotide diversity of Japanese isolates of infectious hematopoietic necrosis virus (IHNV) based on the glycoprotein gene. *Diseases of Aquatic Organisms*. 2006; 71 (3): 267–272. https://doi.org/10.3354/dao071267

32. LaPatra S. E., Groff J. M., Fryer J. L., Hedrick R. P. Comparative pathogenesis of three strains of infectious hematopoietic necrosis virus in rainbow trout *Oncorhynchus mykiss*. *Diseases of Aquatic Organisms*. 1990; 8: 105–112.

33. LaPatra S. E., Fryer J. L., Rohovec J. S. Virulence comparison of different electrophero-types of infectious hematopoietic necrosis virus. *Diseases of Aquatic Organisms*. 1993; 16: 115–120.

34. Wolf K. Fish viruses and fish viral diseases. Ithaca, London: Comstock Publishing Associates; Cornell University Press; 1988. 476 p.

35. Traxler G. S., Roome J. R., Lauda K. A., LaPatra S. Appearance of infectious hematopoietic necrosis virus (IHNV) and neutralizing antibodies in sockeye salmon *Oncorhynchus nerka* during their migration and maturation period. *Diseases of Aquatic Organisms*. 1997; 28 (1): 31–38. https://doi.org/10.3354/dao028031

36. Rudakova S. L. The effect of the infectious hematopoietic necrosis virus on the population of sockeye salmon *Oncorhynchus nerka* (*Salmoniformes, Salmonidae*) from Lake Nachikinskoe. *Journal of Ichthyology*. 2010; 50 (5): 402–407. https://doi.org/10.1134/S0032945210050061

37. Voronin V. N., Kuznetsova E. V., Strelkov Yu. A., Chernysheva N. B. Diseases of Aquacultured Fish in Russia: Manual. Saint Petersburg: GosNIORKH; 2011; 143–146. (in Russ.)

38. Rahkonen R., Vennerström P., Rintamäki P., Kannel R. Terve kala: Tautien ennaltaehkäisy, tunnistus ja hoito. Toinen tarkistettu painos. Helsinki: Riistan-ja kalatalouden tutkimuslaitos; 2012; 38–39. (in Finnish)

39. Vasilkov G. V., Grishchenko L. I., Engashev V. G., Kanaev A. I., Larkova Z. I. Fish Diseases: Reference Guide. Ed. by V. S. Osetrov. 2nd ed., revised and supplemented. Moscow: Agropromizdat; 1989. 288 p. (in Russ.)

40. Scientific Opinion of the Panel on Animal Health and Welfare on a request from the European Commission on possible vector species and live stages of susceptible species not transmitting disease as regards certain fish diseases. *EFSA Journal*. 2007; 584: 1–163. https://doi.org/10.2903/j.efsa.2007.584

41. Rexhepi A., Bërxholi K., Scheinert P., Hamidi A., Sherifi K. Study of viral diseases in some freshwater fish in the Republic of Kosovo. *Veterinarski Arhiv*. 2011; 81 (3): 405–413.

42. Hill B., Reese A., Dixon P., Oidtmann B., Paley R., Peeler E., et al. Epidemiology of different agents causing disease in aquatic animals: *EFSA Supporting Publications*. 2010; 7 (1):37E. https://doi.org/10.2903/sp.efsa.2010.EN-37

43. Apasova L. Yu., Moroz N. V., Rybakov S. S., Yeremeyeva T. B. Purification and concentration of salmon infectious hematopoietic necrosis virus. *Proceedings of the Federal*

Centre for Animal Health. 2009; 7: 234–239. https://www.elibrary.ru/moujpj (in Russ.)

44. Zavyalova E. A., Droshnev A. E., Bulina K. Y., Carpova M. A. Monitoring of epizootic situation on fish diseases: facts and prospects. *Trudi VIEV*. 2018; 80 (1): 182–189. https://doi.org/10.30917/ATT-PRINT-2018-1 (in Russ.)

45. Zavyalova E. A., Droshnev A. E., Bulina K. Yu., Gulyukin A. M. Epizootic situation on fish diseases: research methods, trends, prospects. *Russian Journal "Problems of Veterinary Sanitation, Hygiene and Ecology"*. 2018; (1): 136–142. https://www.elibrary.ru/voiiay (in Russ.)

46. WOAH. World Animal Health Information System: Disease situation. https://wahis.woah.org/#/dashboards/country-or-disease-dashboard

47. Winton J. R. Recent advances in detection and control of infectious hematopoietic necrosis virus in aquaculture. *Annual Review of Fish Diseases*. 1991; 1: 83–93. https://doi.org/10.1016/0959-8030(91)90024-E

48. Oidtmann B. C., Peeler E. J., Thrush M. A., Cameron A. R., Reese R. A., Pearce F. M., et al. Expert consultation on risk factors for introduction of infectious pathogens into fish farms. *Preventive Veterinary Medicine*. 2014; 115 (3–4): 238–254. https://doi.org/10.1016/j.prevetmed.2014.03.017

49. Lapatra S. E., Turner T., Lauda K. A., Jones G. R., Walker S. Characterization of the humoral response of rainbow trout to infectious hematopoietic necrosis virus. *Journal of Aquatic Animal Health*. 1993; 5 (3): 165–171. https://doi.org/10.1577/1548-8667(1993)005<0165:COTHRO>2.3.CO;2

50. Bootland L. M., Leong J. A. C. Infectious haematopoietic necrosis virus. *In: Fish Diseases and Disorders. Vol. 3: Viral, Bacterial and Fungal Infections. Ed. by P. T. K. Woo, D. W. Bruno. 2nd ed.* Wallingford: CABI; 2011; 66–109. https://doi.org/10.1079/9781845935542.0066

51. Jakob E., Barker D. E., Garver K. A. Vector potential of the salmon louse *Lepeophtheirus salmonis* in the transmission of infectious haematopoietic necrosis virus (IHNV). *Diseases of Aquatic Organisms*. 2011; 97 (2): 155–165. https://doi.org/10.3354/dao02414

52. Ritchie G. The host transfer ability of *Lepeophtheirus salmonis* (*Copepoda: Caligidae*) from farmed Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases*. 1997; 20 (3): 153–157. https://doi.org/10.1046/j.1365-2761.1997.00285.x

53. Shors S. T., Winston V. Detection of infectious hematopoietic necrosis virus in an invertebrate (*Callibaetis* sp.). *American Journal of Veterinary Research*. 1989; 50 (8): 1307–1309. PMID: 2782712

54. Yamamoto T., Arakawa C. K., Batts W. N., Winton J. R. Comparison of infectious hematopoietic necrosis in natural and experimental infections of spawning salmonids by infectivity and immunohistochemistry. *In: Viruses of Lower Vertebrates. Ed. by W. Ahne, E. Kurstak*. Berlin, Heidelberg: Springer; 1989; 411–429. https://doi.org/10.1007/978-3-642-83727-2_37

55. Meyers T., Burton T., Bentz C., Starkey N. Common disease of wild and cultured fishes in Alaska. Anchorage: Alaska Department of Fish and Game; 2008. 106 p. https://www.metabunk.org/attachments/fish_disease_book-pdf.16003

56. Rudakova S. L. Factors influenced on prevalence of infectious hematopoietic necrosis virus (IHNV) into adult sockeye salmon populations in spawning lakes of Kamchatka. *The researches of the aquatic biological resources of Kamchatka and the North-West Part of the Pacific Ocean*. 2009; (13): 88–94. https://www.elibrary.ru/knpueh (in Russ.)

57. Pavlov D. K., Pichuyeva A. A. Analysis of fish viral disease epidemic situation worldwide. *Veterinary Science Today*. 2015; (2): 54–58. https://www.elibrary.ru/umtujt (in Russ.)

58. Harmache A., LeBerre M., Droineau S., Giovannini M., Brémont M. Bioluminescence imaging of live infected salmonids reveals that the fin bases are the major portal of entry for *Novirhabdovirus*. *Journal of Virology*. 2006; 80 (7): 3655–3659. https://doi.org/10.1128/JVI.80.7.3655-3659.2006

59. Doronin M. I., Pylnov V. A., Rybakov S. S. Method of latex agglutination for detecting antibodies to infectious hematopoietic necrosis virus in salmon fishes. *Bulletin of Udmurt University. Series Biology. Earth Sciences*. 2015; 25 (2): 135–144. https://www.elibrary.ru/uapuiv (in Russ.)

60. Rodriguez Saint-Jean S., Borrego J. J., Perez-Prieto S. I. Infectious pancreatic necrosis virus: biology, pathogenesis, and diagnostic methods. *Advances in Virus Research*. 2003; 62: 113–165. https://doi.org/10.1016/s0065-3527(03)62003-8

61. Department of Agriculture, Water and the Environment. Aquatic Animal Diseases Significant to Australia: Identification Field Guide. 5th ed. Canberra: Australian Government; Department of Agriculture, Water and the Environment; 2020. 341 p. https://www.agriculture.gov.au/sites/default/files/documents/field-guide-5th-edition.pdf

62. Vanyatinsky V. F., Mirzoeva L. M., Poddubnaya A. V. Fish Diseases: Study Guide. Ed. by V. A. Musselius. Moscow: Pishchevaya promyshlennost'; 1979. 232 p. (in Russ.)

63. Zrnčić S., Radosavljević V. West Balkans Regional Aquatic Animal Disease Diagnostic Manual (TCP/RER/3402). Rome: FAO; 2017. 78 p. https://www.fao.org/3/i6848e/i6848e.pdf

64. Bergmann S. M., Fichtner D., Skall H. F., Schlotfeldt H. J., Olesen N. J. Age- and weight-dependent susceptibility of rainbow trout *Oncorhynchus mykiss* to isolates of infectious haematopoietic necrosis virus (IHNV) of varying virulence. *Diseases of Aquatic Organisms*. 2003; 55 (3): 205–210. https://doi.org/10.3354/dao055205

65. LaPatra S. E., Evilia C., Winston V. Positively selected sites on the surface glycoprotein (G) of infectious hematopoietic necrosis virus. *Journal of General Virology*. 2008; 89 (3): 703–708. https://doi.org/10.1099/vir.0.83451-0

66. LaPatra S. E. Infectious hematopoietic necrosis (2012). *In: AFS-FHS (American Fisheries Society-Fish Health Section). FHS blue book: suggested procedures for the detection and identification of certain finfish and shellfish pathogens*. 2020. https://units.fisheries.org/fhs/fish-health-section-blue-book-2020/section-1-diagnostic

67. Jorgensen P. E. V., Olesen N. J., Lorenzen N., Winton J. R., Ristow S. S. Infectious hematopoietic necrosis (IHN) and viral hemorrhagic septicemia (VHS): Detection of the trout antibodies to the causative viruses by means of plaque neutralization, immunofluorescence, and enzyme-linked immunosorbent assay. *Journal of Aquatic Animal Health*. 1991; 3 (2): 100–108. https://doi.org/10.1577/1548-8667(1991)003<0100:IHNIAV>2.3.CO;2

68. Apasova L. Yu., Rybakov S. S. Use of indirect enzyme-linked immunosorbent assay for detection of infectious hematopoietic necrosis virus. *Proceedings of the*

Federal Centre for Animal Health. 2010; 8: 204–213. https://www.elibrary.ru/ndhhqd (in Russ.)

69. Tarasov V. E., Rudakova S. L., Bochkova E. V., Shepelyakovskaya A. O. Sravnitel'nyi analiz IFA i «zolotogo standarta» pri identifikatsii virusa infektsionnogo nekroza gemopoeticheskoi tkani u polovozreloi nerki = Comparative analysis of ELISA and the golden standard for infectious hematopoetic necrosis virus identification from adult sockeye salmon. *Sovremennye problemy i perspektivy razvitiya rybokhozyaistvennogo kompleksa: sbornik trudov IX Nauchno-prakticheskoi konferentsii molodykh uchenykh s mezhdunarodnym uchastiem, posvyashchennoi 140-letiyu VNIRO (Moskva, 11–12 noyabrya 2021 g.) = Current challenges and prospects of aquaculture development: Proceedings of IX Scientific and practical conference of early-career scientists with international participations, devoted to 140-anniversary of the Russian Federal Research Institute of Fisheries and Oceanography (Moscow, 11–12 November, 2021)*. Moscow: VNIRO; 2021; 163–166. https://www.elibrary.ru/zgbpag (in Russ.)

70. Arnzen J. M., Ristow S. S., Hesson C. P., Lientz J. Rapid fluorescent antibody tests for infectious hematopoietic necrosis virus (IHNV) utilizing monoclonal antibodies to the nucleoprotein and glycoprotein. *Journal of Aquatic Animal Health*. 1991; 3 (2): 109–113. https://doi.org/10.1577/1548-8667(1991)003<0109:RFATFI>2.3.CO;2

71. LaPatra S. E., Roberti K. A., Rohovec J. S., Fryer J. L. Fluorescent antibody test for the rapid diagnosis of infectious hematopoietic necrosis. *Journal of Aquatic Animal Health*. 1989; 1 (1): 29–36. https://doi.org/10.1577/1548-8667(1989)001<0029:FATFTR>2.3.CO;2

72. Doronin M. I., Pilnov V. A., Mudrak N. S. Development of the method of RT-PCR in real time to detect a virus infectious hematopoietic necrosis tissue salmonids. *Science Almanac*. 2015; 8 (10): 1052–1057. https://doi.org/10.17117/na.2015.08.1052 (in Russ.)

73. Dhar A. K., Bowers R. M., Licon K. S., LaPatra S. E. Detection and quantification of infectious hematopoietic necrosis virus in rainbow trout (*Oncorhynchus mykiss*) by SYBR Green real-time reverse transcriptase-polymerase chain reaction. *Journal of Virological Methods*. 2008; 147 (1): 157–166. https://doi.org/10.1016/j.jviromet.2007.08.026

74. Overturf K., LaPatra S., Powell M. Real-time PCR for the detection and quantitative analysis of IHNV in salmonids. *Journal of Fish Diseases*. 2001; 24 (6): 325–333. https://doi.org/10.1046/j.1365-2761.2001.00296.x

75. Purcell M. K., Hart S. A., Kurath G., Winton J. R. Strand-specific, real-time RT-PCR assays for quantification of genomic and positive-sense RNAs of the fish rhabdovirus, *Infectious hematopoietic necrosis virus*. *Journal of Virological Methods*. 2006; 132 (1–2): 18–24. https://doi.org/10.1016/j.jviromet.2005.08.017

76. Purcell M. K., Thompson R. L., Garver K. A., Hawley L. M., Batts W. N., Sprague L., et al. Universal reverse-transcriptase real-time PCR for infectious hematopoietic necrosis virus (IHNV). *Diseases of Aquatic Organisms*. 2013; 106 (2): 103–115. https://doi.org/10.3354/dao02644

77. Arakawa C. K., Deering R. E., Higman K. H., Oshima K. H., O'Hara P. J., Winton J. R. Polymerase chain reaction (PCR) amplification of a nucleoprotein gene sequence of infectious hematopoietic necrosis virus. *Diseases of Aquatic Organisms*. 1990; 8: 165–170.

78. Deering R. E., Arakawa C. K., Oshima K. H., O'Hara P. J., Landolt M. L., Winton J. R. Development of a biotinylated DNA probe for detection and identification of infectious hematopoietic necrosis virus. *Diseases of Aquatic Organisms*. 1991; 11: 57–65. https://doi.org/10.3354/DAO011057

79. Winton J. R., Einer-Jensen K. Molecular diagnosis of infectious hematopoietic necrosis and viral hemorrhagic septicemia. *In: Molecular Diagnosis of Salmonid Diseases. Eds. C. O. Cunningham*. Dordrecht: Springer; 2002; 49–79. https://doi.org/10.1007/978-94-017-2315-2_3

80. Rudakova S. L. Prevention and control of viral diseases in aquaculture. *Current state and development of aquaculture: ecological and ichthyopathological state of reservoirs and breeding facilities, cultivation technologies*: *proceedings of the international conference (Novosibirsk, November 11–13, 2020)*. Novosibirsk: Novosibirsk SAU; 2020; 134–136. https://www.elibrary.ru/mvyfbl (in Russ.)

81. Rudakova S. L., Schelkunova U. P., Novoselova U. A., Recordatova S. A., Kropocheva I. U. Preliminary results of the iodinol use for prevention of infectious hematopoetic necrosis in rainbow trout (experimental data). *Vodnye biologicheskie resursy Rossii: sostoyanie, monitoring, upravlenie: sbornik materialov II Vserossiiskoi nauchnoi konferentsii, posvyashchennoi 90-letiyu Kamchatskogo filiala Vserossiiskogo nauchno-issledovatel'skogo instituta rybnogo khozyaistva i okeanografii (Petropavlovsk-Kamchatskii, 4–6 aprelya 2022 g.) = Aquatic biological resources of Russia: conditions, monitoring, management: Proceedings of the II All-Russia Scientific Conference, devoted to 90th anniversary of the Kamchatka Branch of the Russian Federal Research Institute of Fisheries and Oceanography (Petropavlovsk-Kamchatsky, 4–6 April, 2022)*. Petropavlovsk-Kamchatsky: KamchatNIRO; 2022; 195–199. https://www.elibrary.ru/jhcfaq (in Russ.)

82. Rudakova S. L., Bochkova E. V., Volkova T. V., Saharovskaja L. V. Modification of the method of sockeye salmon egg treatment with iodinol from infectious hematopoietic necrosis virus in Kamchatka Hatchery. *Trudy VNIRO*. 2020; 182: 128–138. https://doi.org/10.36038/2307-3497-2020-182-128-138 (in Russ.)

83. Bovo G., Håstein T., Hill B., LaPatra S. E., Michel C., Olesen N. J., et al. Work package 1 report: Hazard identification for vertical transfer of fish disease agents. Oslo: VESO; 2005. 35 p.

84. Purcell M. K., LaPatra S. E., Woodson J. C., Kurath G., Winton J. R. Early viral replication and induced or constitutive immunity in rainbow trout families with differential resistance to *Infectious hematopietic necrosis virus* (IHNV). *Fish & Shellfish Immunology*. 2010; 28 (1): 98–105. https://doi.org/10.1016/j.fsi.2009.10.005

85. Barroso R. M., Wheeler P. A., LaPatra S. E., Drew R. E., Thorgaard G. H. QTL for IHNV resistance and growth identified in a rainbow trout (*Oncorhynchus mykiss*) × Yellowstone cutthroat (*Oncorhynchus clarki bouvieri*) trout cross. *Aquaculture*. 2008; 277 (3–4): 156–163. https://doi.org/10.1016/j.aquaculture.2008.03.001

86. Miller K. M., Winton J. R., Schulze A. D., Purcell M. K., Ming T. J. Major histocompatibility complex loci are associated with susceptibility of Atlantic salmon to infectious hematopoietic necrosis virus. *Environmental Biology of Fishes*. 2004; 69 (1): 307–316. https://doi.org/10.1023/B:EBFI.0000022874.48341.0f

87. Winton J. R. Immunization with viral antigens: infectious hematopoietic necrosis. *Developments in Biological Standardization*. 1997; 90: 211–220. PMID: 9270850

88. Kurath G. Biotechnology and DNA vaccines for aquatic animals. *Revue Scientifique et Technique (International Office of Epizootics)*. 2008; 27 (1): 175–196. http://dx.doi.org/10.20506/rst.27.1.1793

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Chelate compounds and their use for correction of trace element deficiencies in livestock (review)

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ABSTRACT

Livestock and poultry diseases occurring due to mineral or vitamin deficiencies are widely reported and belong to the factors restraining the development of livestock industry. Almost until the 90s of the last century, the conditions associated with trace element deficiency were prevented and treated using inorganic compounds. In recent decades, scientists have synthesized chelate metal compounds using organic carriers, determining the high bioavailability of these compounds and the efficiency that repeatedly exceeds the efficiency of inorganic compounds. Amino acids are preferably used as organic carriers. In addition to their main function, i.e. replenishing the trace element deficiency, chelate compounds increase the enzymatic activity, the functional activity of the immune system, and are also able to enhance the absorption of other trace elements, showing a synergistic effect. Due to the immunostimulatory activity resulting from increase in the content of sialic acids, properdin, ceruloplasmin, gamma globulin protein fraction, the metal chelates (copper, cobalt, iodine) can be used as immune response modulators. Iron chelate compounds are used for therapy and prevention of iron deficiency anemias not only in veterinary, but also in human medicine. This paper is based on data analysis of Scopus, CyberLeninka, PubMed, RSCI and other databases and systematizes scientific knowledge on the problem of designing and synthesizing metal chelate compounds using organic carriers. The scientific rationale is given for the use of amino acids and organic acids as organic carriers of metal, vitamin and other compounds. The mechanism of biological action of chelate compounds and the pathogenesis of trace element deficiencies in animals are considered, while the advantages of chelate compound use in microelementoses therapy and prevention are specified.

Keywords: review, chelate compounds, organic carriers, biological action, iron deficiency anemia, pathogenesis, prevention, treatment

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Хелатные соединения и их использование для коррекции микроэлементозов сельскохозяйственных животных (обзор литературы)

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РЕЗЮМЕ

Болезни сельскохозяйственных животных и птиц, обусловленные дефицитом минеральных компонентов и витаминов, регистрируются повсеместно и являются одним из факторов, сдерживающих развитие животноводческой отрасли. Профилактика и лечение болезней, связанных с недостатком микроэлементов, практически до 90-х годов прошлого столетия осуществлялись с использованием неорганических соединений. В последние десятилетия учеными синтезированы хелатные соединения металлов с использованием органических носителей, что обусловливает их высокую биодоступность и эффективность, многократно превосходящую эффективность неорганических форм. В качестве органических носителей предпочтительное использование получили аминокислоты. Хелатные соединения, кроме своей основной функции восполнения дефицита микроэлементов, повышают активность ферментов, функциональную активность иммунной системы, а также способствуют усвоению других микроэлементов, проявляя синергический эффект. Благодаря иммуностимулирующей активности за счет увеличения содержания сиаловых кислот, пропердина, церулоплазмина, гамма-глобулиновой фракции белков, хелаты металлов (меди, кобальта, йода) могут применяться в качестве модуляторов иммунного ответа. Хелатные соединения железа

используют для лечения и профилактики железодефицитных анемий не только в ветеринарной, но также и в гуманной медицине. В статье на основе анализа литературы из баз данных Scopus, CyberLeninka, PubMed, РИНЦ и других систематизированы научные знания по проблеме конструирования и синтеза хелатных соединений металлов с использованием органических носителей. Дано научное обоснование использования аминокислот и органических кислот в качестве органических носителей соединений металлов, витаминов и других соединений. Рассмотрен механизм биологического действия хелатных соединений на патогенез микроэлементозов животных, а также описаны преимущества применения хелатных соединений для их терапии и профилактики.

Ключевые слова: обзор, хелатные соединения, органические носители, биологическое действие, железодефицитная анемия, патогенез, профилактика, лечение

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INTRODUCTION

Prior to the development of chelated drug forms, inorganic mineral compounds were used as supplements in animal husbandry and veterinary practice. Inorganic forms of such metals as copper, iron, zinc, manganese, cobalt, etc. were used for treatment and prevention of poultry and animal diseases for many years. All of them had high toxicity and caused multiple adverse effects [1, 2, 3, 4].

The selection of organic carriers and the study of the toxicological characteristics of new chelated drugs open up new opportunities not only for the development of high and waste-free cultivation technology, but also, very importantly, for obtaining high-quality and safe products [5, 6].

According to numerous scientific papers, amino acids and organic acids have proved to be the best organic carriers. During chelation mineral compounds and vitamins are easily integrated into the organic carrier molecule and are practically freely delivered to the body for metabolic processes [7, 8]. Amino acids used as the organic carrier have a number of advantages over other carriers, in particular those having a sulfate form. Such forms of organic compounds are almost completely involved in the metabolic process and participate in the biochemical reactions of synthesis of new organic substrates and energy in the body of animals and birds. This entails an increase in productivity, preservation, better absorption of feed nutrients and an increase in immune status [9]. These organic complexes have a number of advantages over non-organic forms. One of the advantages is low toxicity for livestock and poultry, as well as decreased dosages with the same biological effect [10, 11]. Besides, the use of chelated drug forms in many aspects solves the environmental problem, which is highly acute for ecologists in regions with developed animal husbandry [12].

A very important point is that the chelate complex is not hydrolyzed by enzymes of the digestive tract until it is absorbed in the small intestine and exposed to substances that can slow down their metabolism. Almost all metals, with the exception of silver (I) and copper (I) compounds, are suitable for the chelation process. Livestock and poultry are most sensitive to mineral compounds such as iron, zinc, copper, cobalt and manganese. These minerals have specific activity [13, 14, 15]. Chelated mineral compounds are better absorbed, have a positive effect on the growth and development of food-producing animals and poultry, which ultimately affects the quality indicators of the products obtained [16, 17, 18].

The purpose of this paper was to generalize and systematize scientific knowledge on the problem of constructing and synthesizing chelated metal compounds using organic carriers based on literature analysis. The Scopus, CyberLeninka, PubMed, RSCI and other databases were used to conduct scientific research.

The most important stage in the development of chelated drug forms is the selection of organic carrier. The amino acid glycine is used as a source of organic carriers. This amino acid is a derivative of acetic acid and a representative of fatty acids. Its biological function is producing a calming effect on the processes of arousal in different parts of the central nervous system. It has a nootropic effect. A dipeptide consisting of two glycine molecules is included in the composition of drugs with hemostatic properties. The amino acid glycine is proteinogenic, optically inactive. It occurs in a free state in animals and plants. This acid is found in the compounds such as glutathione, neuropeptides and antibiotics. This amino acid, which is also part of the bacterial cell wall, was isolated from gelatin in the early 19th century. Glycine is the starting compound for the biosynthesis of interchangeable

amino acids, this amino acid is the "supplier" of the amino group in the synthesis of the hemoglobin chromoprotein. Being a part of the polypeptide chain, it participates in the formation of the primary structure of all proteins. It has been proven that glycine participates in the biosynthesis of protoporphyrin – a compound that is a precursor of the pigment chlorophyll and heme. Glycine can be attributed to neurotransmitters, since all the processes that it regulates are reduced to metabolic and receptor actions. The receptors that contain glycine are located in the parts of the spinal cord and brain. Glycine, acting on receptors, reduces the release of glutamic and gamma-aminobutyric acids from them. As a result of the increased release of glutamate, glycine, along with glutamic acid, protects the body from overexcitation processes. Glycine can exhibit an inhibitory effect both with gamma-aminobutyric acid receptors and with its own receptors. Glycine is used as organic carrier in modern pharmacology for development of chelated compounds with alkaline and alkaline earth metals such as lithium, calcium, and magnesium [7, 11].

The research literature contains data on the effect of chelated compounds of amino acids with lithium on growth and development of livestock. As a result of stress, this composition normalizes the work of the hypothalamic-pituitary system, weakening the influence of stress factors on the body. Chelated lithium compounds were subjected to comparative studies. Lithium glycinate and lithium carbonate prevent anemia, have a positive effect on the body growth and development, but lithium glycinate demonstrates a stronger effect in commercial raising of livestock and poultry under stressful situations [8, 19].

Amino acid compounds with magnesium and calcium salts exhibit a high biological effect, and are available as independent medicinal products in the pharmacological industry. Magnesium glycinate promotes better adsorption of magnesium in the intestine, making it more accessible for participation in biological oxidation processes in order to generate energy with adenosine triphosphate, strengthen bone tissue, and relieve tension in muscle tone [8, 9].

L-hydroxyproline was isolated at the beginning of the last century. Currently, this compound is derived from collagen and other proteins as a result of hydrolysis. During the hydroxylation of proline, the interchangeable amino acid oxyproline is synthesized, being involved in the metabolic process, two very important biologically active compounds are formed from it: pyrrole-2-carboxylic and glutamic acids [20]. The amino acid hydroxyproline, in addition to participating in the formation of proteins, is involved in the synthesis of elastin and collagen. The composition of the collagen molecule includes the amino acids hydroxyproline, glycine, and proline. The collagen protein molecule itself has the shape of a three-dimensional spiral. Drugs with anti-inflammatory and antipyretic effects have been developed on the basis of L-proline, 4-hydroxyproline compounds, as well as salts thereof; 4-hydroxyproline is used as the main substrate in the synthesis of drugs with antifungal action. At the cellular level, this compound restores damaged cellular structures by affecting the collagen synthesis, which has found its application in cosmetology. Chelated compounds of 4-hydroxyproline with various salts of lithium, calcium, and magnesium are described in the literature, but their physico-chemical properties and synthesis are not presented. The production of 4-hydroxyproline salts with elements such as lithium, sodium, and magnesium is based on neutralization reaction [2, 21].

Chelated compounds have a wide range of biological effects, ranging from increasing the activity of many important enzymes, as well as ensuring the processes of body protection from adverse external factors [22]. Some compounds, such as copper and zinc, improve the absorption of cobalt, providing the so-called synergistic effect. Excessive protein and iron content slow down the process of its absorption in the gastrointestinal tract [23].

Numerous scientific studies have addressed the role of mineral compounds in humans and animals, daily norms as well as the main sources of intake have been determined. Biogeochemical provinces with a certain content of macro- and microelements in soil and plants, as well as their effect on the physiological state of animals contained in these zones, have been established [24, 25, 26]. Since the middle of the last century, scientists of the Kazan State Academy of Veterinary Medicine named after N. E. Bauman have been conducting scientific work on the study of chelated forms of mineral compounds [5, 8, 11]. The main metal complexes were synthesized on the basis of copper and organic compounds such as lactocasein and lactoalbumin, and copper chelates with destructive proteins were obtained, which were isolated from animal tissues and organs [11, 27, 28].

The positive effect of organometallic compounds on the synthesis of keratin protein and serum proteins has been proven. Metallochelates have a pronounced effect on the production of antibodies in various types of vaccination. Injectable forms of chelates of copper, cobalt, iodine are able to stimulate the protective functions of the body by increasing the content of sialic acids, properdin, cerruloplasmin, gamma globulin fraction of proteins. These data have been confirmed in both laboratory animals and experimental livestock populations [2].

Iron deficiency is the most studied form of micronutrient deficiency. Iron deficiency anemia in animals occurs due to lack of iron being a constituent of the chromoprotein hemoglobin, which provides oxygen transportation [29]. Iron is necessary for the implementation of all vital functions of the body, ensuring its growth, and, accordingly, the volume of circulating blood. Piglets have intensive metabolic processes, so they are sensitive to iron deficiency. Piglets receive iron with maternal milk on the first day of life, with feed, as well as endogenously during the breakdown of red blood cells. The composition of sow milk contains enough biologically active compounds involved in the synthesis of new compounds, adenosine triphosphate, but little iron. Due to the breakdown of red blood cells, maximum one percent of iron enters the bloodstream daily. It is absorbed from the plasma by cells of the reticular-endothelial system [30, 31]. This system does not function well in young animals, the process of iron deposition is disrupted, therefore, its deficiency occurs in the body. The disease is aggravated by the fact that piglets are born with low iron reserves of no more than 50 mg. In this

regard, if there is no external supply of this trace element, the iron deficiency is detected within a week after birth, and anemia is recorded a month later [32]. The severity of the disease is aggravated by the lack of intake of mineral compounds and vitamins into the body.

Considering the pathogenesis of iron deficiency anemia, it is possible to state a decrease in the amount of hemoglobin, as well as a decrease in the activity of iron-containing enzymes, especially cytochromes involved in the biological oxidation chain. Iron, which is part of hemoglobin, forms a complex consisting of iron and oxygen, which is actively involved in metabolic processes. With its deficiency, the phenomenon of hypoxia is observed, which negatively affects the work of all organs.

Compensatory mechanisms develop in conditions of hypoxia, that can lead to organ hypertrophy [33]. In the first days of life, iron deficiency is observed in young animals of almost all animal species, but in calves, foals and lambs this condition is temporary and does not turn into a chronic form. Piglets are more susceptible to this pathology, the most intense clinical symptoms appear one and a half months after birth. The degree of pathological changes occurring in the body will largely depend on the etiological factor, local organotropic effects, the degree of toxic effects on the body, as well as the body's resistance [34].

The manifestation of this disease is characterized by a lag in growth, a decrease in the natural resistance of young farm animals, in particular, piglets are sensitive to iron deficiency. The clinical symptom of iron deficiency anemia is the pale coloration of the visible mucous membranes which subsequently turn yellow. The animals are lethargic, stunted in their growth, the bristles stick up, the skin looks wrinkled. Appetite is either absent or perverted. Digestive disorders are also noted, constipation alternates with diarrhea. Blood tests show a decrease in hemoglobin levels from 10 to 3.5 g/%. The content of erythrocytes remains normal, but their qualitative composition changes, erythroblasts are detected in blood [32, 35].

To make a diagnosis, the amount of iron and hemoglobin in blood and parenchymal organs is determined. A specific marker of anemia is the color index of blood. At the same time, the feeding diet is analysed. Anemia occurring in the setting of infectious and invasive diseases is excluded by means of differential diagnosis [35].

Pharmacotherapeutic intervention in iron deficiency anemia should be aimed at normalizing all links of the pathological process and eliminating all symptoms of the disease [36]. Iron dextran drug products containing carbohydrate-binding colloid iron (III) are of great scientific importance in the treatment and prevention of iron deficiency anemia. These medicinal products are produced in almost all countries of the world. The main difference between all manufactured preparations is that the carbohydrates included therein form different chemical compounds, and the iron content ranges from 50 to 200 mg/mL [37, 38]. The advantage of iron dextrans over drug products containing iron salts is that even one 3 mL dose injected to the animal has a therapeutic effect and prevents the development of iron deficiency anemia. With a significant increase of the dose, the amount of iron in blood may increase leading to the development of hemosiderosis [39, 40].

The opinions of scientists regarding dosages for parenteral administration vary. There are developments on combined antianemic drugs. They include copper chloride, sodium and cobalt salts, and vitamins B which are of great importance. The drugs may also contain raw materials of plant and animal origin, amino acids and biologically active compounds. The compatibility of mineral and vitamin supplements in premixes and compound feeds ensures their bioavailability [41, 42].

CONCLUSION

To date, a fairly large number of study results have been obtained on the development of chelated metal compounds and the rationale of their use for the treatment and prevention of various pathologies of livestock and humans. Currently, amino acids such as glycine, hydroxyproline and others are mainly used for the synthesis of chelated compounds as organic carriers for alkaline and alkaline earth metals (lithium, calcium, magnesium). The effective action of chelated metal compounds is based on metabolic and receptor reactions. The action of chelates depends on a number of factors. Firstly, it depends on which metal ion is included in the composition of the compound, and secondly, on the organic carrier used. Different variants of chelate compositions are used both for the prevention and therapy of pathologies associated with iron, cobalt and other macro- and microelement deficiencies in livestock and poultry: for instance, in case of iron deficiency anemia, lack of cobalt. One of the advantages of chelated compounds is their high bioavailability due to the presence of organic carrier. This predetermined their use as preventive and therapeutic drugs that significantly surpass their non-organic counterparts. In addition, the advantage of chelates is the absence of an accumulation effect in animal tissues and organs, which makes it possible to obtain safe livestock products of high quality. Thus, the development and reasonable administration of new chelated compounds is promising as they can be used to solve a wide range of problems in veterinary medicine.

REFERENCES

1. Arsanukaev D. L. Metabolism of various forms of trace elements in young cattle and sheep: Author's abstract of thesis for degree of Dr. Sci. (Biology). Borovsk; 2006. 50 p. (in Russ.)

2. Deltsov A. A. Farmakoprofilaktika zhelezodefitsitnoi anemii porosyat ferranimalom-75 s kobal'tom = Pharmacoprophylaxis of iron deficiency anemia of pigs by ferranimal-75 with cobalt. *Veterinary Medicine*. 2008; (2–3): 25–27. https://elibrary.ru/mlzyvl (in Russ.)

3. Podobed L. I., Maltsev A. B., Maltseva N. A., Poluboyarov D. V. Methodical guidelines for the use of organosilicon products (silicon chelates) for feeding poultry. Novosibirsk: OOO "Tsentr vnedreniya tekhnologii"; 2012. 68 p. (in Russ.)

4. Ashmead H. D. The absorption and metabolism of iron amino acid chelate. *Archivos Latinoamericanos de Nutrición*. 2001; 51 (Suppl. 1): 13–21. https://pubmed.ncbi.nlm.nih.gov/11688075

5. Strunin B. P., Antipov V. A., Sattarova L. F., Pakhomova T. B., Sapozhnikov Yu. E., Gurevich P. A. Razrabotka metodov analiticheskogo kontrolya preparata «Polizon» = Development of analytical testing methods for "Polizon" drug. *Bulletin of the Kazan Technological University*. 2010; (7): 53–56. https://elibrary.ru/mutqll (in Russ.)

6. Tuaeva E. V. Scientific and practical rationale for the use of chelated forms of trace elements contained in natural feed resources when raising replacement young cattle in the Amur region: Author's abstract of thesis for degree of Dr. Sci. (Agriculture). Dubrovitsy; 2019. 43 p. (in Russ.)

7. Ivanov I. S., Troshin E. I., Krysenko Yu. G., Shishkin A. V., Kulikov A. N. Razrabotka metodik sinteza glitsinatov nekotorykh mikroelementov = Development of methods for synthesizing some trace element glycinates. *Nauchno obosnovannye tekhnologii intensifikatsii sel'skokhozyaistvennogo proizvodstva: materialy Mezhdunarodnoi nauchno-prakticheskoi konferentsii (Izhevsk, 14–17 fevralya 2017 g.). T. 2 = Science-based technologies for intensifying agricultural production: proceedings of the International Scientific and Practical Conference (Izhevsk, February 14–17, 2017). Vol. 2.* Izhevsk: Izhevsk State Agricultural Academy; 2017; 22–24. https://elibrary.ru/zfqdzz (in Russ.)

8. Loginov G. P. Effect of metal chelates with amino acids and protein hydrolysates on productive functions and metabolic processes in animals: Author's abstract of thesis for degree of Dr. Sci. (Biology). Kazan; 2005. 359 p. (in Russ.)

9. Ośko J., Pierlejewska W., Grembecka M. Comparison of the potential relative bioaccessibility of zinc supplements – *in vitro* studies. *Nutrients*. 2023; 15 (12):2813. https://doi.org/10.3390/nu15122813

10. Golovkina E. M., Brykalov A. V. Sintez khelatnykh soedinenii biogennykh elementov s aminokislotami = Synthesis of chelate compounds of biogenic nutrients with amino acids. *Sbornik nauchnykh trudov Stavropol'skogo nauchno-issledovatel'skogo instituta zhivotnovodstva i kormoproizvodstva*. 2009; (1): 75–77. https://elibrary.ru/oogrrb (in Russ.)

11. Kadyrova R. G., Kabirov G. F., Mullakhmetov R. R. Glycylglycine complexable ability study with 3*d*-biogenic metals. *Scientific notes Kazan Bauman State Academy of Veterinary Medicine.* 2014; 218 (2): 102–110. https://elibrary.ru/sexzlx (in Russ.)

12. Stekolnikov A. A., Karpenko L. Yu. Ekologicheskie aspekty primeneniya mineral'no-kormovoi dobavki Khelavit dlya povysheniya kachestva moloka korov = Environmental aspects of Helavit mineral feed additive application for increasing cow milk quality. *Effectivnoe zhivotnovodstvo.* 2019; (2): 22–23. https://elibrary.ru/zamvrb (in Russ.)

13. Budnikova E. N., Ivanova E. A., Kofanova A. V., Chepelev N. A. The use of chelated trace elements in the diets of farm animal. *Aktual'nye voprosy innovatsionnogo razvitiya agropromyshlennogo kompleksa: materialy Mezhdunarodnoi nauchno-prakticheskoi konferentsii (Kursk, 28–29 yanvarya 2016 g.) = Current issues of innovative development of the agro-industrial complex: proceedings of the International Scientific and Practical Conference (Kursk, January 28–29, 2016).* Kursk: Kursk State Agricultural I. I. Ivanov Academy; 2016; 23–26. https://elibrary.ru/wgobnx (in Russ.)

14. Ivanov I. S., Rudenok V. A., Troshin E. I., Kulikov A. N. Influence of the organic form Cu, Co, Zn and Mn on the animal organism. *Legal regulation in veterinary medicine*. 2016; (4): 246–249. https://elibrary.ru/xedibp (in Russ.)

15. Schiavi A., Runci A., Maiorino T., Naso F. D., Barenys M., Fritsche E., et al. Cobalt chloride has beneficial effects across species through a hormetic mechanism. *Frontiers in Cell and Developmental Biology*. 2022; 10:986835. https://doi.org/10.3389/fcell.2022.986835

16. Zuev O. E. Use the helat for increase the assimilation of mineral substances at pigs. *Zootechniya*. 2009; (3): 17–18. https://elibrary.ru/jxdgxz (in Russ.)

17. Nadeev V. P. Effect of chelated compounds of trace elements on productivity and metabolic processes in pigs: Author's abstract of thesis for degree of Dr. Sci. (Biology). Borovsk; 2014. 32 p. (in Russ.)

18. Mizhevikina A. S., Lykasova I. A., Poluboyarov D. V., Odeyanko V. B. Broiler productivity with use of chelated microelements complex, wholesome microoganizms and chondroprotective agents in diets. *Poultry & Chicken Products.* 2017; (1): 40–42. https://elibrary.ru/yiyacv (in Russ.)

19. Ma M., Li L., Zuo G., Xiao J., Chen J., He X., Song Z. Effect of zinc amino acid complexes on growth performance, tissue zinc concentration, and muscle development of broilers. *Biological Trace Element Research*. 2024; 202 (1): 291–306. https://doi.org/10.1007/s12011-023-03661-9

20. Frolov A., Filippova O., Furletov S., Lee V. Organic forms of micronutrient premix for calves. *Dairy and Beef Cattle Farming.* 2010; (3): 18–20. https://elibrary.ru/micjpx (in Russ.)

21. Name J. J., Vasconcelos A. R., Valzachi Rocha Maluf M. C. Iron bisglycinate chelate and polymaltose iron for the treatment of iron deficiency anemia: A pilot randomized trial. *Current Pediatric Reviews*. 2018; 14 (4): 261–268. https://doi.org/10.2174/1573396314666181002170040

22. Ryzhov A. A. Mikroelementnyi premiks Khelavit®: rezul'taty, perspektivy = Trace element premix Helavit®. Results. Prospects. *Farm Animals*. 2015; (1): 39–40. https://elibrary.ru/thaizz (in Russ.)

23. Kavalionak Yu. K., Katovich I. V., Shmurakova E. I. Vliyanie khelatnykh form kobal'ta i medi na pokazateli perekisnogo okisleniya lipidov pri gipomikroelementozakh krupnogo rogatogo skota na otkorme = Effect of cobalt and copper chelates on lipid peroxidation in hypomicroelementoses of cattle on fattening. *Veterinaria i kormlenie*. 2009; (6): 58–59. https://elibrary.ru/urxwcp (in Russ.)

24. Golokhvast K. S. Interaction between organisms and minerals. Vladivostok: FESTU Publishing House; 2010. 115 p. (in Russ.)

25. Ismagilova E. R., Baimatov V. N. Relationship of trace elements in the chain biogeocenotic "Soil-feeding" and the forecast of feed trace element composition of the soil. *Vestnik of Omsk SAU*. 2012; 2012; (2): 23–27. https://elibrary.ru/synqsf (in Russ.)

26. Makarov Yu. A., Gorkovenko N. E. Ecology and animal health: monograph. Blagoveshchensk: Far Eastern SAU; 2006. 204 p. (in Russ.)

27. Toporova L. V., Serebrennikova S. N., Galashov V. V., Lutsyuk V. E., Toporova I. V., Andreev V. V. Effektivnost' organomineral'nykh dobavok v kormlenii zhivotnykh =

Effectiveness of organic mineral supplements in animal feeding. *Head of Animal Breeding*. 2012; (1): 16–26. https://elibrary.ru/pdhunl (in Russ.)

28. Toporova L. V., Toporova I. V., Andreev V. V. Metalloproteinovyi kompleks dlya povysheniya produktivnosti i vosproizvoditel'noi funktsii korov = Metalloprotein complex to increase productivity and reproductive function of cows. *Innovatsionnye puti razvitiya zhivotnovodstva XXI veka: materialy nauchno-prakticheskoi (zaochnoi) konferentsii s mezhdunarodnym uchastiem (Omsk, 11 dekabrya 2015 g.) = Innovative ways of development animal food production XXI century: proceedings of the Scientific and Practical Conference with International Participation (Omsk, December* 11, 2015). Omsk: IP Maksheevoi E. A.; 2015; 97–101. https://elibrary.ru/vpskbj (in Russ.)

29. Matsinovich A. A. Mikroelementozy krupnogo rogatogo skota v usloviyakh Respubliki Belarus': rasprostranenie i diagnostika = Microelementoses of cattle in the Republic of Belarus: distribution and diagnosis. *Transactions of the Educational Establishment "Vitebsk the Order of "the Badge of Honor" State Academy of Veterinary Medicine"*. 2007; 43 (1): 149–152. https://elibrary.ru/uhfwap (in Russ.)

30. Abramov S. S., Zasinets S. V. Latent iron-deficient anemia in calves. *Veterinariya*. 2004; (6): 43–44. https://elibrary.ru/odepxz (in Russ.)

31. Gorodetskii V. V., Godulyan O. V. Iron deficiency conditions and iron deficiency anemia: diagnosis and treatment (guidelines). Moscow: Medpraktika-M; 2006. 28 p. (in Russ.)

32. Andreyeva A. V., Nikolayeva O. N. The dynamics of swine hematological parameters at prevention of alimentary anemia. *Veterinarian*. 2017; (1): 38–41. https://elibrary.ru/xvsspj (in Russ.)

33. Sekhin A. A., Surmach V. N. Primenenie khelatnykh soedinenii mikroelementov dlya molodnyaka svinei = Use of chelated trace element compounds in young pigs. *Zootechnical Science of Belarus.* 2004; 39: 293–296. https://zootech.belal.by/jour/article/view/1381/1274 (in Russ.)

34. Evlash V. V., Pogozhikh N. I., Akmen V. A. Scientific aspects of technologies for anti-anemic products with stabilized heme iron: monograph. Kharkiv: Kharkiv State University of Food Technology and Trade; 2016. 215 p. (in Russ.)

35. Callender S. T. Treatment of iron deficiency. *Clinics in Haematology*. 1982; 11 (2): 327–338. https://pubmed.ncbi.nlm.nih.gov/7042154

36. Stuklov N. I., Semenova E. N. Iron deficiency anemia. Modern diagnostic and treatment strategy. Criteria for therapeutic efficacy. *Clinical Medicine (Russian Journal)*. 2013; 91 (12): 61–67. https://elibrary.ru/sexmdj (in Russ.)

37. Karabanov A. M., Voyt G. A., Pinchuk V. F., Levashkevitch A. L. About new iron dextran preparations for newborn pigs. *Mogilev State A. Kuleshov University Bulletin*. 2004; (2–3): 122–127. https://elibrary.ru/tliczj (in Russ.)

38. Gurevichev P. A. Nekotorye novye zhelezodekstranovye preparaty v veterinarii = Some new iron dextran preparations in veterinary medicine. *Aspects of veterinary medicine and veterinary biology: a collection of scientific works of early-career scientists. Vol. 3*. Moscow: Moscow SAVMB named after K. I. Skryabin; 2006; 31–35. https://elibrary.ru/uqrlup (in Russ.)

39. Kontoghiorghes G. J. Deferiprone and iron-maltol: Forty years since their discovery and insights into their drug design, development, clinical use and future prospects. *International Journal of Molecular Sciences.* 2023; 24 (5):4970. https://doi.org/10.3390/ijms24054970

40. Bai S., Cao S., Ma X., Li X., Liao X., Zhang L., et al. Organic iron absorption and expression of related transporters in the small intestine of broilers. *Poultry Science*. 2021; 100 (8):101182. https://doi.org/10.1016/j.psj.2021.101182

41. Gurkina L. V., Naumova I. K., Lebedeva M. B. Mutual action of biogenic elements and microelements of heavy metals in animals. *Agrarian Journal of Upper Volga Region*. 2016; (1): 32–37. https://elibrary.ru/tpzsij (in Russ.)

42. Kontoghiorghes G. J., Kolnagou A., Demetriou T., Neocleous M., Kontoghiorghe C. N. New era in the treatment of iron deficiency anaemia using trimaltol iron and other lipophilic iron chelator complexes: Historical perspectives of discovery and future applications. *International Journal of Molecular Sciences*. 2021; 22 (11):5546. https://doi.org/10.3390/ijms22115546

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Monitoring of helminth fauna of transhumant cattle in the North Caucasus

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ABSTRACT

The helminth fauna of cattle in the Russian Federation is represented, on average, by 80 parasite species, including 10 trematode species, 13 cestode species and 57 nematode species. In Kabardino-Balkaria and Dagestan, up to 65–100% of cattle population are *Strongylata* and *Anoplocephalata* infected, up to 87% are *Dicrocoelium* infected, up to 40% are *Fasciola* infected, and up to 23% are *Echinococcus* infected. Continuous helminth fauna monitoring tests and studies of the epizootic patterns of helminth infections in transhumant livestock in the North Caucasus are an urgent task. Parasitological examinations were carried out on the farms of the Kabardino-Balkarian Republic that practise vertical transhumance at different altitudes in 2018–2022. The animals of various ages were observed on a year-round basis. The examinations covered transhumant cattle in the subalpine and alpine-subnival subzones of the mountain zone of the Republic, 100 animals per subzone. In the subalpine subzone of the mountain zone of Kabardino-Balkaria, 25 helminth species were detected in the transhumant cattle in the summer and autumn periods, and 7–11 helminth species were detected in the winter and spring periods. The following species prevailed: *Dicrocoelium lanceatum*, *Paramphistomum cervi*, *Trichostrongylus axei*, *Trichostrongylus colubriformis*, *Oesophagostomum radiatum*, *Bunostomum trigonocephalum*, *Nematodirus helvetianus*, *Nematodirus spathiger*, *Ostertagia ostertagi*, *Teladorsagia circumcincta*, *Ostertagiella occidentalis*, *Haemonchus placei*. In the alpine-subnival subzone of the mountain zone of the Republic, 16 helminth species were detected in the transhumant cattle in the summer and autumn periods, and 3–7 helminth species were detected in the winter and spring periods. The following species were found to prevail in this subzone: *Trichostrongylus colubriformis*, *Bunostomum trigonocephalum*, *Dicrocoelium lanceatum*, *Nematodirus helvetianus*, *Ostertagia ostertagi*, *Teladorsagia circumcincta*, *Haemonchus placei*, *Oesophagostomum radiatum*.

Keywords: cattle, transhumance, helminths, fauna, species, extensity, intensity, invasion, North Caucasus, Kabardino-Balkaria

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Мониторинг гельминтофауны крупного рогатого скота при отгонно-пастбищной системе ведения животноводства в условиях Северного Кавказа

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РЕЗЮМЕ

В Российской Федерации гельминтофауна крупного рогатого скота представлена в среднем 80 видами паразитов, среди них 10 видов трематод, 13 – цестод и 57 – нематод. В Кабардино-Балкарии и Дагестане до 65–100% поголовья крупного рогатого скота заражено стронгилятами и аноплоцефалятами, до 87% – дикроцелиями, до 40% – фасциолами, до 23% – эхинококками. Проведение постоянных мониторинговых исследований гельминтофауны и изучение формирования эпизоотического процесса при отгонной системе ведения животноводства на Северном Кавказе является актуальной задачей. Паразитологические исследования выполняли в 2018–2022 гг. в хозяйствах Кабардино-Балкарской Республики, практикующих отгонно-пастбищное

содержание на различных высотах. Наблюдения за животными разного возраста вели круглогодично. Обследованию было подвергнуто по 100 гол. крупного рогатого скота, находящегося на отгонно-пастбищном содержании в субальпийской и альпийско-субнивальной подзонах горной зоны республики. В Кабардино-Балкарии у крупного рогатого скота отгонно-пастбищного содержания в субальпийской подзоне горной зоны в летний и осенний периоды обнаружено 25 видов гельминтов, а в зимний и весенний – 7–11 видов. Доминирующими видами были: *Dicrocoelium lanceatum*, *Paramphistomum cervi*, *Trichostrongylus axei*, *Trichostrongylus colubriformis*, *Oesophagostomum radiatum*, *Bunostomum trigonocephalum*, *Nematodirus helvetianus*, *Nematodirus spathiger*, *Ostertagia ostertagi*, *Teladorsagia circumcincta*, *Ostertagiella occidentalis*, *Haemonchus placei*. В альпийско-субнивальной подзоне горной зоны республики у крупного рогатого скота при отгонно-пастбищном содержании в летний и осенний периоды выявляли 16 видов гельминтов, а зимний и весенний – 3–7 видов. Установлено, что в данной подзоне по распространенности доминируют виды *Trichostrongylus colubriformis*, *Bunostomum trigonocephalum*, *Dicrocoelium lanceatum*, *Nematodirus helvetianus*, *Ostertagia ostertagi*, *Teladorsagia circumcincta*, *Haemonchus placei*, *Oesophagostomum radiatum*.

Ключевые слова: крупный рогатый скот, отгонно-пастбищное содержание, гельминты, фауна, вид, экстенсивность, интенсивность, инвазированность, Северный Кавказ, Кабардино-Балкария

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INTRODUCTION

The North Caucasus is one of the main livestock farming regions of Russia, which is characterized by well-developed sheep farming, dairy and beef cattle farming. Global changes that have taken place in these territories, large numbers of ruminants concentrated within small areas and transhumance create conditions for invasion occurrence [1, 2, 3, 4].

Data on the biology, ecology of agents and the epizootiology of helminthoses in cattle are required to address a variety of theoretical and practical tasks: the detection of infection risk factors in the environment; the assessment of effectiveness of diagnostic, therapeutic, preventive, anti-epizootic measures; the improvement of epizootiological surveillance and strategies aimed at the prevention of parasitic animal diseases [5, 6, 7, 8].

It is known that the development of the epizootic process of many diseases, including helminthoses, is influenced by physical and climatic, as well as economic and ecological factors existing in the regions [9, 10, 11, 12, 13, 14].

Transhumance has a significant impact on animal helminthosis situation [15, 16].

Cattle in the Russian Federation is infected, on average, with 80 helminth species, including 10 trematode species, 13 cestode species and 57 nematode species [17, 18, 19, 20].

In Kabardino-Balkaria and Dagestan, up to 65–100% of cattle population are *Strongylata* and *Anoplocephalata* infected, up to 87% are *Dicrocoelium* infected, up to 40% are *Fasciola* infected, and up to 23% are *Echinococcus* infected [21].

In the Chechen Republic, cattle parasites are represented by 57 species (6 trematode species, 7 cestode species, 44 nematode species) [6, 22, 23, 24].

The specific features of parasite spread depending on the altitudinal zone (subzone), infection occurrence indicators, the epizootic patterns of helminth infections in cattle in the region are under-studied. Therefore, the effectiveness of helminthosis control in animals will depend on making use of knowledge of the agent species composition, epizootiology, seasonal and age-related dynamics, while taking into account the altitudinal zones (subzones) of the region [25].

The aim of the study is the monitoring of helminth fauna and the investigation of the epizootic patterns of helminth infections in transhumant cattle in the North Caucasus.

MATERIALS AND METHODS

Monitoring tests were carried out on the farms of the Republic of Kabardino-Balkaria that practise vertical transhumance at different altitudes in 2018–2022. Laboratory tests were conducted in accordance with GOST R 54627-2011 "Agricultural ruminant animals. Methods of laboratory helminthology diagnostics"[1]. Animal necropsies were performed using K. I. Skryabin's method of complete helminthological necropsy (1928) at the Laboratory for the Study of Invasive Diseases of Farm Animals and Birds of the Caspian Zonal Research Veterinary Institute and at the Chair of Veterinary Medicine of the Kabardino-Balkarian State Agricultural University named after V. M. Kokov.

The animals of various ages were observed on a year-round basis. The examinations covered transhumant cattle in the subalpine and alpine-subnival subzones of the mountain zone, 100 animals per subzone.

[1] https://docs.cntd.ru/document/1200094391

The differentiation of invasion in the animals, including species confirmation, was carried out using the Identification guide to cattle helminths [26].

The data obtained were statistically processed with the Biometrics software (Centre for Biometric Technologies, Russia).

RESULTS AND DISCUSSION

The tests performed revealed that the helminth fauna of transhumant cattle in the subalpine subzone of the mountain zone of the Republic of Kabardino-Balkaria is represented by 25 species in the summer and autumn periods and by 7–11 species in the winter and spring periods (Table 1).

The trematode and cestode species composition in the cattle in this altitudinal subzone includes: *Dicrocoelium lanceatum*, *Fasciola hepatica*, *Paramphistomum cervi*, *Echinococcus granulosus*, *Taenia hydatigena* (larvae), *Moniezia expansa*, *Moniezia benedeni*. Depending on the parasite species, invasion extensity in the animals ranged from 13.0 to 57.0%, and invasion intensity ranged from (2.4 ± 0.3) to (142.6 ± 11.4) parasites/animal.

The tests revealed the presence of the following intestinal and lung nematodes (without intermediate hosts) at different life cycle stages in the cattle: *Ostertagia ostertagi*, *Teladorsagia circumcincta*, *Ostertagiella occidentalis*, *Trichostrongylus axei*, *Trichostrongylus colubriformis*, *Trichostrongylus vitrinus*, *Oesophagostomum radiatum*, *Oesophagostomum venulosum*, *Protosrongylus hobmaeri*, *Bunostomum trigonocephalum*, *Bunostomum phlebotomum*, *Haemonchus placei*, *Cooperia punctata*, *Nematodirus filicollis*, *Nematodirus helvetianus*, *Nematodirus oiratianus*, *Nematodirus spathiger*, *Dictyocaulus viviparous*. Depending on the parasite species, significant differences in invasion extensity, which ranged from 12.0 to 88.0%, and invasion intensity, which ranged from (15.8 ± 2.3) to (254.0 ± 24.3) parasites/animal, were detected.

The helminth fauna of transhumant cattle in the alpine-subnival subzone of the mountain zone is represented

Table 1
Helminth fauna of transhumant cattle in the subalpine subzone of the mountain zone (based on the findings from helminthological dissections of organs and tissues)

No.	Species	Cattle ($n = 100$)		
		Number of infected animals	IE, %	Mean II, parasites/animal
1	*D. lanceatum* (Stiles et Hassall, 1896)	57	57.0	142.6 ± 11.4
2	*F. hepatica* (Linnaeus, 1758)	18	18.0	16.8 ± 2.1
3	*P. cervi* (Zeder, 1790)	43	43.0	104.0 ± 9.7
4	*E. granulosus* (Batsch, 1786)	22	22.0	20.2 ± 3.0
5	*T. hydatigena*, larvae (Pallas, 1766)	13	13.0	9.4 ± 1.0
6	*M. expansa* (Rudolphi, 1810)	20	20.0	3.3 ± 0.5
7	*M. benedeni* (Moniez, 1879)	19	19.0	2.4 ± 0.3
8	*T. axei* (Cobbold, 1879)	88	88.0	243.6 ± 22.5
9	*T. colubriformis* (Giles, 1892)	71	71.0	187.8 ± 19.3
10	*T. vitrinus* (Looss, 1905)	32	32.0	104.7 ± 11.8
11	*Oes. radiatum* (Rudolphi, 1803)	48	48.0	109.2 ± 12.6
12	*Oes. venulosum* (Rudolphi, 1809)	35	35.0	73.4 ± 8.3
13	*B. trigonocephalum* (Rudolphi, 1808)	62	62.0	81.2 ± 7.5
14	*B. phlebotomum* (Railliet, 1900)	36	36.0	60.4 ± 6.7
15	*N. helvetianus* (May, 1920)	69	69.0	159.3 ± 17.5
16	*N. spathiger* (Railliet, 1896)	55	55.0	133.4 ± 14.2
17	*N. oiratianus* (Rajevskaja, 1929)	38	38.0	97.3 ± 10.5
18	*N. filicollis* (Rudolphi, 1802)	25	25.0	66.5 ± 8.8
19	*O. ostertagi* (Stiles, 1892)	80	80.0	254.0 ± 24.3
20	*T. circumcincta* (Stadelman, 1894)	69	69.0	198.6 ± 20.9
21	*O. occidentalis* (Ransom, 1907)	53	53.0	111.0 ± 13.6
22	*P. hobmaeri* (Schulz, Orlow & Kutass, 1933)	12	12.0	21.7 ± 3.1
23	*H. placei* (Place, 1893)	59	59.0	92.0 ± 8.3
24	*C. punctata* (Linstow, 1907)	23	23.0	41.4 ± 3.9
25	*D. viviparus* (Bloch, 1782)	18	18.0	15.8 ± 2.3

IE – invasion extensity, II – invasion intensity.

Table 2
Helminth fauna of transhumant cattle in the alpine-subnival subzone of the mountain zone (based on the findings from helminthological dissections of organs and tissues)

No.	Species	Cattle ($n = 100$)		
		Number of infected animals	IE, %	II range, parasites/animal
1	*D. lanceatum* (Stiles et Hassall, 1896)	49	49.0	17–113
2	*E. granulosus* (Batsch, 1786, Rudolphi, 1801)	14	14.0	3–17
3	*T. hydatigena*, larvae (Pallas, 1766)	6	6.0	2–10
4	*M. benedeni* (Moniez, 1879)	11	11.0	2–6
5	*T. colubriformis* (Giles, 1892)	57	57.0	30–144
6	*Oes. radiatum* (Rudolphi, 1803)	36	36.0	21–115
7	*Oes. venulosum* (Rudolphi, 1809)	27	27.0	24–90
8	*B. trigonocephalum* (Rudolphi, 1808)	53	53.0	21–119
9	*B. phlebotomum* (Railliet, 1900)	32	32.0	30–80
10	*N. helvetianus* (May, 1920)	48	48.0	35–141
11	*N. spathiger* (Railliet, 1896)	34	34.0	14–66
12	*O. ostertagi* (Stiles, 1892)	46	46.0	33–150
13	*T. circumcincta* (Stadelman, 1894)	45	45.0	20–190
14	*H. placei* (Place, 1893)	40	40.0	19–121
15	*D. viviparus* (Bloch, 1782)	11	11.0	4–16
16	*P. hobmaeri* (Schulz, Orlow & Kutass, 1933)	12	12.0	3–11

IE – invasion extensity, II – invasion intensity.

by 16 species (Table 2) in the summer and autumn periods and by 3–7 species in the winter and spring periods.

In the alpine-subnival subzone of the mountain zone, invasion extensity and invasion intensity in the cattle ranged from 6.0 to 57.0% and from 2 to 190 parasites/animal, respectively. The following species were found to prevail in this subzone: *Trichostrongylus colubriformis*, *Bunostomum trigonocephalum*, *Dicrocoelium lanceatum*, *Nematodirus helvetianus*, *Ostertagia ostertagi*, *Teladorsagia circumcincta*, *Haemonchus placei*, *Oesophagostomum radiatum*.

CONCLUSION

Twenty-five helminth species were detected in the cattle in the subalpine subzone of the mountain zone of Kabardino-Balkaria. The helminth species composition was characterized by seasonal variations. Depending on the species, trematode and cestode invasion extensity in the animals ranged from 13.0 to 57.0%, and invasion intensity ranged from (2.4 ± 0.3) to (142.6 ± 11.4) parasites/animal. Significant differences in the extensity of invasion with intestinal and lung nematodes (18 species) with direct life cycle (without intermediate hosts), which ranged from 12.0 to 88.0%, and the intensity of invasion, which ranged from (15.8 ± 2.3) to (254.0 ± 24.3) parasites/animal, were detected in the transhumant cattle in this altitudinal subzone.

The helminth fauna of transhumant cattle in the alpine-subnival subzone of the mountain zone is represented by 16 species with a similarity coefficient of 1. Invasion extensity and invasion intensity in the cattle ranged from 6.0 to 57.0% and from 2 to 190 parasites/animal, respectively. The following species prevailed: *Trichostrongylus colubriformis*, *Bunostomum trigonocephalum*, *Dicrocoelium lanceatum*, *Nematodirus helvetianus*, *Ostertagia ostertagi*, *Teladorsagia circumcincta*, *Haemonchus placei*, *Oesophagostomum radiatum*.

Thus, the current cattle helminthosis situation in the region requires regular monitoring, as well as the improvement of measures aimed at parasitic disease control.

REFERENCES

1. General parasitology and helminthology: a study guide. Compiled by A. N. Tazayan. Persianovskii: Don SAU; 2019. 159 p. (in Russ.)

2. Drobin Yu. D., Shevchenko L. V., Krivonos R. A., Kaloshkina I. M., Chernykh O. Yu., Shevchenko A. A. Epizootic situation on invasive diseases in the North Caucasus Region. *Veterinaria Kubani.* 2019; 2: 3–5. https://elibrary.ru/sldffs (in Russ.)

3. Chernykh O. Yu., Drobin U. A., Shevchenko L. V., Shevchenko A. A. Epizootic situation on invasive diseases in the North Caucasus Region. *Veterinarian.* 2019; (3): 9–11. https://doi.org/10.33632/1998-698X.2019-3-9-12 (in Russ.)

4. Shichalieva M. A., Bittirova M. I., Mantaeva S. Sh., Yusupova Z. H., Chilaev S. Sh. Number and associations of parasites in cattle and goats in the Northern Caucasus Region. *Russian Journal of Parasitology.* 2014; (4): 16–21. https://vniigis.elpub.ru/jour/article/view/277 (in Russ.)

5. Gorochov V. V., Skira V. N., Klenova I. F., Taichinov U. G., Volichev A. N., Peshkov R. A., et al. Epizootic situation on the main helminthoses in the RF. *Theory and Practice of Parasitic Disease Control.* 2009; 10: 137–141. https://vniigis.ru/1_dlya_failov/TPB/Vniigis_2009_konferenciya.pdf (in Russ.)

6. Demilova D. I., Gadaev Kh. Kh. Modern epizootic situation on *Trichostrongylidae* of ruminants in the Chechen Republic. *Theory and Practice of Parasitic Disease Control*. 2022; 23: 173–177. https://doi.org/10.31016/978-5-6046256-9-9.2022.23.173-177 (in Russ.)

7. Uspensky A. V., Malahova E. I., Yershova T. A. Current situation in relation to parasites and measures of struggle against them in Russia and CIS countries (based on materials of coordinating reports). *Russian Journal of Parasitology*. 2014; (2): 43–50. https://vniigis.elpub.ru/jour/article/view/212 (in Russ.)

8. Atayev A. M., Zubairova M. M., Karsakov N. T., Dzhambulatov Z. M., Beliyev S.-M. M., Ashurbekova T. N., Akhmedov M. A. Ecological and faunistic, epizootological characteristics of the agents of parasitic diseases in domestic ruminants of Dagestan. *Problems of Development of the Agro-Industrial Complex of the Region*. 2017; 3: 53–59. https://elibrary.ru/zmqqrx (in Russ.)

9. Gorokhov V. V., Klenova I. F., Puzanova Y. V. State of art epidemic situation and prognosis by major helminthoses of animals within the Russian Federation for 2018 (spring and the beginning of the grazing season). *Russian Journal of Parasitology*. 2018; 12 (2): 23–26. https://doi.org/10.31016/1998-8435-2018-12-2-23-26 (in Russ.)

10. Kriazhev A. L. Epizootic situation on helminthosis of cattle of public and private sectors in the Vologda Region. *Russian Journal of Parasitology*. 2019; 13 (3): 57–62. https://doi.org/10.31016/1998-8435-2019-13-3-57-62 (in Russ.)

11. Atayev A. M., Zubairova M. M., Karsakov N. T., Dzhambulatov Z. M. Parasitic Diseases of Animals. Saint Petersburg: Lan'; 2022. 304 p. (in Russ.)

12. Atabieva J. A., Bichieva M. M., Kolodij I. V., Bittirov A. M., Shikhaliyeva M. A., Sarbasheva M. M., Zhekamuhova M. Z. Prediction epizootic and epidemic situation zoonotic invasion in southern Russia. *Russian Journal of Veterinary Pathology*. 2012; (1): 119–122. https://elibrary.ru/oyrsbz (in Russ.)

13. Bayramgulova G. R., Yunusbaev U. B., Rafikova N. R. The soil as a substratum for development of *Ascaris lumbricoides* L., 1758. *Vestnik of the Orenburg State University*. 2008; (12): 133–134. https://elibrary.ru/vzpqkr (in Russ.)

14. Klimova E. S. Cattle endoparasitoses depending on the farms category. *Russian Journal of Veterinary Pathology*. 2022; (2): 14–18. https://doi.org/10.25690/VETPAT.2022.74.94.003 (in Russ.)

15. Gazimagomedov M. G. Contamination of pastures with different types by infective causative agents in the mountain zone of Dagestan. *Theory and Practice of Parasitic Disease Control*. 2009; 10: 107–109. https://vniigis.ru/1_dlya_failov/TPB/Vniigis_2009_konferenciya.pdf (in Russ.)

16. Almaksudov U. P., Ataev A. M., Zubairova M. M., Karsakov N. T. Infection of sheep and cattle by gastrointestinal strongylates on different types of pastures of the plain zone of Dagestan. *Russian Journal of Parasitology*. 2010; (1): 6–9. https://elibrary.ru/mvpuvf (in Russ.)

17. Gorokhov V. V., Samoylovskaya N. A., Uspensky A. V., Klenova I. F., Peshkov R. A., Puzanova E. V., Moskvin A. S. Current epizootic situation and forecast for 2015 about main helminthosis in animals on the territory of Russia. *Russian Journal of Parasitology*. 2015; (1): 41–45. https://vniigis.elpub.ru/jour/article/view/132 (in Russ.)

18. Domatskiy V. N. Distribution, therapy and prevention of sheep helminthosis in the Russian Federation. *Veterinaria Kubani*. 2021; 2: 21–25. https://elibrary.ru/bocfbq (in Russ.)

19. Vasilevich F. I., Tsepilova I. I., Gorchakova V. I. The fauna of intestinal parasites of small cattle in the conditions of private farms. *Theory and Practice of Parasitic Disease Control*. 2020; 21: 81–86. https://doi.org/10.31016/978-5-9902341-5-4.2020.21.81-86 (in Russ.)

20. Loginova O. A. Helminthiases of cattle in the North West Region of Russia. *Ekologo-biologicheskie problemy ispol'zovaniya prirodnykh resursov v sel'skom khozyaistve: sbornik materialov Mezhdunarodnoi nauchno-prakticheskoi konferentsii molodykh uchenykh i spetsialistov (Ekaterinburg, 7–9 iyunya 2017 g.) = Ecological and biological challenges of using natural resources in agriculture: proceedings of the International Research-to-Practice Conference of Early-Career Researchers and Specialists (Ekaterinburg, 7–9 June 2017)*. Ekaterinburg: Ural'skoe izdatel'stvo; 2017; 255–257. https://elibrary.ru/zpvfet (in Russ.)

21. Arkelova M. R., Gogushev Z. T., Kaloshkin I. V., Bittirov I. A., Bittirov A. M. Assessment of epizootological and probable epidemiological danger of echinococcosis invasion in the Southern Regions of Russia (on example of the Karachay-Circassian Republic). *Veterinaria Kubani*. 2022; 1: 34–36. https://elibrary.ru/ipkyra (in Russ.)

22. Akhmedov M. A., Ataev A. M., Zubairova M. M., Karsakov N. T., Mutuev S. Sh. Epizootic situation on helminthosis in domestic ruminants in the conditions of the Caspian lowland. *Russian Journal of Veterinary Pathology*. 2022; (1): 16–22. https://doi.org/10.25690/VETPAT.2022.36.14.010 (in Russ.)

23. Gadaev H. H. Helminth fauna in farm and wild ruminants on the pastures of Chechen Republic. *Russian Journal of Parasitology*. 2015; (2): 8–12. https://vniigis.elpub.ru/jour/article/view/38 (in Russ.)

24. Gadaev Kh. Kh. Helmintocomplex of respiratory system in young sheep in the North-Eastern Caucasus. *Veterinarian*. 2019; (6): 27–32. https://doi.org/10.33632/1998-698X.2019-6-27-32 (in Russ.)

25. Chilayev S. Sh., Bittirov A. M., Shekikhacheva L. Z. Cattle helminths. *Izvestia Orenburg State Agrarian University*. 2008; (1): 190–191. https://elibrary.ru/mhvunl (in Russ.)

26. Ivashkin V. M., Mukhamadiev S. A. Identification guide to cattle helminths. Moscow: Nauka; 1981. 259 p. (in Russ.)

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Enzyme-linked immunosorbent assay for post-slaughter diagnosis of bovine leukosis

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ABSTRACT

Postmortem diagnosis of enzootic bovine leukosis is made on the basis of the results of tests of biological materials from emergently slaughtered or fallen animals using pathomorphological, histological and molecular genetic methods that have some disadvantages. Results of post-slaughter diagnostic tests for bovine leukosis with enzyme-linked immunosorbent assay are described in the paper. For this purpose, 83 swabs were collected from different carcass parts including 71 swabs from carcasses of the animals that were not pre-slaughter tested and 12 samples from the carcasses of the animals that were pre-slaughter tested with immunodiffusion assay and found bovine leukemia virus-seronegative (control samples). Sterile scalpels, cotton wool, 5 mL tubes with caps were used for swab collection. The samples were taken from incisions in carcasses and internal organs of slaughtered animals with sterile cotton-wool swabs and placed in single-use tubes. Distilled water (or isotonic solution – 0.85% NaCl) was added to the tubes with samples, 0.1 to 0.2 mL per tube depending on the sample size, and the tubes were left at room temperature (22–26 °C) for 1.5–2.0 hours and regularly shaken. Resulting homogeneous substrate was used for enzyme-linked immunosorbent assay carried out in accordance with the instructions for the test-kit for detection of antibodies against bovine leukemia virus. Specific antibodies to bovine leukemia virus gp51 antigen were detected in 6 (8.5%) out of 71 swabs subjected to the laboratory tests. Therewith, the antibodies were detected only in 3 swabs (4.2%) when the swabs were tested with immunodiffusion assay. All 12 control samples from animals that were pre-slaughter tested and found seronegative were negative when tested with enzyme-linked immunosorbent assay. Therefore, the above-said serological method can be used for post-slaughter diagnosis of bovine leukosis together with conventional methods.

Keywords: enzootic bovine leukosis, post-slaughter diagnosis, enzyme-linked immunosorbent assay (ELISA), swabs from carcasses and internal organs, specific antibodies, gp51 antigen of bovine leukemia virus

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Применение иммуноферментного анализа в послеубойной диагностике лейкоза крупного рогатого скота

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РЕЗЮМЕ

Послеубойный диагноз на энзоотический лейкоз крупного рогатого скота ставится на основании результатов исследований биологического материала, полученного от вынужденно убитых или павших животных, выполненных патоморфологическим, гистологическим и молекулярно-генетическим методами, обладающими рядом недостатков. В статье описываются результаты послеубойного диагностического исследования на лейкоз крупного рогатого скота с применением иммуноферментного анализа. Для этого с различных частей туш и органов было отобрано 83 пробы смывов, из них 71 проба – от прижизненно не исследованных животных, а 12 проб (контрольные образцы) – от прижизненно серонегативных в реакции иммунодиффузии к вирусу лейкоза особей. Для взятия проб были использованы стерильные скальпели, вата, пробирки с колпачком объемом 5 мл. С помощью

тампонов из стерильной ваты из надрезов туш и органов послеубойных животных производили взятие смывов, которые помещали в одноразовые пробирки. В пробирки со смывами в зависимости от размера тампона добавляли от 0,1 до 0,2 мл дистиллированной воды (или изотонического раствора – 0,85%-го раствора NaCl), оставляли на 1,5–2,0 ч при комнатной температуре (22–26 °С) и периодически встряхивали. Полученный однородный субстрат использовали для проведения иммуноферментного анализа в соответствии с инструкцией по применению набора для выявления антител к вирусу лейкоза крупного рогатого скота. В результате проведенных лабораторных исследований 71 пробы смывов в 6 (8,5%) из них были выявлены специфические антитела к антигену gp51 вируса лейкоза, при этом при исследовании данных проб в реакции иммунодиффузии антитела выявили только в 3 (4,2%) пробах. Все 12 контрольных образцов от прижизненно серонегативных животных при постановке иммуноферментного анализа дали отрицательный результат. Таким образом, данный серологический метод может применяться в послеубойной диагностике лейкоза крупного рогатого скота наряду с общепринятыми методами.

Ключевые слова: энзоотический лейкоз крупного рогатого скота, послеубойная диагностика, иммуноферментный анализ, смывы с туш и органов, специфические антитела, антиген gp51 вируса лейкоза крупного рогатого скота

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INTRODUCTION

Enzootic bovine leukosis (EBL) is widely spread in many countries as well as in the Russian Federation. Animals infected with EBL virus are the source of the agent at all disease stages [1, 2, 3]. The peculiarity of the disease is that it occurs mainly in a chronic form without clinical symptoms and is characterized by rampant growth of neoplastic blood cells, that, through malignancy and proliferation, affect almost all organs of the animal [4, 5, 6]. The disease progresses through several stages from bovine leukemia virus entry to the animal's body to leukosis clinical manifestations:

1) incubation period (it lasts for 8 to 20 days);

2) asymptomatic virus-carrier state (seropositive animals);

3) hematological stage (changes in composition of blood formed elements);

4) clinical stage (tumor).

The disease is lifetime diagnosed with serological methods (immunodiffusion assay, enzyme-linked immunosorbent assay (ELISA), etc.) based on detection of antibodies to bovine leukemia virus antigens developed by the animals at all stages (except for incubation period). There are other methods that are used for lifetime diagnosis of EBL together with serological ones: clinical, cytomorphological, hematological, bioassay in animals (mainly in sheep), etc. [7, 8, 9, 10, 11, 12, 13]. Polymerase chain reaction (PCR) is also used for laboratory diagnosis of the disease [14, 15].

Post-mortem EBL diagnosis is made based on tests of biological materials collected from emergently slaughtered and fallen animals using pathomorphological, histological and molecular genetic (PCR) methods. The following lesions are detected during pathomorphological examination of carcasses and organs of emergently slaughtered or fallen animals with pathology: proliferative (tumor) masses, enlarged lymph nodes, changes in the internal organ size and organ tissue consistency. Pathological manifestations in organs and body systems vary depending on the bovine leukosis form. For example, in case of lymphoid, undifferentiated and myeloid forms of the disease, the lymph nodes are enlarged with gray-white, firm and waxy cut surfaces, and the spleen may be also enlarged. In case of myeloid form of leukosis, the spleen pulp is red-crimson in color with loose consistency and hemorrhages. In case of hematosarcoma (particularly lymphogranulomatosis), the spleen is enlarged in about 50% of affected animals. Focal (diffuse) proliferative masses of grey-pink or gray-white color in the affected organs (kidneys, liver, skeletal muscles, etc.) are found in animals with any of bovine leukosis forms. Histological analysis is carried out when pathomorphological picture is not obvious. For this purpose, sections of organ pieces (bone marrow, spleen, lymph nodes, etc.) and tissues (connective, muscle, etc.) are prepared using specified methods. The main disadvantages of pathomorphological and histological methods are as follows: these methods are not capable of detecting seropositive for bovine leukemia virus (BLV) animals at early stage and histological analysis and further post-slaughter diagnosis of bovine leukosis are time-consuming (3–4 days) that may affect the quality of tested meat and offal [16, 17].

Molecular-genetic method is important for post-slaughter diagnosis; PCR is used when postmortem picture is not obvious and hampers diagnosis. This method allows for detection of BLV proviral DNA integrated in the host cell genome in organ tissues and muscles. However, PCR has some disadvantages: high cost of analyses, need for maintaining environment temperature, nonspecific reactions, etc.

Serological method was used for post-slaughter diagnosis of bovine leukosis during earlier studies. Antibodies

to BLV antigen were detected with immunodiffusion test in muscle-tissue fluid (plasma, lymph) collected from carcasses and offal of slaughtered animals [18, 19]. Despite of substantial benefits of the proposed method for post-slaughter diagnosis (low cost of the test-kit, easy test running, etc.) it has some disadvantages. The said disadvantages are as follows: time required for immunodiffusion assay (the test results are read after 48 hours), low sensitivity of the test, possible inconclusive results (cross-reactions) [20].

Considering the above, the study was aimed at use of a new technique for post-slaughter diagnosis of bovine leukosis with ELISA.

MATERIALS AND METHODS

Eighty-three samples collected from carcasses and offal of slaughtered animals on the Makhachkala universal market No. 2 were the main materials used for the tests for bovine leukosis. Twelve slaughtered animals used for the test were tested before slaughter and found BLV seronegative based on the veterinary certificates issued by the Veterinary Units and 71 slaughtered animals used for the test were not tested for bovine leukosis with immunodiffusion assay, ELISA, etc. before slaughter.

Swabs were collected from different parts of carcasses and organs for diagnostic tests. Sterile scalpels, cotton wool, 5 mL tubes with caps were used for swabbing. Small tampons were made from the cotton wool and used for taking swabs from incisions in carcasses and organs of the slaughtered animals. Then, the swabs were placed in single-use tubes, the tubes were properly labelled and accompanying documents were prepared for their transportation. The accompanying documents contained information on the sample collection time and place, sample number and other data. In the testing laboratory, distilled water (or isotonic solution – 0.85% NaCl solution) was added to the tubes with swabs, 0.1 to 0.2 mL per tube depending on the tampon size and then the tubes were left at room temperature of 22–26 °C for 1.5–2.0 hours and regularly shaken. Resulting homogeneous substrate from the tubes was used for ELISA testing in accordance with the Instruction on use of ELISA test-kit for detection of antibodies to BLV (Vetbiochem, Russia).

The swabs were taken from carcasses and internal organs (offal) of the slaughtered animals in accordance with Order of the Ministry of Agriculture of the Russian Federation No. 269 of 28 April 2022 on approval of the Veterinary Rules for animal slaughter and Veterinary Rules for veterinary and sanitary examination of meat and products derived from slaughtered (hunted) animals and intended for processing and (or) marketing[1]; serological tests were carried out in accordance with the Methodical Guidelines for bovine leukosis diagnosis approved by the Veterinary Department of the Ministry of Agriculture of the Russian Federation No. 1372/2130 of 23 August 2008[2].

RESULTS AND DISCUSSION

For ELISA tests of 71 samples collected from the cattle not subjected to lifetime tests for bovine leukosis, 100 µL of the buffer for sample dilution were added to each of 75 wells of a strip plate (96-well microplate coated with specific gp51 antigen of BLV). Control sera (C^+ and C^-) in duplicate were added to 4 out of 75 wells, 4 µL per well, and test homogeneous substrate (tissue fluid swab diffused with distilled water) was added to other 71 wells, 4 µL per well. The well contents were thoroughly mixed and the plate was coated with adhesive tape and incubated in a thermostat at temperature of 37 °C for 1 hour. After incubation the plate was washed thrice with preliminary prepared working phosphate-buffered saline (PBS) solution containing Tween-20, by filling the wells with the PBS solution to the top manually (300 µL per well). Then, liquid in the wells was decanted and the plate was dried by tapping against filter paper folded in several layers. The conjugate solution (peroxidase-conjugated anti-bovine IgG monoclonal antibodies) were added to the microplate wells, 100 µL per well, the plate was coated with adhesive tape and incubated in the thermostat at temperature of 37 °C for 1 hour. After incubation the wells were washed thrice with phosphate-buffered saline solution containing Tween-20 (300 µL per well) and dried by tapping against folded filter paper. Then, tetramethylbenzidine solution containing hydrogen peroxide was added to the plate wells, 100 µL per well, and the plate was left at temperature of 22 °C for 10 minutes in a dark place. The reaction was stopped by adding stop-solution (1 N H_2SO_4), 50 µL per well. ELISA results were read by measuring absorbance with a spectrophotometer at wave length of 450 nm.

For final assessment of ELISA results, mean optical density values of positive and negative controls were determined. The relative amounts of anti-BLV antibodies expressed in international ELISA units (EU) in the negative control (C^-) and in test samples were calculated according to the formula:

$$EU = \frac{OD\ (test\ sample)}{OD\ (positive\ control)} \times 100.$$

Specific antibodies to BLV gp51 antigen were detected in 6 (8.5%) out of 71 homogeneous substrate (swab) samples subjected to laboratory tests with ELISA.

Twelve samples from carcasses and offal of the slaughtered animals subjected to lifetime testing for bovine leukosis with immunodiffusion assay and found BLV-seronegative (control samples) were similarly tested with ELISA. All samples were tested negative.

At the next stage, ELISA-tested samples (71 samples) were comparatively tested with immunodiffusion assay and antibodies to BLV antigen were found in 3 (4.2%) samples (swabs). Results of post-slaughter bovine leukosis diagnosis with immunodiffusion assay and ELISA given in the table below show that ELISA is more sensitive as compared to immunodiffusion assay.

Thus, ELISA is able to detect specific antibodies in tissue fluids (plasma and lymph) to BLV gp51 antigen that simplifies and may facilitate post-slaughter bovine leukosis diagnosis.

CONCLUSION

Based on the results of post-slaughter tests of swabs from the animal carcasses and offal for antibodies against BLV antigen contained in tissue fluids (plasma and lymph), bovine leukosis was diagnosed with ELISA in 6 (8.5%) out of 71 swabs and was diagnosed with immunodiffusion assay in 3 (4.2%) out of 71 swabs. Twelve (12) swabs from carcasses and offal of the slaughtered animals subjected

[1] https://www.garant.ru/products/ipo/prime/doc/404684483
[2] https://docs.cntd.ru/document/1200118749

Table
Post-slaughter diagnosis of bovine leukosis with ELISA and immunodiffusion assay

Serological diagnosis of EBL in animals	Number of samples	Specific anti-BLV antigens were detected	No specific anti-BLV antigens were detected
No lifetime tests for bovine leukosis were carried out			
Tested with immunodiffusion assay	71	3 (4.2%)	68 (95.8%)
Tested with enzyme-linked immunosorbent assay		6 (8.5%)	65 (91.5%)
Animals were lifetime tested with immunodiffusion assay and found negative			
Tested with enzyme-linked immunosorbent assay after slaughter	12	0	12 (100%)

to lifetime testing for bovine leukosis with immunodiffusion assay and found BLV-seronegative served as control samples and the said animals were also post-slaughter tested negative with ELISA.

Thus, post-slaughter ELISA tests showed that this test-system can be used for bovine leukosis diagnosis together with conventional methods (postmortem examination, histology, etc.) [21].

REFERENCES

1. Donnik I. M., Gulyukin M. I., Busol V. A., Kovalenko L. V., Kovalenko A. M. Bovine leukemia virus infection – diagnostics, eradication, and anthropozoonotic potential (background) (review). *Agricultural Biology*. 2021; 56 (2): 230–244. https://doi.org/10.15389/agrobiology.2021.2.230eng

2. Donnik I., Petropavlovsky M. Cattle leukosis: modern approach. *Animal Husbandry of Russia*. 2022; (3): 32–34. https://doi.org/10.25701/ZZR.2022.03.03.011 (in Russ.)

3. Gulykin M. I., Barabanov I. I., Ivanova L. A., Stepanova T. V., Kozireva N. G., Simonian G. A., et al. Monitoring of epidemiologic situation with bovine leukemia in production and breeding herds of Russian Federation in 2014–2015. *Veterinaria i kormlenie*. 2016; (4): 5–41. https://elibrary.ru/wfizoz (in Russ.)

4. Gillet N., Florins A., Boxus M., Burteau C., Nigro A., Vandermeers F., et al. Mechanisms of leukemogenesis induced by bovine leukemia virus: prospects for novel anti-retroviral therapies in human. *Retrovirology*. 2007; 4:18. https://doi.org/10.1186/1742-4690-4-18

5. Simonyan G. A. Differential diagnostics of hemoblastosis forms. *Veterinariya*. 2013; (9): 21–25. https://elibrary.ru/rbwctn (in Russ.)

6. Donnik I. M., Ponomareva O. I., Krivonos R. A., Lysenko A. A., Koshchaev A. G., Chernykh O. Yu., et al. Elimination of bovine leukemia in industrial production conditions. *Veterinaria Kubani*. 2021; (2): 3–8. https://elibrary.ru/bycjpo (in Russ.)

7. Trono K. G., Pérez-Filgueira D. M., Duffy S., Borca M. V., Carrillo C. Seroprevalence of bovine leukemia virus in dairy cattle in Argentina: comparison of sensitivity and specificity of different detection methods. *Veterinary Microbiology*. 2001; 83 (3): 235–248. https://doi.org/10.1016/s0378-1135(01)00420-5

8. Zamora-Avila D. E., Zapata-Benavides P., Cedillo-Rosales S., Avalos-Ramírez R., Zarate-Ramos J. J., Riojas-Valdés V., et al. Serological detection of bovine leukemia virus in slaughterhouse workers from San Nicolás de los Garza, Nuevo León, México. *African Journal of Microbiology Research*. 2013; 7 (24): 3042–3048. https://doi.org/10.5897/AJMR12.424

9. Meas S., Seto J., Sugimoto C., Bakhsh M., Riaz M., Sato T., et al. Infection of bovine immunodeficiency virus and bovine leukemia virus in water buffalo and cattle populations in Pakistan. *Journal of Veterinary Medical Science*. 2000; 62 (3): 329–331. https://doi.org/10.1292/jvms.62.329

10. Makarov V. V., Lozovoy D. A. Epizootological features of modern cattle leukemia. *Vestnik of the Russian Agricultural Science*. 2020; (1): 53–58. https://doi.org/10.30850/vrsn/2020/1/53-58 (in Russ.)

11. Choi K. Y., Liu R. B., Buehring G. C. Relative sensitivity and specificity of agar gel immunodiffusion, enzyme immunosorbent assay, and immunoblotting for detection of anti-bovine leukemia virus antibodies in cattle. *Journal of Virological Methods*. 2002; 104 (1): 33–39. https://doi.org/10.1016/s0166-0934(02)00040-x

12. Klintevall K., Näslund K., Svedlund G., Hajdu L., Linde N., Klingeborn B. Evaluation of an indirect ELISA for the detection of antibodies to bovine leukaemia virus in milk and serum. *Journal of Virological Methods*. 1991; 33 (3): 319–333. https://doi.org/10.1016/0166-0934(91)90032-u

13. Bajseitov S. T., Novikova N. N., Vlasenko V. S., Krasikov A. P. Comparative evaluation of diagnostic efficiency of immunodiffusion reaction (IDR), ELISA and indirect reaction of immunofluorescence (IIR) for cattle leukemia. *Vestnik of Omsk SAU*. 2020; (1): 97–102. https://elibrary.ru/itrusm (in Russ.)

14. Yu C., Wang X., Zhou Y., Wang Y., Zhang X., Zheng Y. Genotyping bovine leukemia virus in dairy cattle of Heilongjiang, northeastern China. *BMC Veterinary Research*. 2019; 15 (1):179. https://doi.org/10.1186/s12917-019-1863-3

15. Nekoei S., Hafshejani T. T., Doosti A., Khamesipour F. Molecular detection of bovine leukemia virus in peripheral blood of Iranian cattle, camel and sheep. *Polish Journal of Veterinary Sciences*. 2015; 18 (4): 703–707. https://doi.org/10.1515/pjvs-2015-0091

16. Simonyan G. A. Hematosarcoma is the neoplastic form of the hemoblastosis. *Veterinariya*. 2014; (5): 21–27. https://elibrary.ru/skoksj (in Russ.)

17. Simonyan G. A., Khisamutdinov F. F. Veterinary Hematology. Moscow: Kolos, 1995. 256 p. (in Russ.)

18. Mustafayev A. R. Immunodiffusion assay as a method of bovine leucosis post-mortem diagnosis. *Veterinary Science Today*. 2022; 11 (1): 49–52. https://doi.org/10.29326/2304-196X-2022-11-1-49-52

19. Mustafaev A. R. Method of post-mortem diagnosis of bovine leukemia. Patent No. 2744706 Russian Federation, Int. G01N 33/53 (2006.01), G01N 33/531 (2006.01). Dagestan Agriculture Science Center. No. 2020103451. Date of filing: 27.01.2020. Date of publication: 15.03.2021. Bull. No. 8. (in Russ.)

20. Mustafayev A. R., Baratov M. O. Comparative assessment of immunodiffusion and enzyme-linked immunosorbent assay used for bovine leucosis diagnosis. *Veterinary Science Today.* 2023; 12 (1): 52–56. https://doi.org/10.29326/2304-196X-2023-12-1-52-56

21. Mustafaev A. R. Accelerated method for post-mortem diagnosis of bovine leukemia using enzyme immunoassay. Patent No. № 2803893 Russian Federation, Int. G01N 33/53 (2006.01), G01N 1/28 (2006.01). Dagestan Agriculture Science Center. No. 2022134850. Date of filing: 27.12.2022. Date of publication: 21.09.2023. Bull. No. 27. (in Russ.)

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Gut microbiota and bacterial associations in monkeys with gastrointestinal diseases in the setting of helminth infestation

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ABSTRACT
One of the topical issues of current primatology is spontaneous pathology in monkeys, primarily gastrointestinal infections, which are the leading ones in the morbidity and mortality patterns of the animals raised in captivity. Gastrointestinal pathology in monkeys involves complicated infectious processes, most often of associative type, with the formation of various bacterial and parasitic associations. The study demonstrates the results of gastrointestinal disease and helminth infestation monitoring as well as of the microbial flora spectrum analysis in monkeys in 2017–2022. Mortality of monkeys due to gastrointestinal diseases in the specified period amounted to 60.5%. The postmortem study demonstrated that the leading position in this pathology pattern in monkeys was taken by gastroenterocolitis (62.5%), with dominated chronic atrophic gastroenterocolitis in the acute phase (53.9%). The analysis of the six-year trend in animal mortality showed that the percentage of gastrointestinal diseases remained approximately at the same level every year. Helminth infestations were detected in 22.0% of the diseased animals and in 30.2% of the dead ones. *Trichocephalus trichiurus* was found in 93.3% of the diseased and in 99.7% of the dead monkeys, *Strongyloides* sp. – in 12.2% of the diseased and in 3.3% of the dead animals. Helminths were detected as mono- and less often as mixed infestations. In the isolated microflora, the top position was taken by the representatives of genus *Proteus*. The percentage of pathogenic enterobacteria detections was low, and *Shigella flexneri* was the leader among them. In monkeys that died from gastrointestinal diseases without parasitic infestation, the pathogenic enterobacteria detection rate was 2 times higher than in the infested animals. The microorganisms were isolated as monocultures and in associations. The microorganisms were isolated as monocultures and in associations *Proteus* spp. were detected more often. Gastrointestinal diseases of helminth-bacterial etiology in monkeys require complex therapy of the animals.

Keywords: monkeys, gastrointestinal diseases, pathogenic and opportunistic bacteria, bacterial associations, helminth infestation

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Кишечная микрофлора и бактериальные ассоциации на фоне гельминтозной инвазии при желудочно-кишечных заболеваниях у обезьян

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РЕЗЮМЕ
Одна из актуальных проблем современной приматологии – спонтанная патология обезьян, в первую очередь желудочно-кишечные инфекции, являющиеся ведущими в структуре заболеваемости и смертности животных, разводимых в условиях неволи. Желудочно-кишечная патология обезьян представляет собой сложные инфекционные процессы, чаще всего протекающие по ассоциативному типу, с формированием разных бактериальных и паразитарных сочетаний. В исследовании представлены результаты мониторинга желудочно-кишечных заболеваний, гельминтозной инвазии и анализа

спектра микрофлоры у обезьян, проведенных в течение 2017–2022 гг. Гибель обезьян от заболеваний желудочно-кишечного тракта в указанный период составила 60,5%. При постмортальном исследовании установлено, что в структуре данной патологии у обезьян лидирующая позиция принадлежит гастроэнтероколитам (62,5%), при этом доминировали хронические атрофические гастроэнтероколиты в стадии обострения (53,9%). Анализ динамики гибели животных в течение 6 лет показал, что процент заболеваний желудочно-кишечного тракта из года в год держится примерно на одном уровне. Гельминтозная инвазия выявлена у 22,0% больных и 30,2% погибших животных. Кишечные паразиты *Trichocephalus trichiurus* обнаружены у 93,3% больных и 99,7% погибших обезьян, *Strongyloides* sp. – у 12,2% больных и 3,3% погибших животных. Гельминты выявлены в моноинвазиях, реже – в полиинвазиях. Из выделенной микрофлоры первое место занимают представители рода *Proteus*. Процент выявления патогенных энтеробактерий низкий, но среди них лидирует *Shigella flexneri*. У погибших от желудочно-кишечных заболеваний обезьян без паразитарной инвазии частота обнаружения патогенных энтеробактерий в 2 раза выше, чем у инвазированных животных. Микроорганизмы были выделены в виде монокультур и в ассоциациях. Чаще выявляли сочетания представителей нормофлоры с *Proteus* spp. Желудочно-кишечные заболевания у обезьян гельминто-бактериальной этиологии требуют комплексной терапии животных.

Ключевые слова: обезьяны, заболевания желудочно-кишечного тракта, патогенные и условно-патогенные бактерии, бактериальные ассоциации, гельминтозная инвазия

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INTRODUCTION

Gut microbiota condition is one of the important factors of animal and human health. Microorganisms enter the digestive tract immediately after birth and play an important role in the animal's life. The basis of the normal gut flora is known to consist of bifidobacteria and lactobacilli, *Escherichia coli* with normal fermentation, enterococci. Studies show that changes in the quantitative and qualitative composition of the gut microbiota lead to impaired intestinal function and gastrointestinal (GI) diseases [1, 2, 3, 4]. Etiological agents of intestinal infections can be bacteria, viruses, protozoa, helminths, fungi. However, not only microbial concentration plays a significant role in the development of the intestinal diseases, but also associations of different types of microorganisms in which they enter into symbiosis or antagonism and express pathogenicity factors [5, 6]. Helminths also change the quantitative and qualitative composition of the gut microbiota, forming microparasite community [7, 8, 9].

Monkeys are anatomically and physiologically similar to humans, and show natural susceptibility to many infectious diseases [10, 11, 12]. Observation results demonstrate that monkeys, both in their natural habitat and in captivity, suffer from various diseases typical for other animals and humans. In the Adler apery of the Research Institute of Medical Primatology (now Kurchatov Complex of Medical Primatology, National Research Centre "Kurchatov Institute") monkeys of different species are kept. According to our annual data, mortality of monkeys in more than half the cases is due to gastrointestinal diseases [13, 14]. Intestinal diseases of monkeys are complex infectious processes, most often of an associative type, with the formation of various bacteria and parasite combinations [15, 16]. Intestinal parasites infect various species of monkeys both in the wild and in captivity, causing serious diseases of the digestive tract and in some cases leading to animal death [17, 18, 19, 20]. According to foreign publications, nematodes are the most common helminths in monkeys kept in zoos, and they can be transmitted to humans [21, 22]. In the foreign research publications, there is data about intestinal parasites isolated from primates kept in zoos and reserves or from free-living ones, but these data refer to particular species and small groups of monkeys [23, 24, 25]. Moreover, these papers describe only the results of parasitology research.

The relevance of the presented work is determined by the fact that GI diseases are the major cause of the monkeys' mortality in the apery. At the same time, intestinal parasitic infections and bacterial associations that form intestinal diseases in monkeys are still an understudied problem. Our previous studies demonstrated the circulation of protozoa and helminths in the monkeys in the apery and provided some data on the concomitant microbiota [15, 16]. In this paper, we would like to focus on the gut microbiota of nematode-infested monkeys and on the features of bacterial associations during its formation.

The novelty of the work lies in the fact that our study was the first to report on the microbial landscape and bacterial associations in the setting of the invasion by intestinal parasites *Trichocephalus trichiurus* and *Strongyloides* sp. in non-human primates kept in captivity.

The aim of the study was to examine the structure of the gut microbiota of monkeys with intestinal diseases who died due to GI pathology in the setting of helminth infestation.

Table 1
Characteristics of tested monkeys (2017–2022)

Monkey species	Diseased	Dead	Total
Rhesus macaque (*Macaca mulatta*)	172	731	903
Crab-eating macaque (*Macaca fascicularis*)	152	514	666
Southern pig-tailed macaque (*Macaca nemestrina*)	41	45	86
Green monkey (*Chlorocebus sabaeus*)	14	79	93
Anubis baboon (*Papio anubis*)	12	170	182
Hamadryas baboon (*Papio hamadryas*)	18	438	456
Total	409	1,977	2,386

Table 2
Number of tested dead monkeys (2017–2022)

Monkey species	Number of monkeys						Total
	2017	2018	2019	2020	2021	2022	
Rhesus macaque	117	204	115	105	124	66	731
Crab-eating macaque	82	92	89	89	90	72	514
Southern pig-tailed macaque	6	5	6	13	8	7	45
Green monkey	11	20	17	17	3	11	79
Anubis baboon	19	32	43	17	45	14	170
Hamadryas baboon	57	55	75	66	105	80	438
Total	292	408	345	307	375	250	1,977

Table 3
Characteristics of tested dead monkeys (2017–2022)

Monkey species	Dead		Total
	with GI lesions	without GI lesions	
Rhesus macaque	491	240	731
Crab-eating macaque	289	225	514
Southern pig-tailed macaque	21	24	45
Green monkey	52	27	79
Anubis baboon	101	69	170
Hamadryas baboon	242	196	438
Total	1,196	781	1,977

MATERIALS AND METHODS

The study object included 2,386 monkeys of six species of both sexes aged from 10 days to 35 years, which were kept in the apery. This number included 409 monkeys affected with intestinal diseases and 1,977 dead monkeys (Tables 1, 2).

The test samples included feces collected by a rectal smear from live monkeys, in case of dead animals they included the contents of three parts of the intestine (small intestine, caecum, rectum).

Bacteriological, biochemical and microscopic tests were carried out using routine practical methods[1]. The test material was aseptically collected and delivered to the laboratory, where initial inoculation on the diagnostic nutrient media was performed: Endo agar, *Salmonella-Shigella* agar, 5% blood agar, salt egg yolk agar, *Yersinia* selective agar, chromogenic *Candida* agar. The inoculates were cultivated in the thermostat at 37 °C for 24 hours, dishes with *Yersinia* selective agar – at 28 °C for 48 hours, dishes with *Candida* agar – at 24 °C for 48 hours. Isolation of pure cultures and their further identification were carried out according to generally accepted standards: examination of morphological and tinctorial properties (Gram staining of smears), hemolytic and lecithinase activity, and examination of the biochemical properties. The VITEK®2 Compact system (bioMérieux, France) was also used to determine the enterobacteria species. Slide agglutination method with specific sera was used for determination of the serovars of the isolated *Shigella*, *Salmonella*, *Yersinia* strains.

Parasite tests. Conventional microscopy procedure of native feces preparations was used to detect parasite infestation[2]. To identify helminth eggs, a small amount of feces from different places of the test portion was ground on a slide in a drop of 50% glycerol solution until a uniform transparent smear formation, covered with a coverslip and subjected to microscopy at 10 × 10 and 10 × 40 magnification. The extensity of helminth infestation was determined by the number of infected animals to the total number of the tested ones.

In the study of postmortem material, both microscopy of native preparations and macroscopic examination of the contents of the large intestine were used, as a result of which adult helminths were detected.

Statistical data processing and calculations were carried out using GraphPad Prism 8 software. To assess the significance of the differences in the frequency of helminth and bacteria detections in different monkey species in individual test groups the χ^2 goodness-of-fit test was used. All differences were interpreted as significant at $p < 0.05$. The χ^2 test-for-trend was used to determine changes in frequency indicators depending on the test year. Fisher's exact test was used to determine the statistical significance between helminth infestation extensity and monkey species.

RESULTS AND DISCUSSION

From January 2017 to December 2022, 1,977 dead monkeys were tested; 1,196 (60.5%) of them demonstrated GI lesions during the necropsy (Table 3).

Gastrointestinal diseases were often accompanied with pneumonia, signs of cachexia, exicosis, and dystrophy of internal organs. Analysis of the animal mortality trend over 6 years demonstrated that the percentage of GI diseases remained approximately at the same level every year. As can be seen in Table 4, there was a tendency for a slight decrease in the number of monkeys died of GI diseases in 2022.

[1] Methodological guidelines for the microbiological diagnosis of the enterobacteria-induced diseases: approved by the Ministry of Health of the USSR on 17.12.1984 No. 04-723/3. https://base.garant.ru/71310616/?ysclid=lvdnbim4fh245607194

[2] MUC 4.2.3145-13 Laboratory diagnostics of helminth and protozoa infestations: guidelines (approved by Chief State Medical Officer of the Russian Federation on 26 November 2013). https://docs.cntd.ru/document/1200110752?ysclid=lvdnu57iyo743363677

Table 4
Trend in the monkeys' mortality due to GI diseases, 2017–2022

Monkey species	Number of dead / %						Trend test*	Total / %
	2017 ($n = 292$)	2018 ($n = 408$)	2019 ($n = 345$)	2020 ($n = 307$)	2021 ($n = 375$)	2022 ($n = 250$)		
Rhesus macaque ($n = 731$)	77/65.8	140/68.6	79/68.7	75/71.4	85/68.5	35/53.0	**< 0.0001 (↑↓)**	491/67.2
Crab-eating macaque ($n = 514$)	50/61.0	64/69.6	44/49.4	46/51.7	46/51.1	39/54.2	0.3145	289/56.2
Southern pig-tailed macaque ($n = 45$)	5/83.3	3/60.0	3/50.0	5/38.5	3/37.5	2/28.6	0.5544	21/46.7
Green monkey ($n = 79$)	9/81.8	8/40.0	11/64.7	15/88.2	2/66.7	7/63.6	0.5575	52/65.8
Anubis baboon ($n = 170$)	10/52.6	12/37.5	25/58.1	12/70.6	34/75.6	8/57.1	**0.0429 (↑↓)**	101/59.4
Hamadryas baboon ($n = 438$)	26/45.6	27/49.1	39/52.0	28/42.4	75/71.4	47/58.8	**< 0.0001 (↑↓)**	242/55.3
Total	177/60.6	254/62.3	201/58.3	181/59.0	245/65.3	138/55.2	–	1,196/60.5

* $p < 0.05$ (χ^2 criterion – statistical difference of detections relative to monkey species).
Arrows show the trend of changes in detection frequency over the years upon statistical significance of the test.

Postmortem examination of dead monkeys with GI lesions showed that in 35.3% of cases the GI lesions were in the form of enterocolitis ($n = 422$), in 62.5% – gastroenterocolitis ($n = 748$) and in 0.6% – gastritis ($n = 7$). Furthermore, in 0.6% of cases, the intestinal lesions were associated with infectious pathology, i.e. yersiniosis ($n = 4$), pseudotuberculosis ($n = 3$). Malignant neoplasms were reported in 1.0% of monkeys: gastric adenocarcinoma ($n = 3$), intestinal adenocarcinoma ($n = 9$). According to the data obtained, the GI diseases included dominating chronic atrophic gastroenterocolitis in the acute stage (53.9%), as well as chronic forms of enterocolitis. In case of stomach lesions, only chronic atrophic gastritis was reported in monkeys (Table 5).

As a result of parasite tests, helminth infestation was detected in 22.0% of the diseased monkeys and in 30.2% of the dead ones (Table 6). Two types of intestinal parasites were detected – *Trichocephalus trichiurus* and *Strongyloides* sp. The detection frequency of *Trichocephalus trichiurus* was 93.3% in the diseased monkeys ($n = 84$) and 99.7% in dead ones ($n = 360$). *Strongyloides* sp. were detected in 11 (12.2%) diseased and 12 (3.3%) dead animals. It was established that *Strongyloides* sp. mono-infestations were detected in 6 (6.7%) diseased and 1 (0.3%) dead monkeys, in other cases the helminths were detected as part of mixed infestations.

Table 6 demonstrates that in the crab-eating macaques with intestinal diseases the helminth detection frequency was higher than in the dead ones. As for Anubis baboons, *Trichocephalus trichiurus* was detected somewhat more often in the intestines of the dead animals. The frequency of infection with these parasites in the diseased and dead rhesus monkeys was almost the same. The same situation was reported in hamadryas baboons. Over the 6-year period, a small number of southern pig-tailed macaques and green monkeys were examined, however, the resulted data demonstrated that helminths were more often detected in the diseased animals of these species than in the dead ones. It was noted that *Strongyloides* sp. were found only in 3 species: rhesus macaques, green monkeys and hamadryas baboons. Thus, the results of the work showed that *Trichocephalus trichiurus* often infected

Table 5
GI diseases and lesions in monkeys (2017–2022)

GI diseases	Number of animals / %	Lesions, number / %			
		acute	chronic atrophic	chronic with complications	CAGE (exacerbation)
Enterocolitis	422/35.3	25/5.9	368/87.2	29/6.9	–
Gastroenterocolitis	748/62.5	25/3.3	69/9.2	9/1.2	645/86.2
Gastritis	7/0.6	0	7	0	–
Infectious pathology	7/0.6	–	–	–	–
Malignant neoplasms	12/1.0	–	–	–	–
Total	1,196/100	50/4.2	444/37.1	38/3.2	645/53.9

CAGE – chronic atrophic gastroenterocolitis.

Table 6
Helminth infestation extensity in monkeys (2017–2022)

Monkey species	Diseased, infested / %	Dead monkeys with GI lesions, infested / %	$p < 0.05$
Rhesus macaque	23/13.4	72/14.7	0.2497
Crab-eating macaque	14/9.2	10/3.5	**< 0.0001**
Southern pig-tailed macaque	23/56.1	9/42.9	**< 0.0001**
Green monkey	9/64.3	16/30.8	0.0662
Anubis baboon	8/66.7	73/72.3	**0.0134**
Hamadryas baboon	13/72.2	181/74.8	**< 0.0001**
Total	90/22.0	361/30.2	

non-human primates, and this coincided with the data of the foreign studies [26, 27, 28].

In 2017–2022, as a result of bacteriological tests of the feces of the diseased monkeys and intestinal contents of the dead ones, 1,468 microorganisms were detected and isolated gut microbiota was characterized by species diversity; 242 microorganisms were isolated from the feces of the diseased monkeys. The proportion of gram-negative gut microbiota was 80.6% ($n = 195$), gram-positive – 18.6% ($n = 45$), yeast-like fungi – 0.8% ($n = 2$). Representatives of the *Enterobacteriaceae* family were found in 43.5% of the diseased animals with pathogenic enterobacteria isolated in 1.9% of monkeys ($n = 8$) and opportunistic ones – in 41.6% ($n = 170$). Coccal microorganisms detected in 9.1% of animals included *Staphylococcus* spp. (6.6%), hemolytic *Enterococcus* spp. (2.2%), gram-positive diplococci (0.3%); 1,226 microorganisms were isolated from the dead monkeys, of which 95.4 % belonged to the gram-negative gut microbiota ($n = 1,170$), 2.8% belonged to the gram-positive gut microbiota ($n = 34$); the proportion of yeast-like fungi amounted to 1.8% ($n = 22$). In 96.0% of the dead animals, enterobacteria prevailed in the isolated gut microbiota ($n = 1,148$), of which the proportion of pathogenic ones amounted to 7.2% ($n = 83$), and opportunistic – to 92.8% ($n = 1,065$). Gram-positive cocci were found in the intestines of 1.5% of the dead monkeys ($n = 18$), while *Staphylococcus* spp. was detected in 0.7% of the animals, hemolytic *Enterococcus* spp. in 0.3%, and gram-positive diplococcus in 0.5%. Pathogenic and opportunistic gut microbiota was not detected in 282 dead and 220 diseased monkeys (23.6 and 53.8%, respectively). No bacterial growth was reported after inoculation of the samples from 3 diseased monkeys onto the nutrient media (0.7%).

The study results demonstrated that representatives of the genus *Proteus* dominated in the gut microbiota, their detection was three times more likely in the dead animals than in the diseased ones (55.0 and 16.4%, respectively).

Klebsiella spp., *Staphylococcus* spp., hemolytic *Enterococcus* spp., *Enterobacter* spp., *Pseudomonas aeruginosa* were more often isolated from the diseased animals. *Providencia* spp., *Enterobacter* spp., *Shigella flexneri*, *Morganella morganii, Klebsiella* spp., *Citrobacter* spp. were more often detected in the dead animals with GI diseases (Fig. 1).

Analysis of the bacterial gut microbiota composition of monkeys indicated (Fig. 2) that non-helminth-infested animals with the highest frequency demonstrated *Klebsiella* spp. (7.8% – in the diseased animals, 5.5% – in the dead animals) and *Enterobacter* spp. (5.0% – in the diseased animals, 7.6% – in the dead animals). At the same time, *Staphylococcus* spp. (6.9%), hemolytic *Escherichia coli* (6.0%), *Pseudomonas aeruginosa* (4.4%) were more often isolated from the diseased monkeys without parasite infestation, and *Providencia* spp. (5.6%), *Citrobacter* spp. (5.2%), *Morganella morganii* (4.4%) were most frequent in the dead parasite-free animals. As for helminth infested animals, *Staphylococcus* spp. (5.6%), *Klebsiella* spp. (4.5%), *Bacillus* spp. (4.5%) were most often detected in the diseased monkeys, and *Providencia* spp. (16.9%), *Morganella morganii* (6.7%) were found in the dead ones.

It was noted that pathogenic enterobacteria were not detected in the helminth infested diseased animals, while in the helminth-free monkeys *Shigella flexneri* were isolated in 2.5% of cases ($n = 8$). In dead monkeys without helminth infestation, the frequency of the detection of pathogenic enterobacteria (*Shigella flexneri*, rare *Salmonella* serovars, *Yersinia* spp.) was 2 times higher than in the infested animals – 8.3% ($n = 69$) and 3.9% ($n = 14$), respectively. As a result, we assume that presence of the intestinal helminths can reduce the number of bacterial pathogens, occupying their niche in the gut biocenosis.

When analyzing the trend in the frequency of isolation of gut microbiota bacteria over six years, a consistently high annual percentage of *Proteus* spp. detections was established (Table 7).

Monitoring of the isolation of pathogenic and opportunistic bacteria in monkeys during 2017–2022 showed decrease in the frequency of detections of *Klebsiella* spp., *Morganella morganii*, *Shigella* spp., *Pseudomonas* spp., hemolytic *Enterococcus* spp. and increase in the frequency of detections of hemolytic *Escherichia coli*, *Enterobacter* spp., *Providencia* spp., *Serratia* spp., *Yersinia* spp., yeast-like fungi of genus *Candida*. The decrease in the frequency of detections of *Klebsiella* spp. and *Shigella flexneri* might be associated with the use of *Klebsiella* phage and intestinal phage in the treatment of the animals. The highest percentage of detections of *Citrobacter* spp., *Klebsiella* spp., *Morganella morganii*, *Pseudomonas aeruginosa*, *Staphylococcus* spp., *Bacillus* spp., as compared with other years, was recorded in 2019.

The microorganisms were isolated both as mono-infestations and mixed infestations. Combinations of normal flora representatives with *Proteus* spp. were more often detected. Thus, in the diseased helminth-infested monkeys, the association of *Escherichia coli* + *Proteus* spp. was reported in 10.0% of cases, *Escherichia coli* + *Enterococcus* + *Proteus* spp. – in 4.5% of cases, *Escherichia coli* + *Enterobacter* spp. + *Staphylococcus* spp. – in 2.2% of cases. The highest percentage of simultaneous detections of *Escherichia coli* + *Proteus* spp. was also reported in the diseased helminth non-infested monkeys (9.4%). Combinations of *Escherichia coli* + *Enterococcus* spp. + *Proteus* spp. were reported in 4.1% of animals; bacterial associations of normal flora involving *Escherichia coli* + *Enterococcus* spp. + *Klebsiella* spp. and *Escherichia coli* + *Enterococcus* spp. + *Staphylococcus* spp. were detected in 3.5% of monkeys. Combinations of hemolytic *Escherichia coli* with *Enterococcus* spp. were reported in 2.5% of the animals, normal flora with *Enterobacter* spp. – in 2.2% of the cases. Associations of *Escherichia coli* + *Serratia* spp., *Escherichia coli* + *Klebsiella* spp., *Escherichia coli* +

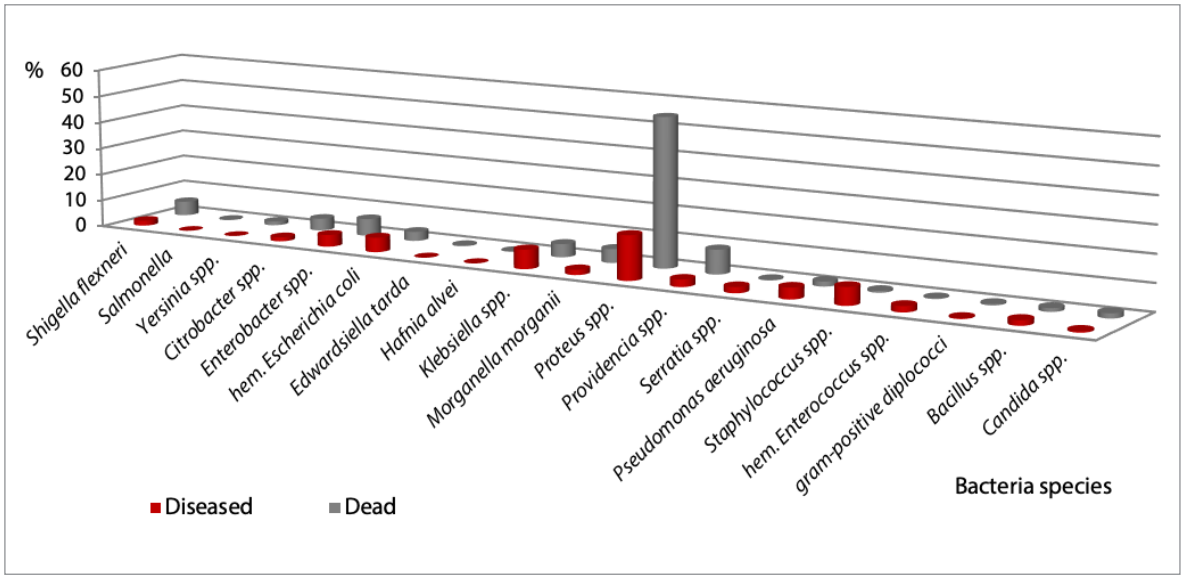

Fig. 1. Gut microbiota composition (or species diversity) in tested monkeys (2017–2022)

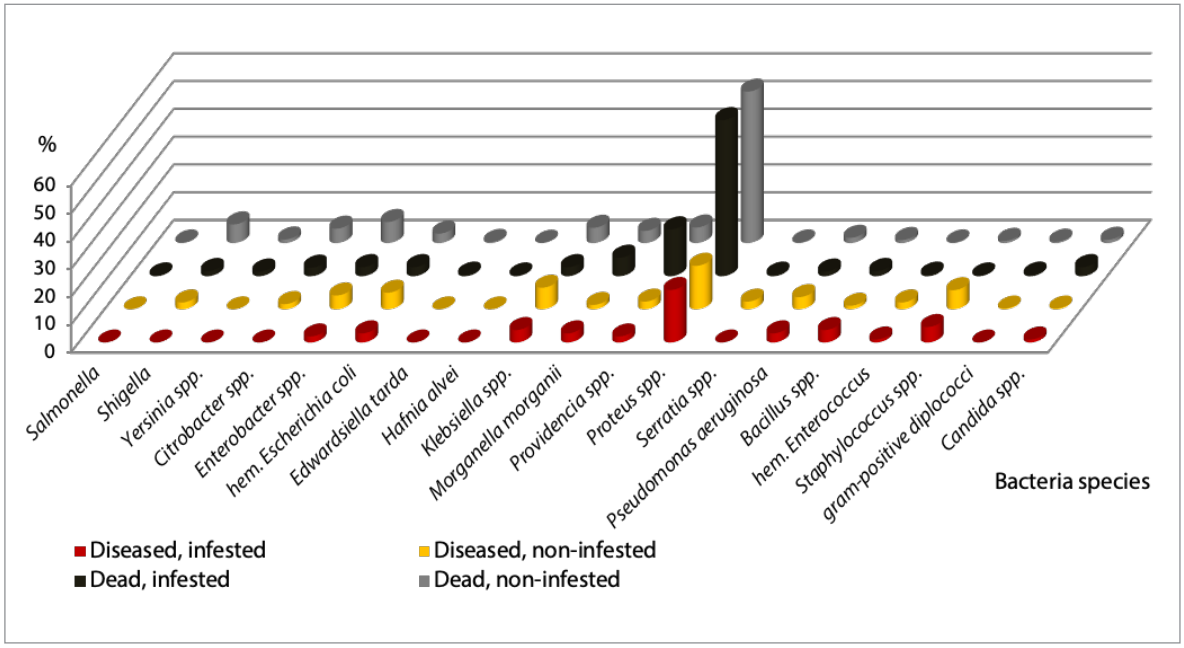

Fig. 2. Effect of helminth infestation on the frequency of detection of the pathogenic and opportunistic bacteria in monkeys (2017–2022)

Enterococcus spp. + *Pseudomonas aeruginosa* were detected in 1.9% of the tested monkeys. The remaining variants of microbial associations were identified in rare cases.

In the dead monkeys the bacterial associations were more diverse. Thus, two-component associations were found in 53.0% of cases, three-component associations in 36.7%, four-component associations in 6.7%, five-component associations in 2.5% and six-component associations in 0.3% of cases. As in the diseased monkeys, *Proteus* spp. were most often detected concurrently with the normal flora in the dead monkeys (39%). Combinations involving normal flora with other opportunistic bacteria were much less common. Thus, co-growth of *Escherichia coli* and *Enterococcus* spp. with the following bacteria were observed: *Providencia* spp. – in 3.5% of cases, *Enterobacter* spp. – in 2.9% of cases, *Shigella flexneri* – in 2.4% of cases, *Citrobacter* spp. – in 2.0% of cases, *Morganella morganii* – in 1.8% of cases, and *Klebsiella* spp. – in 1.3% of cases. In rare cases, *Proteus* spp. were isolated from the intestines of the dead animals together with *Citrobacter* spp., *Enterobacter* spp., *Yersinia* spp. without normal flora. The proportion of concurrent detections of *Proteus* spp. + *Enterococcus* spp. amounted to 1.0%.

Associations of yeast-like fungi of genus *Candida* in the intestines of the dead monkeys with one representative of the opportunistic microbiota were detected more often than with two ones (13 and 4 cases, respectively). Incidence of *Candida* spp. in association with *Proteus* spp. (11 monkeys) was higher than with *Klebsiella* spp. (3 monkeys) and *Pseudomonas aeruginosa* (2 monkeys). Therefore, combinations of pathogenic and opportunistic bacteria with yeast-like fungi in helminth-infested

Table 7
Frequency of microbiota detection in GI diseased and dead monkeys (2017–2022)

Detected microorganisms	Quantity / %						Trend test*	Total / %
	2017 (*n* = 177)	2018 (*n* = 254)	2019 (*n* = 201)	2020 (*n* = 181)	2021 (*n* = 245)	2022 (*n* = 138)		
Citrobacter spp.	8/4.5	13/5.1	17/8.5	13/7.2	3/1.2	6/4.3	0.1953	60/5.0
Enterobacter spp.	7/4.0	15/5.9	14/7.0	17/9.4	20/8.2	20/14.5	**0.0007 (↑)**	93/7.8
Hemolytic *Escherichia coli*	9/5.1	8/3.1	8/4.0	13/7.2	14/5.7	12/8.7	**0.0445 (↑↓)**	64/5.4
Edwardsiella tarda	2/1.1	0	0	1/0.6	2/0.8	0	0.7845	5/0.4
Hafnia alvei	1/0.6	0	1/0.5	0	0	0	0.9010	2/0.2
Klebsiella spp.	11/6.2	13/5.1	27/13.4	24/13.3	4/1.6	8/5.8	0.3492	87/7.3
Morganella morganii	6/3.4	15/5.9	21/10.4	9/5.0	13/5.3	5/3.6	0.7308	69/5.8
Proteus spp.	86/48.6	168/66.1	121/60.2	103/56.9	171/69.8	77/55.8	0.0914	726/60.7
Providencia spp.	7/4.0	17/6.7	24/11.9	19/10.5	28/11.4	22/16.0	**0.0002 (↑↓)**	117/9.8
Salmonella of rare serovars	2/1.1	1/0.4	0	0	0	0	**0.0279 (↓)**	3/0.3
Serratia spp.	1/0.6	1/0.4	1/0.5	0	0	10/7.2	**0.0002 (↓↑)**	13/1.1
Shigella flexneri	0	30/11.8	17/8.5	9/5.0	9/3.7	6/4.3	0.3172	71/5.9
Yersinia spp.	0	3/1.2	2/1.0	6/3.3	0	6/4.3	**0.0330 (↓↑)**	17/1.4
Pseudomonas aeruginosa	3/1.7	11/4.3	18/9.0	5/2.8	2/0.8	0	0.0186 (↑↓)	39/3.3
Bacillus spp.	0	6/2.4	10/5.0	3/1.7	0	5/3.6	0.7586	24/2.0
Hemolytic *Enterococcus*	7/4.0	1/0.4	0	1/0.6	2/0.8	2/1.4	0.1159	13/1.1
Staphylococcus spp.	4/2.3	3/1.2	11/5.5	1/0.6	12/4.9	4/2.9	0.2040	35/2.9
Gr+ diplococcus	1/0.6	5/2.0	0	0	0	0	**0.0183 (↑↓)**	6/0.5
Candida spp.	0	2/0.8	7/3.5	1/0.6	1/0.4	13/9.4	**0.0001 (↑)**	24/2.0

* $p < 0.05$ (χ^2 criterion – statistical difference of detections relative to monkey species).
Arrows show the trend of changes in detection frequency over the years upon statistical significance of the test.

monkeys can aggravate the course of GI diseases due to the simultaneous involvement of pathogenicity factors of various microorganisms and parasites in the development of the infectious process.

As for the species composition of the microbiota, the following *Enterobacteriaceae* species were isolated from monkeys*: Citrobacter freundii*, *C. diversus*, *C. amalonaticus*, *Enterobacter aerogenes*, *E. agglomerans*, *E. cloacae*, *E. gergoviae*, *Klebsiella pneumoniae*, *K. oxytoca*, *K. ozaenae*, *Proteus vulgaris*, *Pr. mirabilis*, *Pr. penneri*, *Providencia stuartii*, *P. rettgeri*, *P. alcalifaciens*, *Serratia marcences*, *S. odorifera*; *Enterococcus*: *Enterococcus faecalis*, *E. faecium*; *Staphylococcus*: *Staphylococcus aureus*, *S. haemolyticus*; as well as yeast-like fungi: *Candida krusei*, *C. glabrata*, *C. tropicalis*.

In conclusion, it can be noted that an important role in the development of GI diseases in monkeys is also played by weakened immunity due to various external factors, including stress, breaches of veterinary and sanitary, zootechnical and animal hygiene rules of feeding and keeping monkeys, which lead to normal gut microbiota disorders and opportunistic microbiota activation. Thus, GI diseases of helminth and bacterial etiology in monkeys require complex therapy. When keeping monkeys in captivity, there is a risk of parasite and pathogenic microorganism transmission to handlers due to human-animal contact. The detected *Trichocephalus trichiurus* and *Strongyloides* sp. are dangerous to humans, therefore it is necessary to comply with safety requirements when working with

the diseased monkeys (regular deworming of animals, daily cleaning of cages and enclosures, and strict compliance with the personal hygiene rules). Knowledge about the parasitic and bacterial agents of spontaneous intestinal infections in monkeys is necessary for proper and safe breeding and keeping of these rare animals in captivity and for the practical use of monkeys in biomedical research.

CONCLUSIONS

The following conclusions were made based on the study results.

1. *Trichocephalus trichiurus* are prevalent in the non-humane primates kept in the apery.

2. Etiology of GI diseases in monkeys involves various associations of diverse bacteria with prevailing representatives of the *Enterobacteriaceae* family.

3. The dominant microorganisms were *Proteus* spp., which were isolated from 16.4% of the monkeys with GI diseases, and from 55.0% of the dead animals.

4. The percentage of pathogenic enterobacteria detections was low (diseased monkeys – 1.9%, dead monkeys – 7.2%), but *Shigella flexneri* was the leader among them.

5. In helminth non-infested monkeys, the pathogenic enterobacteria detection frequency was higher than in the infested ones.

6. Associative GI diseases of helminth and bacterial etiology require complex therapy of monkeys.

7. When keeping monkeys in captivity, there is a risk of parasite and pathogenic microorganism transmission to the handlers due to human-animal contact.

REFERENCES

1. Denisenko O. V., Gapon M. N., Ternovskay L. N., Tverdohlebova T. I. The species composition of conditionally pathogenic microflora at the large intestine dysbacterioses in residents of Rostov-on-Don. *Kuban Scientific Medical Bulletin*. 2013; (1): 74–77. https://elibrary.ru/qccqbr (in Russ.)

2. Egorova S. A., Makarova M. A., Kaftyreva L. A. Opportunictic *Enterobacteriacae* as the cause of the acute diarrhea and gut disbiosis. *Russian Journal of Infection and Immunity*. 2011; 1 (2): 181–184. https://doi.org/10.15789/2220-7619-2011-2-181-184 (in Russ.)

3. Ivanova E. I., Kolesnikova L. I., Rychkova L. V., Savelkaeva M. V., Nemchenko U. M., Rakova E. B. Microecological and associative structure of intestinal biocenosis in children with functional gastrointestinal disorders. *Bulletin of the East Siberian Scientific Center of the Academy of Medical Sciences.* 2016; 1 (5): 22–25. https://elibrary.ru/wxbrkl (in Russ.)

4. Ivanova E. I., Popkova S. M., Rakova E. B., Nemchenko U. M., Savelkayeva M. V., Gorbunova E. L. Study of the genus *Candida* fungi associations with some opportunistic microorganisms in persons with functional disorders of the gastrointestinal tract. *Bulletin of the East Siberian Scientific Center of the Academy of Medical Sciences*. 2011; (3-1): 196–198. https://elibrary.ru/oomfaj (in Russ.)

5. Skuratovich E. G. Microbiocenosis of the gastrointestinal tract in young deer European: age dynamics for the first year of life. *Problems of Productive Animal Biology*. 2019; (3): 96–105. https://elibrary.ru/zpewab (in Russ.)

6. Ivanyuk V. P., Bobkova G. N. Changes of microbiocenosis of pigs' intestine with helminth infestation. *Vestnik of the Bryansk State Agricultural Academy*. 2017; (1): 19–22. https://elibrary.ru/xvktpb (in Russ.)

7. Katkov A. E., Romanova E. M. Osobennosti mikrobiotsenoza kishechnika na fone strongiloidnoi invazii = Intestinal microbiocenosis specifics in the setting of *Strongyloides* infestation. *Vestnik of Ulyanovsk State Agricultural Academy*. 2007; 2 (5): 61–66. https://elibrary.ru/rvweev (in Russ.)

8. Aslanova M. M., Zagaynova A. V., Kuznetsova K. Y., Rakova V. M., Smetanina N. V. Study of the composition of the intestinal microbiota by parasitological indicators in the population belonging to different health groups. *Medical Parasitology and Parasitic Diseases*. 2019; (2): 19–25. https://doi.org/10.33092/0025-8326mp2019.2.19-25 (in Russ.)

9. Lee S. C., Tang M. S., Lim Y. A. L., Choy S. H., Kurtz Z. D., Cox L. M., et al. Helminth colonization is associated with increased diversity of the gut microbiota. *PLoS Neglected Tropical Diseases*. 2014; 8 (5):e2880. https://doi.org/10.1371/journal.pntd.0002880

10. Lapin B. A., Dzhikidze E. K., Fridman E. P. Medical primatology guide. Moscow: Meditsina; 1987. 192 p. (in Russ.)

11. Blersch R., Archer C., Suleman E., Young C., Kindler D., Barrett L., Henzi S. P. Gastrointestinal parasites of vervet monkeys (*Chlorocebus pygerythrus*) in a high latitude, semi-arid region of South Africa. *Journal of Parasitology*. 2019; 105 (4): 630–637. https://doi.org/10.1645/19-19

12. Obanda V., Maingi N., Muchemi G., Ng'ang'a C. J., Angelone S., Archie E. A. Infection dynamics of gastrointestinal helminths in sympatric non-human primates, livestock and wild ruminants in Kenya. *PLoS ONE*. 2019; 14 (6):e0217929. https://doi.org/10.1371/journal.pone.0217929

13. Kalashnikova V. A. Detection of *Campylobacter jejuni* and *Helicobacter pylori* associated with gastrointestinal diseases of monkeys. *Russian Journal of Veterinary Pathology*. 2009; (4): 8–12. https://www.vetpat.ru/jour/article/view/1197 (in Russ.)

14. Ardasheliya S. N., Kalashnikova V. A., Dzhikidze E. K. Etiologic structure of bacterial intestinal infections in monkeys of Adler breeding center. *Bulletin of Experimental Biology and Medicine*. 2011; 151 (6): 734–737. https://doi.org/10.1007/s10517-011-1428-3

15. Egorova T. P., Arshba I. M., Demerchyan A. V., Lenshina Y. I. Species diversity and structure of parasitic-bacterial combinations in intestinal diseases of monkeys. *Veterinariya*. 2023; (2): 31–35. https://doi.org/10.30896/0042-4846.2023.26.2.31-35 (in Russ.)

16. Egorova T. P. Data on intestinal parasites of lower monkeys in the Adler apery. *Parazitologiya*. 2010; 44 (4): 343–350. https://elibrary.ru/oizsdv (in Russ.)

17. Adrus M., Zainudin R., Ahamad M., Jayasilan M.-A., Abdullah M. T. Gastrointestinal parasites of zoonotic importance observed in the wild, urban, and captive populations of non-human primates in Malaysia. *Journal of Medical Primatology*. 2019; 48 (1): 22–31. https://doi.org/10.1111/jmp.12389

18. Boundenga L., Ngoubangoye B., Moukodoum N., Dibakou S. E., Moussadji C., Hugot J. P. Diversity of parasites in two captive chimpanzee populations in southern Gabon. *Infection, Genetics and Evolution*. 2021; 91:104807. https://doi.org/10.1016/j.meegid.2021.104807

19. Islam S., Rahman M. K., Uddin M. H., Rahman M. M., Chowdhury M. N. U., Hassan M. M., et al. Prevalence and diversity of gastrointestinal parasites in free-ranging rhesus macaques (*Macaca mulatta*) in different land gradients of Bangladesh. *American Journal of Primatology*. 2022; 84 (1):e23345. https://doi.org/10.1002/ajp.23345

20. Klyueva A. K., Egorova T. P., Deltsov A. A. Parasite fauna of Javanese macaques (*Macaca fascicularis*). *Veterinariya, Zootekhniya i Biotekhnologiya*. 2021; 11: 72–77. https://doi.org/10.36871/vet.zoo.bio.202111010 (in Russ.)

21. Vonfeld I., Prenant T., Polack B., Guillot J., Quintard B. Gastrointestinal parasites in non-human primates in zoological institutions in France. *Parasite.* 2022; 29:43. https://doi.org/10.1051/parasite/2022040

22. Kumar S., Sundararaj P., Kumara H. N., Pal A., Santhosh K., Vinoth S. Prevalence of gastrointestinal parasites in bonnet macaque and possible consequences of their unmanaged relocations. *PLoS ONE*. 2018; 13 (11):e0207495. https://doi.org/10.1371/journal.pone.0207495

23. N'da K. M., Dahourou L. D., Ndiaye P. I., Lindshield S., Gbati O. B., Traore A. Gastrointestinal parasites of baboons (*Papio papio*) in Niokolo-Koba National Park, Senegal. *Open Veterinary Journal*. 2022; 12 (4): 481–488. https://doi.org/10.5455/OVJ.2022.v12.i4.9

24. Tabasshum T., Liza F. T., Rabbe M. F., Mukutmoni M., Alam M. M., Begum A. Occurrence of gastrointestinal (GI) parasites in captive Olive Baboon and Common Langur in Bangladesh. *Animal Diseases*. 2022; 2:4. https://doi.org/10.1186/s44149-022-00037-9

25. Petrášová J., Modrý D., Huffman M. A., Mapua M. I, Bobáková L., Mazoch V., et al. Gastrointestinal parasites of indigenous and introduced primate species of Rubondo Island National Park, Tanzania. *International Journal of Primatology.* 2010; 31: 920–936. https://doi.org/10.1007/s10764-010-9439-x

26. Valenta K., Twinomugisha D., Godfrey K., Liu C., Schoof V. A. M., Goldberg T. L., Chapman C. A. Comparison of gastrointestinal parasite communities in vervet monkeys. *Integrative Zoology.* 2017; 12 (6): 512–520. https://doi.org/10.1111/1749-4877.12270

27. Kouassi R. Y. W., McGraw S. W., Yao P. K., Abou-Bacar A., Brunet J., Pesson B., et al. Diversity and prevalence of gastrointestinal parasites in seven non-human primates of the Taï National Park, Côte d'Ivoire. *Parasite.* 2015; 22:1. https://doi.org/10.1051/parasite/2015001

28. Tandan S., Kshetri S., Paudel S., Dhakal P., Kyes R. C., Khanal L. Prevalence of gastrointestinal helminth parasites in rhesus macaques and local residents in the central mid-hills of Nepal. *Helminthologia*. 2023; 60 (4): 327–335. https://doi.org/10.2478/helm-2023-0037

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Clinical efficacy studies of the vaccine against feline panleukopenia, calicivirus infection and viral rhinotracheitis Carnifel PCH in kittens

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ABSTRACT

Viral infections in cats can cause serious diseases and even death. Vaccines protect animals from diseases by inducing antibody production and cellular immune response. Primary and booster vaccinations are performed in accordance with the recommendations for the use of vaccines prescribed by the manufactures depending on the minimum duration of immunity. In case of feline panleukopenia, antibody titers correlate with the protection against infection, as for feline calicivirus infection and feline rhinotracheitis, there is no such correlation or it is less clear. Vaccination of cats against these diseases has been performed in the Russian Federation for many years, nevertheless, the feline panleukopenia virus (FPV), feline calicivirus (FCV) and feline herpesvirus (FHV) are still the main common cause of morbidity and mortality among cats. Virus-carrying cats play an important role in the transmission of respiratory viruses such as FHV and FCV in the feline population, and the long-term persistence of FPV in the body, stability in the environmental conditions and resistance to disinfecting agents can be a potential cause of the infection in susceptible kittens. Due to variety of antigenically different FCV strains, the use of the vaccines containing two or more viral strains may induce a broader heterologous protection. The purpose of this work was to evaluate the effectiveness of the vaccine against feline panleukopenia, feline calicivirus infection and feline viral rhinotracheitis developed at the Federal Center for Animal Health (Vladimir) subordinate to the Rosselkhoznadzor, containing 2 heterologous FCV strains (Pers strain genotype I and Fauna strain genotype II), FPV Sheba strain and FHV Lavr strain. The product was developed and tested for its quality in accordance with the requirements of the Russian Federation law. Clinical studies were conducted using 8–12 week-old kittens from different litters born from seronegative, non-vaccinated cats and kept in the household, in a veterinary hospital and animal shelters. The product has successfully passed comprehensive quality control and is registered in the territory of the Russian Federation.

Keywords: feline panleukopenia, feline calicivirus infection, feline viral rhinotracheitis, prevention, vaccine safety and efficacy

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УДК 619:616.98:578:636.8:615.371

Клинические исследования по оценке эффективности вакцины против панлейкопении, калицивироза и вирусного ринотрахеита кошек «Карнифел PCH» при иммунизации котят

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РЕЗЮМЕ

Вирусные инфекции у кошек приводят к серьезным заболеваниям, нередко становясь причиной летального исхода. Вакцины защищают животных от заболеваний, индуцируя образование антител и клеточный иммунный ответ. Первичная и бустерная вакцинация проводится в соответствии с рекомендациями по применению вакцинных препаратов, предоставленными организациями-разработчиками, исходя из минимальной продолжительности

иммунитета. При панлейкопении кошек титры антител коррелируют с защитой от инфекции, что касается калицивироза и ринотрахеита, то аналогичная взаимосвязь отсутствует или менее четкая. Вакцинация кошек против данных заболеваний доступна на территории Российской Федерации уже много лет, тем не менее вирус панлейкопении (FPV), калицивирус (FCV) и герпесвирус (FHV) продолжают оставаться основными распространенными причинами заболеваемости и смертности среди представителей семейства кошачьих. Кошки-вирусоносители играют важную роль в передаче таких респираторных вирусов, как FHV и FCV, в кошачьей популяции, а длительная персистенция FPV в организме, устойчивость в окружающей среде и к дезинфектантам приводит к заражению восприимчивых котят. Ввиду того, что существует множество обладающих антигенным разнообразием штаммов FCV, введение вакцин, содержащих два штамма вируса или более, будет приводить к более широкому спектру перекрестной защиты. Целью данной работы было оценить эффективность разработанной на базе подведомственного Россельхознадзору ФГБУ «Федеральный центр охраны здоровья животных» (г. Владимир) вакцины против панлейкопении, калицивироза и вирусного ринотрахеита кошек, состоящей из 2 гетерологичных штаммов FCV (штамм «Перс» генотип I и штамм «Фауна» генотип II), штамма «Шеба» FPV и штамма «Лавр» FHV. Разработку и контроль качества препарата осуществляли согласно требованиям законодательства Российской Федерации. Клинические исследования проводили с использованием котят 8–12-недельного возраста из разных пометов, рожденных от серонегативных, невакцинированных кошек и содержавшихся в домашних условиях, ветеринарном госпитале и приютах для животных. Препарат успешно прошел всесторонний контроль качества и зарегистрирован на территории Российской Федерации.

Ключевые слова: панлейкопения кошек, калицивирусная инфекция кошек, вирусный ринотрахеит кошек, профилактика, безвредность и эффективность вакцины

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INTRODUCTION

Infections caused by the *Feline panleukopenia virus* (FPV), *Feline calicivirus* (FCV) and *Feline herpesvirus 1* (FHV), the main pathogens of cats, are widespread in all countries of the world and increasingly reported in Russia every year. Cats without specific immunity can become infected and even die. The viruses can be transmitted not only by diseased animals, but also by virus-carrying animals (possible healthy carriers) without apparent clinical signs, shedding the viruses into environment with feces, urine, and nasal discharge [1, 2].

Feline viral rhinotracheitis (*Feline herpesvirus 1*, FHV) is a contagious viral disease caused by FHV involving upper respiratory tract, causing conjunctivitis and keratitis. If the infections are complicated by secondary bacterial infections, the disease would be more severe and ultimately cause the death of the animal. About 80% of cats remain FHV-infected for the rest of their lives and the virus may become reactivated periodically, often due to stress or immunosuppression [2, 3].

Feline panleukopenia (*Feline panleukopenia virus*, FPV) is a highly contagious disease of viral etiology characterized by fever, acute hemorrhagic enteritis, leukopenia, dehydration and high mortality rates (from 25 to 100%) [1, 4, 5].

Feline calicivirus infection (*Feline calicivirus*, FCV) is a highly contagious viral disease characterized mainly by the involvement of the oral and upper respiratory mucosa. According to literature data, FCV was detected in 18–30% of cases suffering from upper respiratory diseases. Virus carriers are also common (up to 75%), especially among stray cats [2, 6, 7]. In addition to the common symptoms of oral ulcers, rhinitis and conjunctivitis, the disease signs may also include lameness, edema of the head and limbs, pneumonia, tongue and palate necrosis, gastrointestinal involvement. Various sources describe a systemic infection that causes death of up to 60% of diseased animals. FCV is characterized by a high genetic variability and antigenic diversity of strains, which significantly reduces the effectiveness of available vaccines [7, 8].

FPV, FHV and FCV are ubiquitous and infect domestic cats of all breeds and ages, as well as zoo and wild felines.

Live and inactivated mono- and combined vaccines are used to prevent viral diseases of cats. The vaccination effectiveness strongly relies on the presence of maternally derived antibodies (MDA), which usually persist in kittens up to 8–12 weeks of age, and sometimes even longer. There is a critical period for kitten vaccination, or «window of susceptibility», when MDA neutralize the vaccine virus, but do not protect against infection with field virulent viruses. The presence of MDA in high titers in kittens blocks the development of post-vaccination immunity and, as a result, affects the immunization outcomes [2, 9, 10, 11]. The level of MDAs will differ between litters and individual animals within litters, depending on the antibody levels in the colostrum of the queens and the amount of colostrum ingested. Therefore, it is common practice to perform the first core vaccination at 8–9 weeks of age (or earlier in higher risk or sheltered kittens), and to administer additional doses at 2–4-week intervals until the age of 12–16 weeks or older with the expectation that one of these vaccinations will occur after the blocking effect of the MDA has waned, and before exposure to virulent agents. Three international

expert groups: the Feline Vaccination Advisory Panel of the American Association of Feline Practitioners (AAFP), the Vaccination Guidelines Group of the World Small Animal Veterinary Association (WSAVA VGG) and the European Advisory Board on Cat Diseases (ABCD) provided veterinary practitioners with recommendations on the use of vaccines for cats. Despite some differences, all three groups recommend re-vaccinations for core vaccines at intervals of more than one year. The current recommendation for vaccination against FHV and FCV infection is primary vaccination with two injections at an interval of 3–4 weeks and revaccination after 1 year. Subsequent boosters should be administered every 3 years, except for higher-risk situations [4, 10, 11, 12, 13, 14, 15].

Unlike vaccines against feline panleukopenia, which provide long-term complete protection for cats, vaccines against feline viral rhinotracheitis and calicivirus infection significantly reduce the frequency of clinical cases, but do not confer full protection, and vaccine-induced immunity may decrease over time, requiring regular revaccination [16, 17, 18]. Therefore, along with the FPV antigen, FCV and FHV are considered the major components of the vaccine that all cats should receive regardless of age and gender [2, 4, 19].

Vaccines against feline calicivirus infection do not provide complete protection due to considerable antigenic variability amongst FCV strains [8, 20], this means poor efficacy of the vaccines and inability to completely prevent infection with field virulent strains and further transmission of the virus among cats [7, 8]. For several decades, commercial vaccines for cats have been based on FCV F9 or 255 vaccine strains or a combination of two G1 and 431 vaccine strains, however, some publications state that due to frequent FCV mutations, these vaccines are not always effective [16, 17, 21]. In addition, vaccines against feline calicivirus infection or viral rhinotracheitis do not prevent infection, but rather reduce the severity of clinical signs and sometimes viral shedding [6, 9, 10, 21, 22, 23, 24]. Although commercial combined vaccines against feline panleukopenia, viral rhinotracheitis and calicivirus infection are used worldwide, significantly reducing both morbidity and mortality rates, nevertheless, these viral diseases are still common among cats in various countries, including the territory of the Russian Federation. The development of the vaccines against these infectious feline diseases requires taking into account the wide variety of FCV genotypes, as well as the genetic and antigenic variability of the strains. That is why it is urgently needed to update the strain composition of core vaccines against feline panleukopenia, viral rhinotracheitis and calicivirus infection.

Thus, the specific protection against FPV, FCV, FHV, and prevention of the diseases caused by these viruses among felines are of paramount importance to ensure the favourable animal health situation in the country.

Based on the above, the Federal Centre for Animal Health, subordinate to the Rosselkhoznadzor, was tasked to develop and register a safe and effective vaccine for cats against feline panleukopenia, calicivirus infection and viral rhinotracheitis in the Russian Federation.

MATERIALS AND METHODS

Carnifel PCH vaccine against feline panleukopenia, calicivirus infection and viral rhinotracheitis was developed and tested for quality in accordance with the requirements of Federal Law No. 61-FZ "On circulation of medicines" and Order No. 101 of the Ministry of Agriculture of the Russian Federation "On approval of the Rules for preclinical studies, clinical studies and bioequivalence studies of veterinary medicinal products".

Vaccine. The active ingredients of the Carnifel PCH vaccine include inactivated FPV (Sheba strain), FCV (Pers strain genotype I and Fauna strain genotype II) and FHV (Lavr strain). Aluminum hydroxide is used as an adsorbent. All components of the vaccine undergo comprehensive input quality control, including control of sterility and innocuity in Crandell-Rees Feline Kidney Cells (CRFK) using three consecutive passages.

Animals. Clinical studies were conducted using 8–12-week-old kittens (n = 37) from different litters born from seronegative, non-vaccinated cats and kept in a household, in a veterinary hospital and animal shelters.

Animal handling complied with the ethical standards adopted by the European Convention ETS No. 123 and approved by the Bioethics Commission of the Federal Centre for Animal Health.

Serological tests. Kitten sera obtained before vaccination and at 7, 14, 21, 28, 35, 42 days post vaccination (dpv) were tested. Then sera were collected every month for a year, and tested for antibodies to FPV by haemagglutination inhibition test (HI test), and to FCV and FHV by virus neutralization test (VNT). Before tests sera were inactivated by heating at 56 °C for 30 minutes.

Haemagglutination inhibition test (HI test). For the purposes of the test 25 µL of heat-inactivated serum was subjected to 2-fold serial dilutions started at 1:10 with phosphate buffered saline solution (pH 6.8) in a 96-well U-bottom microplate. Then an equal volume of FPV containing 8 haemagglutination units was added to the diluted sera. Following one-hour incubation, 0.8% porcine erythrocytes were added to each plate well and incubated overnight at 4 °C. The reaction was interpreted after the red blood cells completely settled in the control wells (in the form of a button). The reaction result was considered positive if the tested serum contained FPV-specific antibodies at a titer of ≥ 1:40 (HI titer of ≥ 5.3 $\log_2$) and higher. The antibody titer was expressed as the highest serum dilution causing complete inhibition of hemagglutination.

Virus neutralization test (VNT). To determine the level of neutralizing FCV and FHV antibodies, monolayers of CRFK cells were used. The antibody titer was determined by serial dilution of serum sample, which was then added to the standard amount of the virus: 50 µL of diluted serum and 50 µL of an infectious culture medium containing 100 $TCID_{50}$ of the selected virus strain, mixed and incubated for 2 hours at 37 °C and 5% CO_2. Then, the antibody-virus mixture was inoculated into CRFK monolayer cells in 96-well microplates with CellBIND-treated surface. Each serum dilution was added to 4 wells. The cultures were incubated for 5 days at 37 °C and 5% CO_2. The reaction was interpreted using a microscope. The neutralization titer was expressed as the reciprocal of the highest dilution at which cell infection was blocked (VNT).

Statistical analysis of the results. Microsoft Excel software statistical methods were used to process the obtained data. The mean group titers and standard deviation were determined. The specific antibody titer was calculated using Kaerber formula and expressed as $\log_2$.

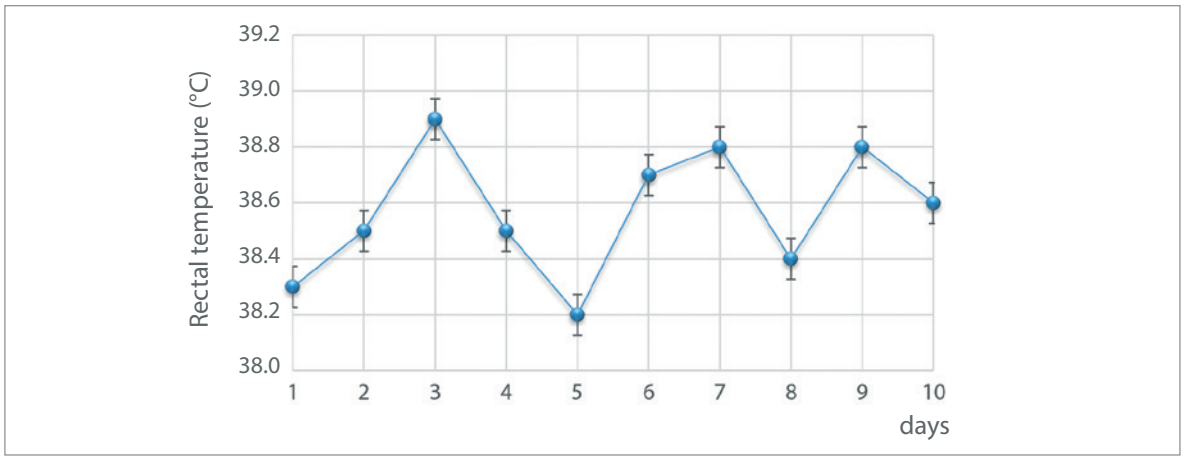

Fig. 1. Body temperature of animals (kittens)

RESULTS AND DISCUSSION

For clinical studies to evaluate the effectiveness of vaccination against FPV, FCV and FHV infections with Carnifel PCH, outbred 8–12 week-old kittens from different litters (n = 37) were used. The kittens were vaccinated subcutaneously twice at a 21 day interval at a dose of 1.0 cm^3. The animals were monitored and their body temperature was measured during 10 days post vaccination.

Figure 1 shows the dynamics of the average body temperature in animals after vaccination. It was established that during 10 days after the first immunization, the body temperature of the kittens remained within the normal range; no depression and loss of appetite were observed.

Within the observation period, it was found that during 21 days post first immunization and after booster vaccination, kittens remained healthy, no changes in behavior and clinical symptoms of FPV, FCV and FHV infection were noted, which suggests the safety of the vaccine used.

Testing of sera collected from kittens before vaccination showed that the animals were seronegative to FCV and FHV (VNT results); FPV specific antibodies (HI test results) were determined at a titer of ≤ 1:20 (4.3 log_2).

Figure 2 shows the dynamics of the humoral immune response development in kittens to the vaccination with Carnifel PCH. It was established that the immune system of animals actively reacted to the antigens included in the vaccine, the concentration of FCV, FHV and FPV antibodies increased gradually over time. The level of FPV antibodies was above the threshold value ≥ 1:40 by 14 dpv; the maximum titers (1:640–1:1,280) were recorded at 42 dpv and persisted throughout the entire study period.

After booster vaccination, at 35 dpv all kittens had high titers of specific antibodies to FCV, FHV and FPV. For example the mean group titer of virus neutralizing antibodies to FHV (Lavr strain) was 6.3 log_2, to FCV (Fauna strain) – 6.5 log_2, to FCV (Pers strain) – 7.2 log_2; the mean group titer of specific antibodies to FPV (Sheba strain) was at the level of 10.3 log_2 (by HI test).

The mean group titers determined at 7, 14, and 21 dpv significantly differed from the same value at 35 dpv ($p \geq 0.1$). At the same time, the mean titers detected at 35 and 42 dpv were statistically identical ($p \geq 0.05$). Biologically this meant that the period up to 35 dpv corresponded to the active phase of the humoral immunity development, the period after 35 dpv corresponded to the stabilization phase. Based on the data obtained, it was concluded that after double vaccination with Carnifel PCH, a strong humoral immune response in kittens develops by 35 dpv, i. e. 14 days post booster vaccination.

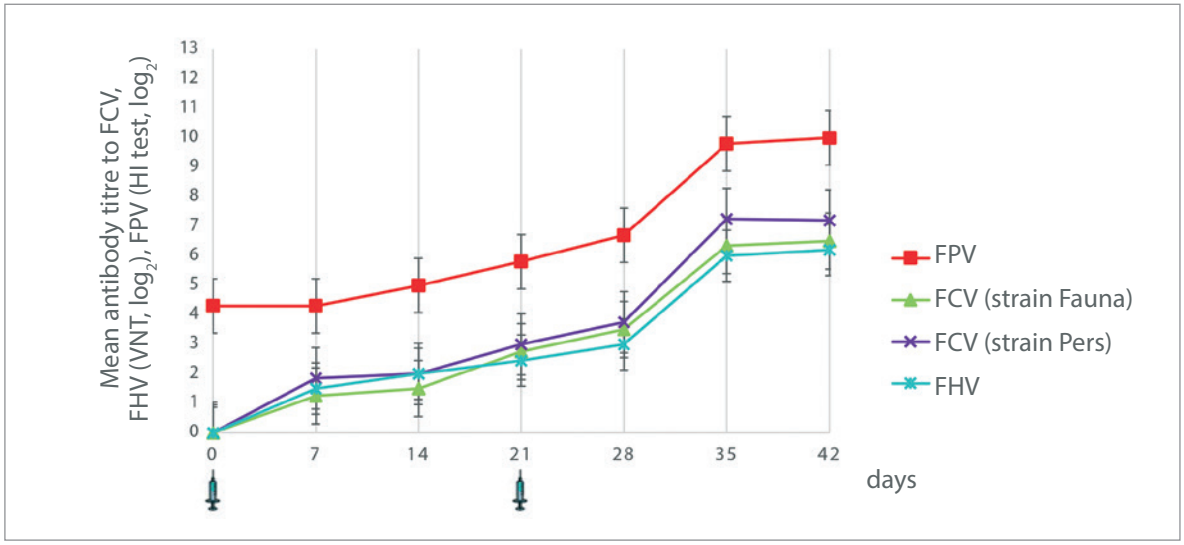

Fig. 2. Development of humoral immune response in kittens following vaccination with Carnifel PCH

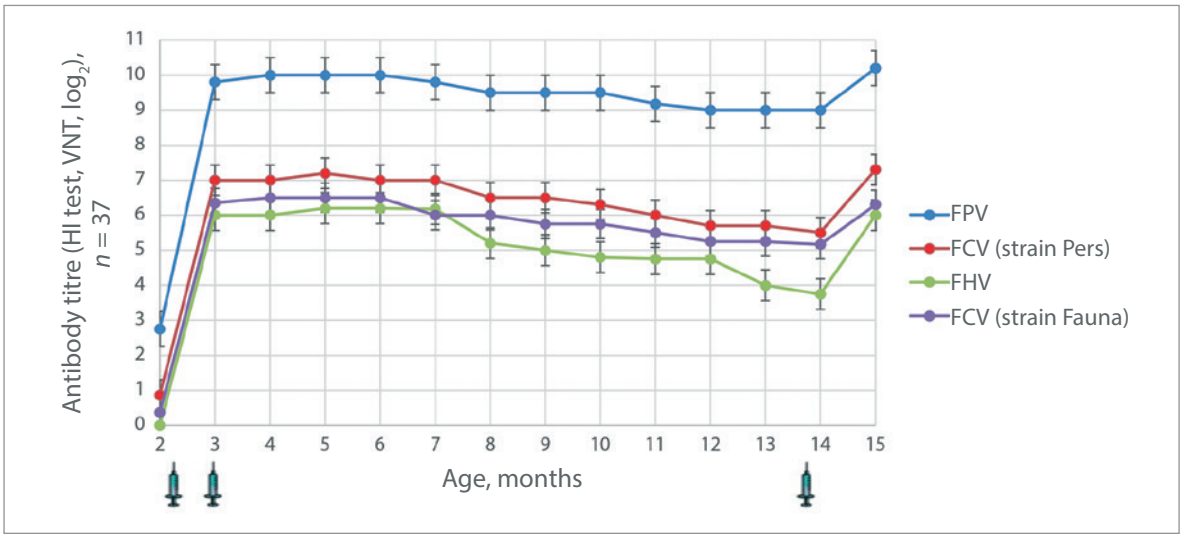

Fig. 3. Duration of immunity in kittens following vaccination with Carnifel PCH

To evaluate the strength of post-vaccination immunity against FPV, FCV and FHV, blood samples were collected from kittens every month during a year. Tests of collected sera showed that during 12 months the mean titer of FHV specific antibodies was in the range from 4.0 to 6.5 $\log_2$, to FCV (Pers strain) – from 5.5 to 7.0 $\log_2$, to FCV (Fauna strain) – from 5.0 to 6.0 $\log_2$ (by VNT), to FPV – from 9.0 to 10.0 $\log_2$ (by HI test).

Figure 3 demonstrates that kittens showed a slight decrease in the level of specific antibodies, which by 14 months of age was the following (mean group titer): to FHV – 3.8 $\log_2$, to FCV (Pers strain) – 5.5 $\log_2$, to FCV (Fauna strain) – 5.2 $\log_2$, to FPV – 9.0 $\log_2$ (by HI test). The kittens were revaccinated in a year with Carnifel PCH once subcutaneously at a dose of 1.0 cm^3, and in 30 days more blood samples were collected for testing. The results showed that the mean group titer of FHV specific antibodies increased by 2.3 $\log_2$, to FCV (Pers strain) – by 1.6 $\log_2$, to FCV (Fauna strain) – by 1.1 $\log_2$ (by VNT), to FPV – by 1.2 $\log_2$ (by HI test).

Thus, based on the data obtained, it was found that Carnifel PCH induced seroconversion after booster subcutaneous vaccination at a dose of 1.0 cm^3 with 21 day interval between the doses; the duration of immunity was at least 12 months.

Most cat vaccination guidelines recommend using a basic vaccination scheme: primary vaccination and subsequent revaccination in a year [4, 10, 11, 14, 25]. The same vaccination scheme of cats was used in our study, which proved its effectiveness for Carnifel PCH vaccination. The vaccine induced strong immunity and specific antibodies to FPV, FHV and FCV at high titers after revaccination.

CONCLUSION

Based on the conducted studies, it was concluded that the vaccine causes the development of an immune response in cats against FPV, FCV and FHV 14 days post double vaccination with a 21-day interval between the doses. The duration of the immunity against these diseases is at least 12 months. When studying the dynamics of FPV, FCV and FHV post-vaccination immunity strength in kittens vaccinated with Carnifel PCH vaccine, its specific effectiveness was proven. The results of serology by HI test and VNT demonstrated strong immune response.

During the tests, it was shown that the developed vaccine has a good tolerability in kittens at 8–12 weeks of age. Double vaccination of animals with a 21-day interval between the doses at a dose of 1.0 cm^3 induces antibodies to FCV, FHV and FPV in high titers. It was found that Carnifel PCH vaccine against FPV, FCV and FHV is safe, non-reactogenic and potent and can be recommended for cats to prevent these infections.

The risk of cat infection with infectious diseases is high at any age, so it is important to understand the need for immunization, which is a tool to keep the diseases under control. Vaccination of even a single animal significantly contributes to the prevention of infectious disease spread in the feline population. The higher the percentage of vaccinated animals in a population, the lower the risk of epizootics.

REFERENCES

1. Truyen U., Parrish C. R. Feline panleukopenia virus: Its interesting evolution and current problems in immunoprophylaxis against a serious pathogen. *Veterinary Microbiology*. 2013; 165 (1–2): 29–32. https://doi.org/10.1016/j.vetmic.2013.02.005

2. Diagnosis and prevention of infectious diseases of dogs and cats: manual for veterinary practitioners. Ed. by T. I. Aliper. Moscow: ZooVetKniga; 2017; 85–100. (in Russ.)

3. Walter J., Foley P., Yason C., Vanderstichel R., Muckle A. Prevalence of feline herpesvirus-1, feline calicivirus, *Chlamydia felis*, and *Bordetella bronchiseptica* in a population of shelter cats on Prince Edward Island. *Canadian Journal of Veterinary Research*. 2020; 84 (3): 181–188. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7301681

4. Stuetzer B., Hartmann K. Feline parvovirus infection and associated diseases. *The Veterinary Journal*. 2014; 201 (2): 150–155. https://doi.org/10.1016/j.tvjl.2014.05.027

5. Jakel V., Cussler K., Hanschmann K. M., Truyen U., König M., Kamphuis E., Duchow K. Vaccination against Feline Panleukopenia: implications from a field study in kittens. *BMC Veterinary Research*. 2012; 8:62. https://doi.org/10.1186/1746-6148-8-62

6. Masubuchi K., Wakatsuki A., Iwamoto K., Takahashi T., Kokubu T., Shimizu M. Immunological and genetic

characterization of feline caliciviruses used in the development of a new trivalent inactivated vaccine in Japan. *Journal of Veterinary Medical Science*. 2010; 72 (9): 1189–1194. https://doi.org/10.1292/jvms.09-0436

7. Porter C. J., Radford A. D., Gaskell R. M., Ryvar R., Coyne K. P., Pinchbeck G. L., Dawson S. Comparison of the ability of feline calicivirus (FCV) vaccines to neutralise a panel of current UK FCV isolates. *Journal of Feline Medicine and Surgery*. 2008; 10 (1): 32–40. https://doi.org/10.1016/j.jfms.2007.06.011

8. Schultz B. S., Hartmann K., Unterer S., Eichhorn W., Majzoub M., Homeier-Bachmann T., et al. Two outbreaks of virulent systemic feline calicivirus infection in cats in Germany. *Berliner und Münchener Tierärztliche Wochenschrift*. 2011; 124 (5–6): 186–193. https://doi.org/10.2376/0005-9366-124-186

9. Bergmann M., Speck S., Rieger A., Truyen U., Hartmann K. Antibody response to feline calicivirus vaccination in healthy adult cats. *Viruses*. 2019; 11 (8):702. https://doi.org/10.3390/v11080702

10. Radford A. D., Addie D., Belák S., Boucraut-Baralon C., Egberink H., Frymus T., et al. Feline calicivirus infection. ABCD guidelines on prevention and management. *Journal of Feline Medicine and Surgery*. 2009; 11 (7): 556–564. https://doi.org/10.1016/j.jfms.2009.05.004

11. Richards J. R., Elston T. H., Ford R. B., Gaskell R. M., Hartmann K., Hurley K. F., et al. The 2006 American Association of Feline Practitioners Feline Vaccine Advisory Panel report. *Journal of the American Veterinary Medical Association*. 2006; 229 (9): 1405–1441. https://doi.org/10.2460/javma.229.9.1405

12. Day M. J., Horzinek M. C., Schultz R. D. Compiled by the Vaccination Guidelines Group (VGG) of the World Small Animal Veterinary Association (WSAVA). *Journal of Small Animal Practice*. 2007; 48 (9): 528–541. https://doi.org/10.1111/j.1748-5827.2007.00462.x

13. Scherk M. A., Ford R. B., Gaskell R. M., Hartmann K., Hurley K. F., Lappin M. R., et al. 2013 AAFP feline vaccination advisory panel report. *Journal of Feline Medicine and Surgery*. 2013; 15 (9): 785–808. https://doi.org/10.1177/1098612X13500429

14. Thiry E., Addie D., Belák S., Boucraut-Baralon C., Egberink H., Frymus T., et al. Feline herpesvirus infection: ABCD guidelines on prevention and management. *Journal of Feline Medicine and Surgery*. 2009; 11 (7): 547–555. https://doi.org/10.1016/j.jfms.2009.05.003

15. Schultz R. D. Duration of immunity for canine and feline vaccines: A review. *Veterinary Microbiology*. 2006; 117 (1): 75–79. https://doi.org/10.1016/j.vetmic.2006.04.013

16. Poulet H., Jas D., Lemeter C., Coupier C., Brunet S. Efficacy of a bivalent inactivated non-adjuvanted feline calicivirus vaccine: Relation between *in vitro* cross-neutralization and heterologous protection *in vivo*. *Vaccine*. 2008; 26 (29–30): 3647–3654. https://doi.org/10.1016/j.vaccine.2008.04.082

17. Huang C., Hess J., Gill M., Hustead D. A dual-strain feline calicivirus vaccine stimulates broader cross-neutralization antibodies than a single-strain vaccine and lessens clinical signs in vaccinated cats when challenged with a homologous feline calicivirus strain associated with virulent systemic disease. *Journal of Feline Medicine and Surgery*. 2010; 12 (2): 129–137. https://doi.org/10.1016/j.jfms.2009.08.006

18. Lappin M. R. Feline panleukopenia virus, feline herpesvirus-1 and feline calicivirus antibody responses in seronegative specific pathogen-free kittens after parenteral administration of an inactivated FVRCP vaccine or a modified live FVRCP vaccine. *Journal of Feline Medicine and Surgery*. 2012; 14 (2): 161–164. https://doi.org/10.1177/1098612X11432240

19. Richards J. R., Elston T. H., Ford R. B., Gaskell R. M., Hartmann K., Hurley K. F., et al. The 2006 American Association of Feline Practitioners Feline Vaccine Advisory Panel report. *Journal of the American Veterinary Medical Association*. 2006; 229 (9): 1405–1441. https://doi.org/10.2460/javma.229.9.1405

20. Baulch-Brown C., Love D., Meanger J. Sequence variation within the capsid protein of Australian isolates of feline calicivirus. *Veterinary Microbiology*. 1999; 68 (1–2): 107–117. https://doi.org/10.1016/S0378-1135(99)00066-8

21. Berger A., Willi B., Meli M. L., Boretti F. S., Hartnack S., Dreyfus A., et. al. Feline calicivirus and other respiratory pathogens in cats with Feline calicivirus-related symptoms and in clinically healthy cats in Switzerland. *BMC Veterinary Research*. 2015; 11:282. https://doi.org/10.1186/s12917-015-0595-2

22. Wensman J. J., Samman A., Lindhe A., Thibault J.-C., Berndtsson L. T., Hosie M. J. Ability of vaccine strain induced antibodies to neutralize field isolates of caliciviruses from Swedish cats. *Acta Veterinaria Scandinavica*. 2015; 57:86. https://doi.org/10.1186/s13028-015-0178-z

23. Spiri A. M., Riond B., Stirn M., Novacco M., Meli M. L., Boretti F. S., et al. Modified-live feline calicivirus vaccination reduces viral RNA loads, duration of RNAemia, and the severity of clinical signs after heterologous feline calicivirus challenge. *Viruses*. 2021; 13 (8):1505. https://doi.org/10.3390/v13081505

24. Hofmann-Lehmann R., Hosie M. J., Hartmann K., Egberink H., Truyen U., et al. Calicivirus infection in cats. *Viruses*. 2022; 14 (5):937. https://doi.org/10.3390/v14050937

25. Sparkes A. Feline vaccination protocols: is a consensus emerging? *Schweizer Archiv für Tierheilkunde*. 2010; 152 (3): 135–140. https://doi.org/10.1024/0036-7281/a000032

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On occurrence of some avian bacterial diseases and biosafety provision

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ABSTRACT

The proportion of infectious diseases in general avian pathology is known to vary significantly, while bacterial infections play a critical role in avian disease occurrence and incidence. Most of them are registered in the country's large-scale poultry holdings, poultry farms and backyards and pose a serious risk in terms of epidemic and veterinary-sanitary aspects. This paper presents the results of analysis of avian colibacillosis and salmonellosis occurrence in 2018–2022, taking into account the number of outbreaks and diseased poultry for each disease. A retrospective analysis showed that these infections are registered annually in poultry farms of the Russian Federation, within a 5-year-period the number of poultry with colibacillosis ranged from 66.18% in 2018 to 0.15% in 2021 of the total number of diseased birds, and the number of *Salmonella*-infected poultry ranged from 65.91% in 2019 to 0.57% in 2021. In 2018–2020 219,020 samples of poultry meat and poultry products were tested for *Salmonella*, while *Salmonella enteritidis, Salmonella typhimurium, Salmonella infantis* were detected in 0.80% cases. It should be noted that in accordance with the requirements of Technical Regulations TR CU 021/2011 and TR EAEU 051/2021, no *Salmonella* is allowed in 25 g of poultry meat. According to the VESTA automated system, during the study period, incompliances with microbiological safety parameters were detected in 16.11% of poultry meat and poultry product samples, of which 10.98% of the samples contained mesophilic aerobic and facultative anaerobic microorganisms, and 5.13% contained *Escherichia coli*. The data obtained indicate the need for a retrospective analysis of the occurrence of some avian bacterial infections in order to study the animal disease situation in poultry farms for the purpose of improving the set of measures to ensure the disease freedom in poultry industry, while addressing the laboratory test results.

Keywords: colibacillosis, salmonellosis, occurrence analysis, poultry meat and poultry products, biosafety parameters

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К вопросу о заболеваемости птицы отдельными бактериальными болезнями и обеспечение биобезопасности

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РЕЗЮМЕ

Известно, что доля инфекционных болезней в общей патологии птицы значительно варьирует, причем в структуре неблагополучия и заболеваемости бактериальные инфекции имеют решающее значение. Большая их часть регистрируется в крупных птицеводческих хозяйствах, на птицефабриках и в личных подсобных хозяйствах нашей страны и представляет серьезную опасность в эпизоотическом и ветеринарно-санитарном отношении. В данной работе представлены результаты анализа заболеваемости птицы колибактериозом и сальмонеллезом за период с 2018 по 2022 г. с учетом количества неблагополучных пунктов и заболевшей птицы по каждой болезни. Ретроспективный анализ показал, что в птицеводческих хозяйствах Российской

Федерации данные бактериозы регистрируются ежегодно, за 5 лет количество заболевшей колибактериозом птицы варьировало от 66,18% в 2018 г. до 0,15% в 2021 г. от общего количества заболевшей птицы, а количество заболевшей сальмонеллезом птицы – от 65,91% в 2019 г. до 0,57% в 2021 г. В 2018–2020 гг. на наличие сальмонелл исследовано 219 020 проб мяса птицы и птицеводческой продукции, из них в 0,80% случаев обнаружены *Salmonella enteritidis*, *Salmonella typhimurium*, *Salmonella infantis.* Следует обратить внимание, что в соответствии с требованиями технических регламентов ТР ТС 021/2011 и ТР ЕАЭС 051/2021 не допускается присутствие сальмонелл в 25 г мяса птицы. По данным автоматизированной системы «Веста», за исследуемый период несоответствия по микробиологическим показателям безопасности выявлены в 16,11% проб мяса птицы и птицепродуктов, из них в 10,98% образцов содержались мезофильные аэробные и факультативно анаэробные микроорганизмы, в 5,13% – бактерии группы кишечной палочки. Полученные данные свидетельствуют о необходимости проведения ретроспективного анализа заболеваемости птицы отдельными бактериальными инфекциями для изучения эпизоотической ситуации в птицеводческих хозяйствах с целью совершенствования комплекса мероприятий по обеспечению ветеринарного благополучия птицеводства, при этом следует обращать внимание на результаты лабораторных исследований.

Ключевые слова: колибактериоз, сальмонеллез, анализ заболеваемости, мясо птицы и птицеводческая продукция, показатели биологической безопасности

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INTRODUCTION

Commercial poultry farming is known to remain the leading branch of agricultural production, providing the population with valuable dietary food. Despite the fact that veterinary specialists pay special attention to the prevention and control of zooanthroponous avian diseases, as well as ensuring veterinary and sanitary safety of poultry products, infectious diseases are still detected in poultry farms of the Russian Federation. Researchers agree that avian infectious diseases pose a potential risk of mass distribution in the population all over the country's territory and result in decreased weight gains and egg yield, livestock reduction, increased microbial contamination and deterioration of poultry product quality. According to some authors, infectious diseases such as Newcastle disease, avian influenza, Marek's disease, Gumboro disease, avian infectious bronchitis, avian infectious laryngotracheitis, colibacillosis, salmonellosis, pasteurellosis and others take a significant place in the formation of the nosological profile of avian infectious pathology and deteriorate the animal health situation in commercial poultry industry. Many foodborne pathogens are widespread in nature, may persist in the environment for a long time and remain facultative parasites for warm-blooded, including food-producing animals. According to veterinary reports, bacterial diseases (colibacillosis, salmonellosis, pasteurellosis, etc.) play a significant role in poultry disease occurrence and incidence and develop when maintenance and feeding conditions are incompliant and the overall animal body resistance is decreased; they can also be secondary infections or a result of the virus latently circulating in poultry body, which exacerbates the development of infectious process. Such pathogenic bacteria as *Salmonella*, *Listeria*, pathogenic *Escherichia coli* strains and other microorganisms occupying a specific place among pathogens common to animals (including birds) and humans and being risk factors for occurrence of foodborne toxicoinfections, still present the greatest danger to poultry farms and poultry processing establishments [1, 2, 3, 4, 5, 6, 7, 8, 9, 10].

According to the statistics of the Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing (Rospotrebnadzor), salmonellosis is one of the most common zoonotic bacterial infections transmitted through food (predominantly through poultry products for most pathogens) and the main cause of group morbidity among the population. For instance, in 2022 the Rospotrebnadzor registered 27 outbreaks of foodborne salmonellosis in 22 Subjects of the Russian Federation, whereas 1,204 people were affected, and 36 major salmonellosis outbreaks were recorded within 11 months of 2023. Level of infection in people due to consumption of substandard products (meat, eggs) varies within significant limits.

Colibacillosis (escherichiosis, colisepticemia, dysentery) detected in birds is an acute infectious disease caused by enteropathogenic *Escherichia coli*, which occurs in the form of septicemia and is characterized by diarrhea. *Escherichia coli* originally called *Bacterium coli* was first identified in 1885 by the Austrian scientist Theodor Escherich after whom it received its name. *Escherichia coli* are small, polymorphic, gram-negative rod-shaped bacteria with rounded ends, they have no spores, are well cultivated on conventional nutrient media and belong to facultative anaerobes. Diseased and convalescent birds are the source of infection, and the pathogen can also be transmitted by wild birds and rodents. The routes of infection can be aerogenic, alimentary and transovarial. According to the researchers, chickens, turkeys, ducklings and goslings up to 90 days of age are most susceptible to the infection, adult birds rarely get diseased, humans may be susceptible. The pathogen persists in the environment for up to 4 months, dies within 1–2 minutes when heated to 100 °C, it is sensitive to conventional disinfectants. Clear 2% active chlorine solution, 5% chloraminum B solution, 3% caustic soda hot (45–50 °C) solution, 2% formaldehyde

solution, 20% suspension of freshly slaked lime (by double whitewashing with an interval of one hour) are used. The ante-mortem diagnostic examination of young birds reveals depression, cyanosis of the head skin, dyspnea, wheezing, diarrhea, fever, intoxication signs. During post-mortem examination cyanosis of muscle tissue is observed, the liver is enlarged, there are fibrin films on the liver surface, multiple small spot hemorrhages are found on the serous membranes of internal organs, and the lungs are hyperemic. Peritonitis and enteritis are recorded in adult birds [1, 11, 12, 13, 14, 15, 16]. It should be noted that the final diagnosis is confirmed by laboratory tests conducted in accordance with regulatory documents.

Young poultry and wild birds are most susceptible to *Salmonella* infection, resulting in salmonellosis (paratyphoid) – an infectious disease that occurs mainly in the gastrointestinal form and, less often, may be systemic. *Salmonella* agents belong to the *Enterobacteriaceae* family of intestinal bacteria, the *Salmonella* genus. Salmonellas are morphologically rod-shaped bacilli with rounded ends that do not form spores and capsules, are well stained with aniline dyes, gram-negative, cultured on conventional nutrient media, classified as aerobic or facultative anaerobes. The pathogen is quite resistant to environmental factors and sensitive to conventional disinfectants. Thus, bacteria can survive in soil within 1–9 months, in frozen meat – within 6–13 months, in eggs – within up to 13 months, in egg powder – within up to 9 months; *S. enteritidis*, *S. typhimurium*, *S. gallinarum*, *S. pullorum*, *S. infantis* are the major species in the etiological structure of avian salmonellosis. Diseased and convalescent birds can be a reservoir of the pathogen, that is, they can be *Salmonella* carriers for a long time, which is especially dangerous as this is a latent source of infection. The disease can be acute, subacute, chronic and sometimes asymptomatic (in adult chickens, ducks and geese). Diseased birds demonstrate lethargy, inappetite, weight loss, conjunctivitis, rhinitis, diarrhea, arthritis, dyspnea, comb and wattle cyanosis; ducks and geese demonstrate swelling of the head. During post-mortem examination enlarged liver, small necrotic foci in the spleen and kidneys are found in adult birds, inflammations of ovaries, oviduct, and cloaca are frequently observed as well. In chicks the serous membrane of the intestine is red, the mucous membranes of the digestive tract are catarrhally inflamed, there are streaky hemorrhages in places, the liver is enlarged, with fibrinous depositions on the capsule and multiple small necrotic foci. Liver degeneration is noted in goslings, whereas in ducklings the liver is enlarged and has many small necrotic foci [2, 5, 13, 17, 18, 19]. *Salmonella* bacteria cause food toxicoinfections in humans; poultry meat, edible eggs and other poultry products are the pathogen transmission factors.

The Department of Microbiology of the All-Russia Scientific Research Veterinary Institute of Poultry Science (A. N. Borisenkova, T. N. Rozhdestvenskaya, O. B. Novikova) has developed a control system for prevention of bacterial diseases in commercial poultry farming, which establishes the main technological links and includes 11 main aspects: diagnostic monitoring (serological tests, microbiological tests of faeces samples, cloaca smears); microbiological monitoring during chicken hatching and rearing; epizootological monitoring of the production technological cycle; antibiotic therapy; probiotic-based prevention, disinsection, desacarization; deratization; specific prevention; HACCP-based analysis of critical control points and risk management (microbiological control of feed, technological facilities, product yield) [2, 20, 21].

Thus, colibacillosis and salmonellosis are the most common avian bacterial diseases currently posing a risk to poultry farming and, in particular, to food product consumers. Therefore, the analysis of colibacillosis and salmonellosis occurrence in poultry farms in the country, as well as the results of poultry meat and poultry product laboratory tests are essential for development of the veterinary and sanitary service activities to ensure poultry disease freedom and biosafety, and that determined the direction of our research.

MATERIALS AND METHODS

Based on statistical data of the Ministry of Agriculture of the Russian Federation, a retrospective analysis of the colibacillosis and salmonellosis occurrence in poultry farms in the country in 2018–2022 was carried out, taking into account the detected outbreaks and the number of infected birds for each disease. The results of laboratory tests of *Salmonella* contamination of poultry meat and poultry products in 2018–2020 are analyzed.

RESULTS AND DISCUSSION

It was established that in 2018–2022, 827,442 poultry in 190 outbreak areas got infected with colibacillosis in the Russian Federation, 176 poultry in 18 outbreak areas got infected with salmonellosis.

In 2018 colibacillosis was registered in 87 outbreak areas, salmonellosis – in 7 outbreak areas, while the number of chicks and young birds infected with colibacillosis amounted to 547,561 birds, with salmonellosis – 33 birds, the percentage of the total number of diseased birds for each disease was 66.18 and 18.75%, respectively.

In 2019 the number of poultry infected with colibacillosis amounted to 242,410 birds in 103 outbreak areas (29.30% of the total number of colibacillosis-infected poultry), salmonellosis – 116 birds in 6 outbreak areas (65.91% of the total number of salmonellosis-infected poultry). In 2020, 2021 and 2022 no new colibacillosis-infected areas were identified in the Russian Federation, while the number of diseased birds was 33,560 (4,06%), 1,204 (0,15%) and 2,707 (0.33%) respectively.

In 2020 the number of salmonellosis-infected areas decreased to 4 and the number of diseased birds amounted to 22 (12.50% of the total number of diseased birds with salmonellosis). In 2021 no new salmonellosis-infected areas were identified in the Russian Federation, while there was one diseased bird accounting for 0.57% of the total number of diseased birds. In 2022 salmonellosis was registered in one outbreak area, where the number of diseased poultry was 4 birds (2.27% of the total number of salmonellosis-infected poultry).

Thus, the data provided indicate that these nosological units are registered annually in the Russian Federation, but as a result of veterinary and sanitary measures, the number of colibacillosis-infected poultry in the RF Subjects decreased from 66.18% in 2018 to 0.33% in 2022, and the number of salmonellosis-infected poultry decreased from 65.91% in 2019 to 2.27% in 2022 (Fig.). At the same time, the data obtained suggest that bacterial diseases still occupy a specific place in the nosological profile of poultry infectious pathology and may pose a danger to poultry

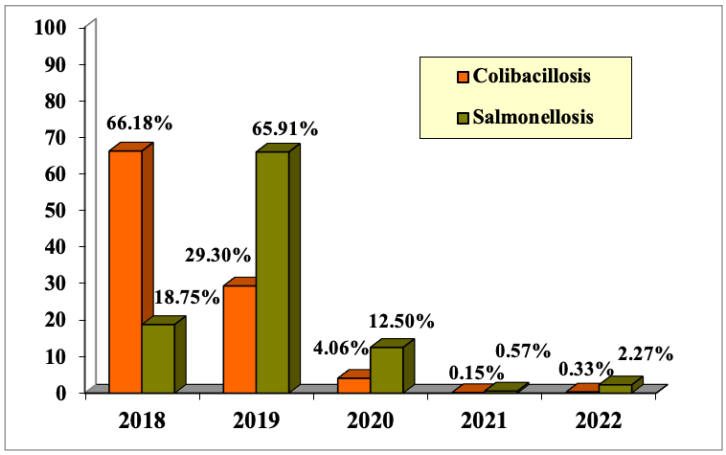

Fig. Avian colibacillosis and salmonellosis occurrence in the Russian Federation in 2018–2022 (mean percentage of the total number of diseased poultry for each disease)

Table 1
Results of tests of poultry meat and poultry products for *Salmonella* contamination in 2018–2020

Product name	Number of collected samples	Number of positive samples
Poultry meat	120,923	1,716 ± 15
Chicken eggs	87,259	21 ± 1
Melange, egg powder	10,838	20 ± 1
Total	219,020	1,757 ± 17

$p \leq 0.05$

Table 2
Results of tests of poultry meat and poultry products for mesophilic aerobic and facultative anaerobic microorganisms and coliforms (Total Viable Count)

Parameter	Number of collected samples	Number of positive samples	%
Total Viable Count	2,340	257 ± 12	10.98
Coliforms	2,340	120 ± 6	5.13

$p \leq 0.05$

establishments. Therefore, in order to ensure the animal disease freedom of poultry population and timely implement a complex of preventive, veterinary and sanitary measures, it is necessary to constantly monitor and analyze the data on colibacillosis and salmonellosis occurrence in poultry.

At the next stage of the study, a retrospective analysis of data on *Salmonella* contamination of poultry meat and poultry products obtained during laboratory tests in 2018–2020 was carried out. Results of tests of poultry products for *Salmonella* contamination are presented in Table 1.

According to the data obtained, 219,020 samples of poultry meat and poultry products were collected for testing, which included 120,923 samples of poultry meat, 87,259 samples of chicken eggs, 10,838 samples of melange and egg powder. It was established that *Salmonella* bacteria were detected in 1,716 ± 15 poultry meat samples, which is 1.42% of the total number of meat samples collected for testing. *Salmonella* was detected in chicken eggs in 0.02% of cases, the proportion of egg product samples (melange and egg powder) containing bacteria was 0.18%. Based on analysis of data on *Salmonella* contamination in 2018–2020, the following *Salmonella* serovars were most often found in poultry products: *S. enteritidis*, *S. typhimurium*, *S. infantis*.

The data from the automated VESTA system (laboratory testing of regulated product samples) in 2018–2020 were used for the retrospective analysis of the level of contamination with mesophilic aerobic and facultatively anaerobic microorganisms and coliforms (total viable count) and *Escherichia coli* bacteria (coliforms) in poultry meat and poultry products. It should be noted that these microbiological safety parameters are specified in the Technical Regulations of the Customs Union "On Food Safety" (TR CU 021/2011) and the Eurasian Economic Union "On Safety of Poultry Meat and Processed Products Thereof" (TR EAEU 051/2021): the total viable count in fresh meat shall not exceed 10 CFU/g (cm^3), in chicken and quail eggs – not more than 100 CFU/g (cm^3); no coliforms are allowed in 1.0 g (cm^3) of fresh meat and 0.1 g (cm^3) of chicken and quail eggs.

During the specified period 2,340 samples were tested. As the data in Table 2 show, a significant number of samples do not meet the requirements of regulatory documents according to the VESTA automated system.

CONCLUSION

According to the Ministry of Agriculture of the Russian Federation, the retrospective analysis of colibacillosis and salmonellosis occurrence in poultry farms in the country in 2018–2022 showed that 827,442 birds got infected with colibacillosis in 190 outbreak areas, salmonellosis was registered in 176 birds in 18 outbreak areas during the entire study period. In 2018–2020 219,020 samples of poultry meat and poultry products were subjected to bacteriological testing for the presence of *Salmonella*. It was found that *Salmonella* bacteria were detected in 1.42% of poultry meat samples of the total number of meat samples collected for testing, in 0.02% of chicken egg samples, in 0.18% of egg product (melange and egg powder) samples. Despite the seemingly small percentage of bacterial detections, it should be remembered that *Salmonella* is a pathogenic microorganism and its presence is not allowed in 25 g of meat products, therefore, the aspects of bacterial avian disease prevention have not lost their relevance to date.

Based on the results of our own research and literature data, we came to the conclusion that deterioration of the animal health situation in poultry farms may affect the public health due to consuming unsafe *Salmonella*-contaminated poultry products. Therefore, it is necessary to ensure assessment of the animal health situation in poultry farms, epizootic monitoring in regions with developed poultry farming, improvement of measures for the prevention and control of poultry infectious diseases and compliance with veterinary and sanitary requirements at each production stage to achieve the biological safety of poultry meat and poultry products.

REFERENCES

1. Gerasimova A. O., Novikova O. B., Savicheva A. A. Avian colibacillosis – current aspects. *Veterinary Science Today*. 2023; 12 (4): 284–292. https://doi.org/10.29326/2304-196X-2023-12-4-284-292

2. Ruzina A. V., Rozhdestvenskaya T. N., Pankratov S. V. Epizootological and epidemiological aspects of avian

salmonellosis. *Poultry & Chicken Products*. 2022; (5): 35–37. https://elibrary.ru/ddbzya (in Russ.)

3. Spiridonov A. N., Petrova O. N., Irza V. N., Karaulov A. K., Nikiforov V. V. Epizootic situation on infectious avian diseases based on analysis of data from veterinary reports. *Veterinary Science Today*. 2015; (4): 18–28. https://elibrary.ru/vodnwv

4. Kurmakaeva T. V., Baranovich E. S., Yenshin A. V., Sautkin A. V. The importance of individual bacterial diseases for farmers engaged in poultry breeding and processing. *Dopolnitel'noe professional'noe obrazovanie APK: nauchnoe i konsul'tatsionnoe obespechenie (informatsionnoe i konsul'tatsionnoe obespechenie pri realizatsii gosudarstvennoi agrarnoi politiki): materialy 4-i Mezhdunarodnoi nauchno-prakticheskoi konferentsii (8 fevralya 2023 g.) = Advanced professional training in the agro-industrial complex: scientific and advisory support (information and advisory support in the implementation of state agricultural policy): proceedings of the 4th International Scientific and Practical Conference (8 February 2023).* Moscow: Russian Academy of Personnel Support for the Agroindustrial Complex; 2023; 223–233. https://elibrary.ru/vhtdpy (in Russ.)

5. Kozak S. Prevention of toxic infections during poultry processing. *Animal Husbandry of Russia*. 2021; (7): 15–18. https://doi.org/zzr.ru/zzr-2021-07-005 (in Russ.)

6. Seregin I. G., Baranovich E. S., Kurmakaeva T. V., Gusarova M. L. Infektsionnye bolezni, vyyavlyaemye pri vyrashchivanii i pererabotke ptitsy = Infectious diseases detected during poultry raising and processing. *BIO*. 2019; (6): 14–17. https://elibrary.ru/kgakzy (in Russ.)

7. Sochnev V. V., Pashkina Yu. V., Kozyrenko O. V., Pashkin A. V., Nikulin V. V., Filippov V. N., et. al. Evidence-based epizootology (scientific research methodology): study guide. Ed. by A. G. Samodelkin, V. V. Sochnev. 4th ed., revised and supplemented. Nizhny Novgorod; Nizhny Novgorod State Agricultural Academy; BIKAR; 2016. 160 p. https://www.elibrary.ru/download/elibrary_28775604_13415048.pdf (in Russ.)

8. Shilkina L. V., Kozyrenko O. V., Kolobov E. A., Piuncina V. V., Pomazov E. A., Jezlova N. V., et al. Episootic particularities of forming of nozoprofile of infectious and invasive pathology in Nizhniy Novgorod area conditions. *Veterinarian*. 2012; (6): 2–4. https://elibrary.ru/ptzucn (in Russ.)

9. Seryogin I. G., Nikitchenko D. V., Abdullaeva A. M. About illness of foodborne diseases. *RUDN Journal of Agronomy and Animal Industries.* 2015; (4): 101–107. https://doi.org/10.22363/2312-797X-2015-4-101-107 (in Russ.)

10. Hafez H. M., El-Adawy H. Foodborne diseases of poultry and related problems. *Journal of Food Nutrition and Metabolism*. 2019; 1 (1): 2–5. https://doi.org/10.31487/j.JFNM.2018.01.005

11. Novikova O. B., Pavlova M. A., Bartenev A. A. O probleme kolibakterioza v ptitsevodstve = On collibacillosis in poultry industry. *Effektivnoe zhivotnovodstvo.* 2018; (6): 64–66. https://elibrary.ru/uyqkce (in Russ.)

12. Rozhdestvenskaya T. N., Ruzina A. V., Pankratov S. V., Tomina E. V. Kolibakterioz ptits: faktory patogennosti vozbuditelya i profilaktika bolezni = Avian colibacillosis: pathogenicity factors and disease prevention. *Sovremennye nauchnye razrabotki i peredovye tekhnologii dlya promyshlennogo ptitsevodstva: sbornik statei nauchno-prakticheskoi konferentsii (12–14 ijulya 2023 g.) = Modern scientific developments and advanced technologies for industrial poultry farming: a collection of papers of a scientific and practical conference (July 12–14, 2023).* Saint Petersburg: Mediapapir; 2023; 100–104. https://www.elibrary.ru/uvolms (in Russ.)

13. Zhitenko P. V., Seregin I. G., Nikitchenko V. E. Meat inspection and poultry processing technology: study-book. Moscow: Akvarium; 2001. 350 p. (in Russ.)

14. Oliveira G. da S., McManus C., dos Santos V. M. Control of *Escherichia coli* in poultry using the *in ovo* injection technique. *Antibiotics*. 2024; 13 (3):205. https://doi.org/10.3390/antibiotics13030205

15. Manges A. R. *Escherichia coli* and urinary tract infections: the role of poultry-meat. *Clinical Microbiology and Infection*. 2016; 22 (2): 122–129. https://doi.org/10.1016/j.cmi.2015.11.010

16. Mitchell N. M., Johnson J. R., Johnston B., Curtiss R. 3rd, Mellata M. Zoonotic potential of *Escherichia coli* isolates from retail chicken meat products and eggs. *Applied and Environmental Microbiology*. 2015; 81 (3): 1177–1187. https://doi.org/10.1128/AEM.03524-14

17. Novikova O. B., Pavlova M. A. System of control of bacterial diseases of birds in modern conditions of industrial poultry. *Innovations in Agricultural Complex: problems and perspectives*. 2017; (4): 153–159. https://elibrary.ru/zvzphb (in Russ.)

18. Alexandrova Ya. R., Kozak S. S., Borovkov M. F., Baranovich E. S., Kozak Yu. A. Detection of salmonella in biological material of animals, poultry and livestock products. *Vestnik Chuvash State Agrarian University*. 2023; (1): 45–49. https://doi.org/10.48612/vch/859n-x394-vdea (in Russ.)

19. Antunes P., Mourão J., Campos J., Peixe L. Salmonellosis: the role of poultry meat. *Clinical Microbiology and Infection*. 2016; 22 (2): 110–121. https://doi.org/10.1016/j.cmi.2015.12.004

20. Javadov E. J., Novikova O. B., Kraskov D. A., Berezkin V. A. Bolezni ptits, vyzyvaemye uslovno-patogennoi mikrofloroi = Avian diseases caused by some opportunistic microorganisms. *Effectivnoe zhivotnovodstvo.* 2023; (6): 8–12. https://doi.org/10.24412/cl-33489-2023-6-8-12 (in Russ.)

21. Ruzina A. V. Microbiological monitoring of the spread of avian salmonellosis in poultry farms of the Russian Federation. *Proceedings of the All-Russian Research Institute of Experimental Veterinary Sciences named after Ya. R. Kovalenko.* 2023; 83: 121–127. https://www.elibrary.ru/gmmgst (in Russ.)

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Adjuvant properties of chitosan derivatives administered to mice with anti-rabies vaccine

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ABSTRACT
Searching for a preparation that would meet all the requirements for modern adjuvants remains a matter of critical importance for specific immunoprophylaxis. Much information is available now on chitosan positive effect, including its effect on the immune response. The article provides results of the preclinical tests for different affordable chitosan-based products. For the test purposes, we took the following three products manufactured by LLC Bioprogress (Shchelkovo, Russia): water-soluble chitosan (succinate) – 2% solution edible chitosan (water-soluble) – 2% solution; edible chitosan (acid-soluble) – 2% solution, as well as anti-rabies vaccine RABIKOV manufactured by Shchelkovo Biocombinat (Russia). Immunogenic properties of chitosan-based products were tested in 85–100-day-old female white laboratory mice weighing 21–35 g. The animals were divided into 37 groups (6 mice in each group). Chitosan-based products were administered subcutaneously or intramuscularly, either together with the anti-rabies vaccine or without it. Animals from the control groups received either saline solution or the vaccine only. There was also a group of intact animals. The experiment demonstrated that the water-soluble chitosan (succinate) administered subcutaneously, acid-soluble edible chitosan (at a concentration of 1:64 and more), and water-soluble edible chitosan (at a concentration of $1:10^8$) administered subcutaneously and intramuscularly increase the level of post-vaccination anti-rabies antibodies. Thus, the tested chitosan-based products do not have any negative impact on the laboratory animals and have immunogenic properties.

Keywords: chitosan, adjuvant, vaccination, immunoprophylaxis, RABIES vaccine, RABIKOV vaccine, cytotoxicity, immunity, antibodies, preclinical tests

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Адъювантные свойства производных хитозана при введении мышам антирабической вакцины

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РЕЗЮМЕ
Для специфической иммунопрофилактики особенно актуальным остается вопрос поиска препарата, который бы отвечал всем требованиям, предъявляемым к современным адъювантам. В литературе много сведений о положительном влиянии хитозана, в том числе и на иммунную систему. В статье представлены результаты доклинических испытаний препаратов на основе различных форм хитозана, которые являются экономически доступными. В качестве испытуемых были взяты три препарата производства ООО «Биопрогресс» (г. Щелково, Россия): хитозан водорастворимый (сукцинат) – 2%-й раствор; хитозан пищевой (водорастворимый) – 2%-й раствор; хитозан пищевой (кислоторастворимый) – 2%-й раствор; а также вакцина против бешенства «Рабиков» производства ФКП «Щелковский биокомбинат» (Россия). Изучение иммуногенных свойств препаратов хитозана проводили на 85–100-суточных самках белых лабораторных мышей массой 21–35 г. Животные были поделены на 37 групп по 6 мышей в каждой. Препараты хитозана применяли подкожно или внутримышечно сочетанно с антирабической вакциной или без таковой. Животным контрольных групп вводили либо физиологический раствор, либо только вакцину. Также была сформирована группа интактных животных. Показано, что хитозан водорастворимый (сукцинат) при подкожном введении, хитозан пищевой (кислоторастворимый) в концентрации 1:64 и выше и хитозан пищевой (водорастворимый) в концентрации $1:10^8$ при подкожном и внутримышечном способах введения повышают уровень поствакцинальных антирабических антител. Таким образом, исследуемые препараты на основе хитозана не оказывают негативного влияния на организм лабораторных животных и обладают иммуногенными свойствами.

Ключевые слова: хитозан, адъювант, вакцинация, иммунопрофилактика, вакцина «Рабиес», вакцина «Рабиков», цитотоксичность, иммунитет, антитела, доклиническое исследование

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INTRODUCTION

Scientists from all over the world are looking for the best options to ensure effective and safe specific prevention of animal diseases. Despite the progress made in the vaccine production, searching for a low-cost and safe adjuvant that would enhance the immune response remains a matter of great importance.

Modern adjuvants shall boost immunity (cell-mediated and/or humoral), be easily metabolized and be widely affordable [1, 2].

Chitosan properties are being actively studied worldwide [3–20]. Its derivatives are widely used in the pharmaceutical manufacturing and in veterinary medicine. Chitosan-based products have shown a variety of biological properties, including antimicrobial and cholesterol-lowering ones. The chitosan properties increase the dissociation rate of poorly soluble medicinal products, enhance their absorption, influence the drug release and create drugs of prolonged action [2]. It was also noted that chitosan solution boosts both the humoral and cell-mediated immune response after subcutaneous administration of vaccines [15]. It is also possible to inject chitosan as a part of medicinal products and vaccines [21].

The available literature provides information on chitosan as an antigen sorbent and a stimulator of the post-vaccination immune response [3, 7, 8, 9, 10, 12, 13, 14, 17, 18, 19, 20, 22]. Progress made in studying the properties of chitosan derivatives suggests that they may meet the basic requirements for modern adjuvants.

Previously, in order to find optimal concentrations of chitosan-based products for administration to animals, we analyzed the cytotoxicity of various dilutions of the tested samples in the continuous bovine kidney cell line PT-80 [6, 11].

The purpose of this research is to study adjuvant properties of chitosan derivatives in various concentrations, administered to mice together with an anti-rabies vaccine.

MATERIALS AND METHODS

Chitosan. The following chitosan-containing products manufactured by Bioprogress (Shchelkovo, Russia) on the basis of saline solution (NaCl 0.9%), were taken as starting materials:

– water-soluble chitosan (succinate), 2% solution – Preparation No. 1;

– edible chitosan (water-soluble), 2% solution – Preparation No. 2;

– edible chitosan (acid-soluble), 2% solution – Preparation No. 3.

Anti-rabies vaccines for animals:

– anti-rabies vaccine RABIKOV manufactured by the Shchelkovo Biocombinat (Russia);

– anti-rabies vaccine RABIES (Intervet International, B. V., the Netherlands) was used to compare protective properties with the domestically produced RABIKOV vaccine as part of the implementation of import substitution measures.

Experiment design. Thirty-seven homogeneous groups of female white lab mice were formed for experimental purposes. The mice weighed 21–35 g and were 85–100 days old. Six mice were included in each group. The mice were injected with the tested products (at a volume of 0.3 cm^3), according to the scheme given in the table.

Before the experiment, all the laboratory animals stayed in quarantine for 14 days. The experimental mice were subjected to daily clinical examination and control weighing. On day 28 postadministration, the animals were decapitated and pathological material (organs and blood serum) was taken for further tests.

All animal experiments were conducted in strict compliance with Interstate Standards GOST 33215-2014, GOST 33216-2014 as adopted by the Interstate Council for Standardization, Metrology and Certification, as well as in accordance with Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

The mass index of organs is calculated as the ratio of the organs mass to animal body mass (organ mass index = m_{organ}/m_{animal}).

Calculating titers of rabies virus-neutralizing antibodies. In order to observe adjuvant properties of chitosan-based preparations, we measured the level of rabies virus-neutralizing antibodies in sera from laboratory animals using diffusion precipitation test, according to the instruction for "Diffusion precipitation test kit for rabies diagnosis" produced by the All-Russian Research and Technological Institute of Biological Industry (Russia).

Processing statistics. The obtained statistics were processed using the standard Microsoft Excel 2007 program

adopted in biology and medicine. The results were considered reliable at the probability level of $p < 0.05$.

RESULTS AND DISCUSSION

Preliminary tests in PT-80 cell line have shown that edible chitosan (acid-soluble) at a concentration of 1:4 has a cytotoxic effect [6], therefore, during the experiment 1:64 and $1:10^8$ concentrations were used in the lab animals.

During the observation, no deviations in mice behavior were reported, and there were no specific death cases. Further autopsy revealed no pathological lesions at the injection sites (inflammation, granulation, etc.) or in the internal organs [6].

The liver is an organ that increases in size due to acute toxicity and decreases in size due to chronic toxicity. Therefore, the organ mass index was calculated.

Figure 1 shows that the mean liver mass index in all mice groups ranged from 0.045 to 0.050. This suggests that the tested preparations do not have any acute toxicity effects.

The spleen is the largest lymphoid organ. Therefore, to assess the immunity response to the administration of the tested preparations, the organ mass index was calculated for experimental mice.

The mean spleen mass index calculated (Fig. 2) for groups 1–8 and 10–16, either corresponded to the data obtained for groups 19, 20 and 37, or exceeded them ($p \leq 0.05$). This may suggest that the mice's immune system is stimulated by the chitosan-containing preparations. However, in group No. 9, the mean spleen mass index was lower than in the control groups. It can be assumed that intramuscularly administered edible chitosan (water-soluble) at a concentration of 1:64 does not have any pronounced immunostimulating effect.

To confirm chitosan adjuvant properties, the next step was to measure the level of rabies virus-neutralizing antibodies in the sera from laboratory mice using diffusion precipitation test. The experiment results are given in Figure 3.

The data obtained show that the titres of rabies virus-neutralizing antibodies post-vaccination without the tested preparations (groups No. 19 and 20) were 1:32; whereas those groups that were subcutaneously vaccinated together with water-soluble chitosan (succinate) in all the tested concentrations (groups No. 2, 4 and 6) showed the antibody level of 1:64. Regardless of the administration route, edible chitosan (acid-soluble) at all the tested concentrations (groups No. 13–16) and edible chitosan (water-soluble) at a concentration of $1:10^8$ (groups No. 11 and 12) stimulate antibody production.

Water-soluble chitosan (succinate) was administered intramuscularly at all the tested concentrations (groups No. 1, 3 and 5), edible chitosan (water-soluble) was administered subcutaneously (concentration 1:4, group No. 8) and intramuscularly (concentration 1:64, group No. 9) reduced the level of rabies virus-neutralizing antibodies down to 1:8 – 1:16. In this regard, it can be assumed that the tested preparations (at the given concentrations and administered using the mentioned routes) suppress the immune response, since natural chitosan salts are practically insoluble at pH above 6, which may be problematic for the delivery of vaccine antigens that are soluble and stable at neutral pH or higher [2].

Table
Routes of administration of the tested products to the mice groups

Preparation	Dilution	Group number	Administration route
Experimental groups			
Preparations with vaccine RABIKOV			
Preparation No. 1	1:4	1	intramuscularly
		2	subcutaneously
	1:64	3	intramuscularly
		4	subcutaneously
	$1:10^8$	5	intramuscularly
		6	subcutaneously
Preparation No. 2	1:4	7	intramuscularly
		8	subcutaneously
	1:64	9	intramuscularly
		10	subcutaneously
	$1:10^8$	11	intramuscularly
		12	subcutaneously
Preparation No. 3	1:64	13	intramuscularly
		14	subcutaneously
	$1:10^8$	15	intramuscularly
		16	subcutaneously
Control groups			
Saline solution			
Saline solution (NaCl 0.9%)	–	17	intramuscularly
	–	18	subcutaneously
Vaccine control			
Vaccine RABIKOV	–	19	subcutaneously
Vaccine RABIES	–	20	subcutaneously
Tested products without a vaccine			
Preparation No. 1	1:4	21	intramuscularly
		22	subcutaneously
	1:64	23	intramuscularly
		24	subcutaneously
	$1:10^8$	25	intramuscularly
		26	subcutaneously
Preparation No. 2	1:4	27	intramuscularly
		28	subcutaneously
	1:64	29	intramuscularly
		30	subcutaneously
	$1:10^8$	31	intramuscularly
		32	subcutaneously
Preparation No. 3	1:64	33	intramuscularly
		34	subcutaneously
	$1:10^8$	35	intramuscularly
		36	subcutaneously
No products administered (intact)	–	37	–

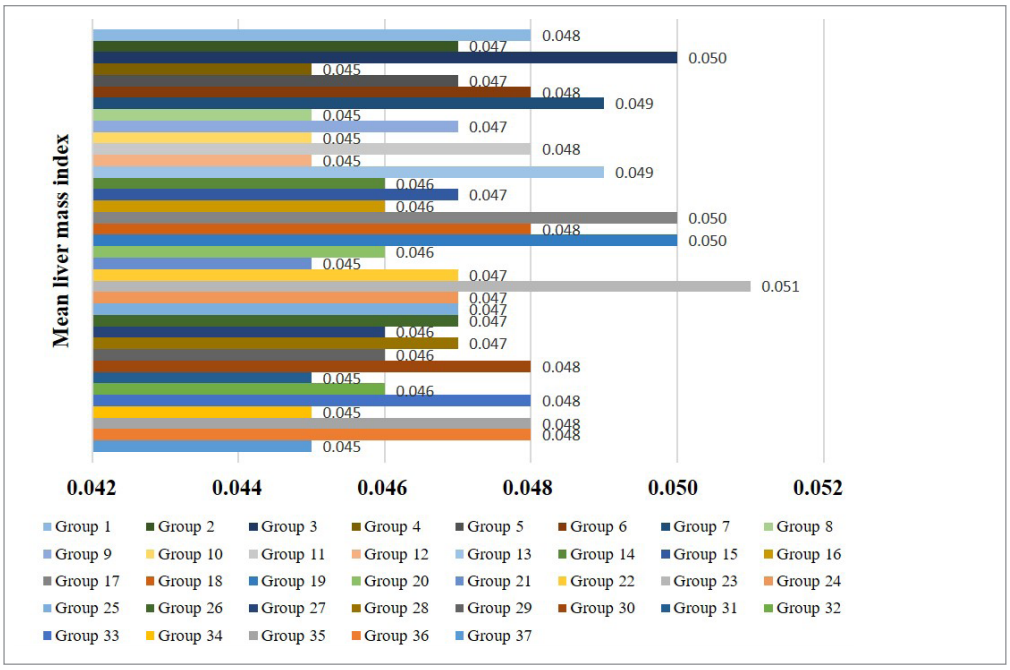

Fig. 1. Liver/body weight ratio in the laboratory mice after administration of the tested forms and concentrations of the chitosan-based products

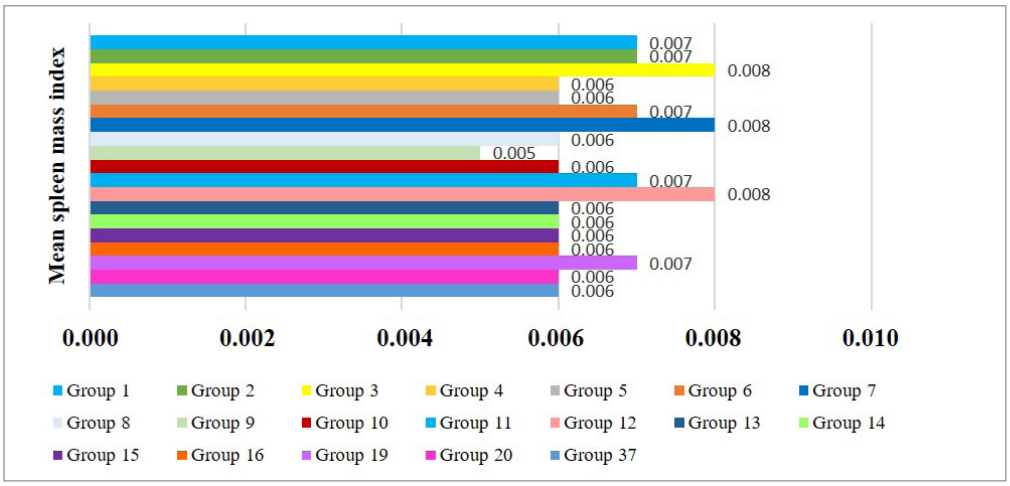

Fig. 2. Spleen/body weight ratio in the laboratory mice after administration of the tested forms and concentrations of the chitosan-based products

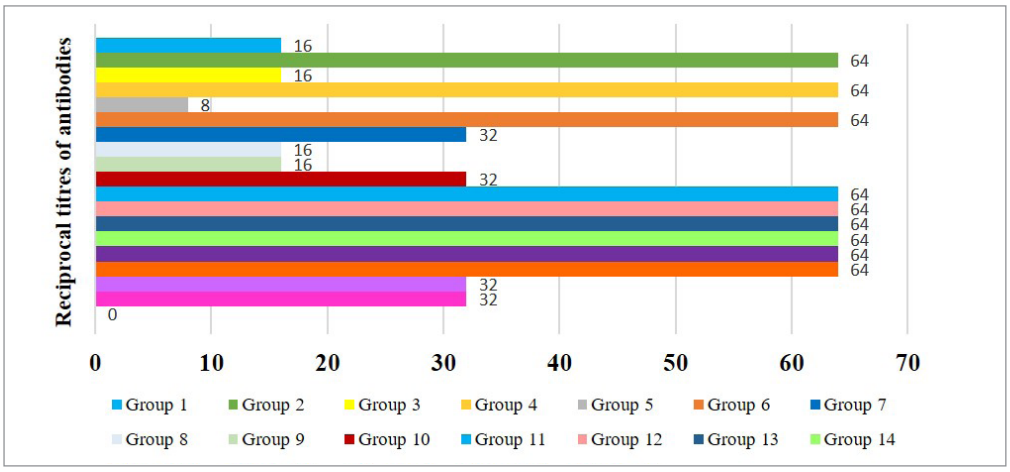

Fig. 3. Post-vaccination antibody titres against rabies virus antigen as demonstrated by the diffusion precipitation test

CONCLUSION

Thus, it has been shown that the tested chitosan-containing preparations do not have a negative impact on the laboratory animals and have immunogenic properties.

The following preparations can be recommended as affordable adjuvants: water-soluble chitosan (succinate) for subcutaneous administration; edible chitosan (acid-soluble) at a concentration of 1:64 and above, as well as edible chitosan (water-soluble) at a concentration of $1:10^8$ (administered subcutaneously and intramuscularly). Alongside it, our test results as well as results provided by other researchers [2] show that water-soluble chitosan (succinate) administered intramuscularly and edible chitosan (water-soluble) administered subcutaneously at a concentrations of 1:4 and administered intramuscularly at a concentration of 1:64 reduce the vaccine efficacy.

REFERENCES

1. Avdeeva Zh. I., Alpatova N. A., Bondarev V. P., Volkova R. A., Lonskaya N. I., Lebedinskaya E. V., et al. Vaccines with adjuvants. Preclinical studies. *BIOpreparations. Prevention, Diagnosis, Treatment*. 2015; (1): 15–20. https://elibrary.ru/ubekft (in Russ.)

2. Samuilenko A. Ya., Grin S. A., Eremets V. I., Albulov A. I., Eremets N. K., Borovoy V. N., et al. Adjuvants. Moscow: August Borg; 2016. 171 p. https://elibrary.ru/zsdher (in Russ.)

3. Albulov A. I., Frolova M. A., Krasochko P. A., Krasochko P. P., Grin A. V., Eliseev A. K. Ispol'zovanie khitozana v kachestve ad"yuvanta pri proizvodstve vaktsinnykh preparatov = The use of chitosan as an adjuvant in vaccine production. *Sovremennye perspektivy v issledovanii khitina i khitozana (Roskhit-23): materialy shestnadtsatoi Vserossiiskoi konferentsii s mezhdunarodnym uchastiem (Vladivostok, 2–6 oktyabrya 2023 g.) = Current perspectives on chitin and chitosan (Roskhit-23): Proceedings of the Sixteenth All-Russian Conference with International Participation (Vladivostok, 2–6 October 2023)*. Vladivostok: Far Eastern Federal University; 2023; 155–158. https://doi.org/10.24866/7444-5553-8 (in Russ.)

4. Varlamov V. P., Il'ina A. V., Shagdarova B. Ts., Lunkov A. P., Mysyakina I. S. Chitin/Chitosan and its derivatives: Fundamental problems and practical approaches. *Biochemistry (Moscow)*. 2020; 85 (Suppl. 1): 154–176. https://doi.org/10.1134/S0006297920140084

5. Dobroskok K. B., Lipatova M. S., Yarygina E. I. The effect of chitosan succinate on the formation of a monolayer of PT-80 cell culture. *Sbornik nauchnykh trudov desyatoi Vserossiiskoi mezhvuzovskoi konferentsii po klinicheskoi veterinarii v formate Purina Partners (Moskva, 18 dekabrya 2020 g.) = Collection of scientific papers of the Tenth All-Russian Interuniversity Conference on Clinical Veterinary Medicine in Purina Partners format (Moscow, December 18, 2020)*. Moscow: NPO "Sel'skokhozyaistvennye tekhnologii"; 2020; 453–460. https://elibrary.ru/slcelz (in Russ.)

6. Dobroskok K. B., Yarygina E. I. Doklinicheskie issledovaniya razlichnykh form khitozana na biologicheskikh sistemakh: kul'ture kletok PT-80 i laboratornykh myshakh = Preclinical studies of various forms of chitosan on biological systems: PT-80 cell culture and laboratory mice. *Aktual'nye voprosy biologii, biotekhnologii, veterinarii, zootekhnii, tovarovedeniya i pererabotki syr'ya zhivotnogo i rastitel'nogo proiskhozhdeniya: materialy natsional'noi nauchno-prakticheskoi konferentsii (Moskva, 1 aprelya 2021 g.). Chast' II = Current issues of biology, biotechnology, veterinary medicine, animal science, commodity science and processing of raw materials of animal and plant origin: proceedings of the national scientific and practical conference (Moscow, April 1, 2021). Part II*. Moscow: Moscow State Academy of Veterinary Medicine and Biotechnology – MVA by K. I. Skryabin; 2021; 14–16 (in Russ.)

7. Kurashova S. S., Dzagurova T. K., Ishmukhametov A. A., Egorova M. S., Balovneva M. V., Sotskova S. E., Tkachenko E. A. Carbohydrate-based adjuvants for vaccine production. *BIOpreparations. Prevention, Diagnosis, Treatment*. 2018; 18 (2): 81–91. https://doi.org/10.30895/2221-996X-2018-18-2-81-91 (in Russ.)

8. Minkova O. A., Yarygina E. I., Bachinskaya V. M. Vliyanie preparata khitozana v sostave vaktsiny na prodolzhitel'nost' immunnogo otveta u kur = Effect of the chitosan-based vaccine on the immunity duration in chickens. *Vaktsiny novogo pokoleniya dlya profilaktiki osobo opasnykh boleznei sel'skokhozyaistvennykh zhivotnykh: sbornik trudov Mezhdunarodnoi nauchno-prakticheskoi konferentsii (Moskva, 10 oktyabrya 2023 g.). Pod. obshch. red. S. V. Pozyabina, L. A. Gnezdilovoi = Next-generation vaccines for control of highly dangerous diseases in farm animals: proceedings of the International Scientific and Practical Conference (Moscow, October 10, 2023). Ed. by S. V. Pozyabin, L. A. Gnezdilova*. Moscow: Sel'skokhozyaistvennye tekhnologii; 2023; 224–230. https://elibrary.ru/erolzp (in Russ.)

9. Khantimirova L. M. Preparation of chitosan and its derivatives, study of their physico-chemical characteristics and immunoadjuvant activity in the composition of inactivated influenza vaccines: Author's abstract of thesis for degree of Cand. Sci. (Biology). M.; 2019. 24 p. (in Russ.)

10. Khantimirova L. M., Kashirina O. S., Chernikova M. I., Vasiliev Y. M. Comparative immunogenicity evaluation of characterized chitosan-based and other adjuvants for inactivated influenza vaccines. *Epidemiology and Vaccinal Prevention*. 2016; 15 (1): 86–92. https://doi.org/10.31631/2073-3046-2016-15-1-86-92 (in Russ.)

11. Yarygina E. I., Kalmykova M. S., Tretyakova I. V. Study of cytotoxic effect of drugs based on chitosan for transplantable cell cultures. *Veterinary, Zootechnics and Biotechnology*. 2019; (8): 40–43. https://elibrary.ru/vnvdpa (in Russ.)

12. Malik A., Gupta M., Gupta V., Gogoi H., Bhatnagar R. Novel application of trimethyl chitosan as an adjuvant in vaccine delivery. *International Journal of Nanomedicine*. 2018; 13: 7959–7970. https://doi.org/10.2147/ijn.s165876

13. Choi B., Jo D.-H., Anower A. K. M. M., Islam S. M. S., Sohn S. Chitosan as an immunomodulating adjuvant on T-cells and antigen-presenting cells in herpes simplex virus type 1 infection. *Mediators of Inflammation*. 2016; 2016:4374375. https://doi.org/10.1155/2016/4374375

14. Zaharoff D. A., Rogers C. J., Hance K. W., Schlom J., Greiner J. W. Chitosan solution enhances both humoral and cell-mediated immune responses to subcutaneous vaccination. *Vaccine*. 2017; 25 (11): 2085–2094. https://doi.org/10.1016/j.vaccine.2006.11.034

15. Younes I., Rinaudo M. Chitin and chitosan preparation from marine sources. Structure, properties and applications. *Marine Drugs*. 2015; 13 (3): 1133–1174. https://doi.org/10.3390/md13031133

16. Vázquez J. A., Rodríguez-Amado I., Montemayor M. I., Fraguas J., González M. del P., Murado M. A. Chondroitin sulfate, hyaluronic acid and chitin/chitosan production using marine waste sources: characteristics, applications

and eco-friendly processes: A review. *Marine Drugs*. 2013; 11 (3): 747–774. https://doi.org/10.3390/md11030747

17. Muzzarelli R. A. A. Chitins and chitosans as immunoadjuvants and non-allergenic drug carriers. *Marine Drugs*. 2010; 8 (2): 292–312. https://doi.org/10.3390/md8020292

18. Vasiliev Y. M. Chitosan-based vaccine adjuvants: incomplete characterization complicates preclinical and clinical evaluation. *Expert Review of Vaccines*. 2015; 14 (1): 37–53. https://doi.org/10.1586/14760584.2015.956729

19. Gong Y., Tao L., Wang F., Liu W., Jing L., Liu D., et al. Chitosan as an adjuvant for a *Helicobacter pylori* therapeutic vaccine. *Molecular Medicine Reports*. 2015; 12 (3): 4123–4132. https://doi.org/10.3892/mmr.2015.3950

20. Xia Y., Fan Q., Hao D., Wu J., Ma G., Su Z. Chitosan-based mucosal adjuvants: Sunrise on the ocean. *Vaccine*. 2015; 33 (44): 5997–6010. https://doi.org/10.1016/j.vaccine.2015.07.101

21. Kamskaya V. E. Chitosan: structure, properties and using. *Scientific review. Biological sciences*. 2016; (6): 36–42. https://science-biology.ru/ru/article/view?id=1020 (in Russ.)

22. Wen Z. S., Xu Y. L., Zou X. T., Xu Z. R. Chitosan nanoparticles act as an adjuvant to promote both Th1 and Th2 immune responses induced by ovalbumin in mice. *Marine Drugs*. 2011; 9 (6): 1038–1055. https://doi.org/10.3390/md9061038

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Studying immunotherapeutic properties of the conjugate based on BCG antigens with betulonic acid in guinea pigs infected with *Mycobacterium scrofulaceum*

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ABSTRACT

The paper reports on the research into the immunotherapeutic properties of a conjugate based on BCG antigens with betulonic acid after experimental infection of guinea pigs with *Mycobacterium scrofulaceum* culture, belonging to nontuberculosis mycobacteria type II according to the Runyon classification. Fifteen guinea pigs were used for the experimental purposes, divided into 3 groups. *Mycobacterium scrofulaceum* was subcutaneously injected into animals of groups 1 and 2 ($n = 10$) at a dose of 5 mg. Fourteen days later, a conjugate based on BCG antigens with betulonic acid was subcutaneously injected into animals of group 2 ($n = 5$) at a dose of 500 μg/mL of protein. Five intact animals were used as controls. During the experiment, neutrophil bactericidal activity was assessed, and histopathological examination of inguinal lymph nodes was done. The experiment showed that the inoculation of *Mycobacterium scrofulaceum* into guinea pigs activates cationic proteins and neutrophil myeloperoxidase, and on experiment day 42 (preceded by mycobacteria withdrawal from the body) their concentration reduced to the level of the control group. The vaccine administration induced a more active intracellular phagocyte metabolism during the entire observation period, which resulted in the elimination of nontuberculosis mycobacteria in animals as early as day 7 after treatment with the conjugate. The elimination was confirmed by the absence of mycobacterial antigen in blood smears tested in indirect immunofluorescence, as well as by histopathological changes in inguinal lymph nodes demonstrated as a reduction of germinal centers within lymphoid follicles.

Keywords: non-tuberculosis mycobacteria, guinea pigs, Bacillus Calmette-Guerin (BCG), betulonic acid, neutrophils, inguinal lymph nodes

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Изучение иммунотерапевтических свойств конъюгата антигенов БЦЖ с бетулоновой кислотой на морских свинках, инфицированных *Mycobacterium scrofulaceum*

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РЕЗЮМЕ

В настоящей работе представлены результаты изучения иммунотерапевтических свойств препарата из антигенного комплекса БЦЖ, конъюгированного с бетулоновой кислотой, после экспериментального заражения морских свинок культурой *Mycobacterium scrofulaceum,* относящейся к нетуберкулезным микобактериям II типа по классификации Раньона. С этой целью проведен опыт на 15 морских свинках, из которых было сформировано 3 группы. Животным 1-й и 2-й групп ($n = 10$) подкожно инокулировали *Mycobacterium scrofulaceum* в дозе 5 мг, после чего особям 2-й группы ($n = 5$) через 14 сут подкожно вводили конъюгат антигенов БЦЖ с бетулоновой кислотой в дозе 500 мкг/мл белка. Пять интактных особей служили контролем. При проведении экспериментов оценивали функциональное состояние бактерицидных систем нейтрофилов, а также выполняли патогистологические исследования паховых лимфатических узлов. В результате было установлено, что сенсибилизация морских свинок *Mycobacterium scrofulaceum* активизирует

деятельность катионных белков и миелопероксидазы нейтрофилов, и по мере выведения микобактерий из организма к 42-м сут от начала эксперимента их концентрация снижалась до уровня контрольной группы. Введение препарата индуцировало более выраженное усиление внутриклеточного метаболизма фагоцитов в течение всего срока наблюдения, способствуя элиминации нетуберкулезных микобактерий из организма животных уже на 7-е сут после обработки конъюгатом, что подтверждалось отсутствием микобактериального антигена в мазках крови при исследовании в реакции непрямой иммунофлуоресценции, а также патогистологическими изменениями в паховых лимфатических узлах, которые выражались уменьшением выраженных центров размножения в лимфатических фолликулах.

Ключевые слова: нетуберкулезные микобактерии, морские свинки, бацилла Кальмета – Герена (БЦЖ), бетулоновая кислота, нейтрофилы, паховые лимфатические узлы

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INTRODUCTION

Out of more than 190 currently known species of *Mycobacterium* genus a significant number belongs to non-tuberculous mycobacteria and over 60 species are pathogenic to animals and humans [1, 2].

Non-tuberculous mycobacteria may be found ubiquitously in the environment and they pose a serious problem for *in vivo* and postmortem diagnosis of bovine tuberculosis as they cause false positive response to administration of tuberculin due to antigenic determinants in the allergen, which are common to non-tuberculous and pathogenic mycobacteria. In addition, visible and microscopic changes induced by non-tuberculous mycobacteria are in some cases difficult to distinguish from lesions caused by *Mycobacterium tuberculosis* and *Mycobacterium bovis* [2, 3, 4, 5, 6].

Owing to a drop in bovine tuberculosis transmission and strong diagnostic measures taken to detect residual infection in the territories where disease control programs are in place, there has been an increase in mycobacterioses caused by non-tuberculous mycobacteria [7, 8, 9, 10]. Despite the growing interest, little data has been published so far on non-tuberculous mycobacterial infections, and the available literature is mainly focused on the *Mycobacterium avium* complex and its subspecies [11, 12, 13, 14, 15].

To solve the problem of non-specific reactions induced by non-tuberculous mycobacteria, specific immunoprophylactic or immunotherapeutic tools may be an extra option to complement lifetime differential tests (simultaneous, palpebral tests, etc.). Several recent studies suggest that cross-reactive response to non-tuberculous mycobacteria [16, 17, 18, 19] is induced by BCG vaccination, as well as by immunization with areactogenic conjugates based on protective antigens, isolated from the BCG vaccine, with polyions [20]. Conversely, some scientists claim that previous contacts with non-tuberculous mycobacteria may have an antagonistic effect, reducing vaccination effectiveness; however, this concern is only about live BCG vaccine and did not affect protective properties of inactivated subunit tuberculous vaccines [21, 22, 23, 24].

From our perspective, conjugates based on BCG antigens with betulin and its derivatives (betulonic and betulinic acids) may look promising in this regard. In particular, molecular docking has shown that betulonic acid in most cases exhibits the highest inhibitory activity against protein targets that are structural parts of *Mycobacterium tuberculosis* and/or *Mycobacterium bovis* [25].

In connection with the above, the purpose of this work is to study the immunotherapeutic efficacy of an experimental conjugate based on BCG antigens with betulonic acid.

MATERIALS AND METHODS

The experiment was conducted in Agouti guinea pigs in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes as of 18 March 1986, and was approved by the local independent ethical committee of the organization for the care and use of laboratory animals. Experimental animals were grouped based on common characteristics (weight – 400–500 g, age – 4–5 months).

From 14- to 21-day scotochromogenic mycobacteria *Mycobacterium scrofulaceum* (Runyon II: Scotochromogens) was used to infect experimental animals. It was administered subcutaneously into the left groin, at a dose of 5 mg/mL. *Mycobacterium* cultures were administered to 10 animals, further divided into 2 groups: group 1 – infected with *Mycobacterium scrofulaceum* ($n = 5$); group 2 – infected with *Mycobacterium scrofulaceum* and on day 14

after the administration, they were treated with conjugate of BCG antigen with betulonic acid ($n = 5$). The other five intact guinea pigs were used as controls.

The experimental conjugate of BCG antigenic complexes with betulonic acid was designed in accordance with the author's development. The preparation was administered subcutaneously to animals at a dose of 500 μg/mL of protein. Betulonic acid was synthesized at the Department of Organic and Environmental Chemistry of the Institute of Chemistry of the University of Tyumen and was kindly provided for research by Professor, Dr. Sci. (Chemistry) I. V. Kulakov.

Mycobacterial antigen in blood samples was detected using indirect immunofluorescence in accordance with the methodological recommendations of N. N. Novikova et al. [26]. Myeloperoxidase activity and number of neutrophil cationic proteins were measured using benzidine test and bromophenol blue test with phagocyte distributed depending on the number of cytoplasmic granules (1st, 2nd and 3rd degrees), followed by calculation of average cytochemical coefficients (ACC) using standard methods.

Before the start of the experiment and on day 21 post infection, allergy tests were performed using intradermal administration of purified tuberculin for mammals. Blood was sampled for serological tests on day 21 and 42 after administration of scotochromogenic mycobacteria; and on days 14, 28 and 42 to assess functional status of neutrophils.

The laboratory animals were euthanized under ether anesthesia followed by total exsanguination on day 45 after the beginning of the experiment. For histological tests pieces of inguinal lymph nodes were taken (from regional lymph nodes, i.e. the closest ones to the site of mycobacteria inoculation, as well as from the lymph nodes on the opposite side). The sampled pieces were placed into cassettes and submerge in 10% neutral buffered formalin, and then the tissue was paraffin-embedded using MICROM EC 350 (Thermo Fisher Scientific Inc., USA). Rotary Microtome HM 340E (produced by Thermo Fisher Scientific Inc., USA) was used to cut sample sections, ranging between 5 and 7 μm. Histological tissue preparations were stained with hematoxylin and eosin, and then examined microscopically.

Standard methods of variational statistics were used, such as calculation of arithmetic means (M) and calculation of errors of arithmetic means (m), to process the obtained data. Student's t-test was used to assess significance of differences (p) between the two mean values of Mx and My. The differences in the results were considered statistically significant at a significance level of $p \leq 0.05$.

RESULTS AND DISCUSSION

Inoculation of *Mycobacterium scrofulaceum* to guinea pigs enhanced oxygen-independent mechanisms of neutrophils, as evidenced by a 1.60 and 1.74-fold increase in phagocytes with a large number of cytoplasmic granules (3rd degree) containing cationic proteins in group 1 and 2, respectively ($p < 0.01$), as compared to the control group. Following these changes, average cytochemical coefficients also increased by a factor of 1.65 (Table 1).

Delayed-type hypersensitivity response to a tuberculin test conducted on day 21 post infection of guinea pigs was observed only in 60% of animals who had not received experimental preparation (group 1). Nevertheless, mycobacterial antigen was detected in all animals of this group using indirect immunofluorescence. Mean induration size in the reactors was 4.33 ± 0.33 mm.

Table 1
Level of neutrophil cationic proteins in animals at different moments post inoculation of *Mycobacterium scrofulaceum*, $M \pm m$

Cytochemical parameters	Group of animals		
	Control	Experimental group 1	Experimental group 2
Day 14 after inoculation of *Mycobacterium*			
1st degree, %	5.00 ± 0.58	11.33 ± 3.33	10.00 ± 3.05
2nd degree, %	9.66 ± 1.67	16.66 ± 2.40	10.00 ± 1.15
3rd degree, %	33.00 ± 1.15	52.66 ± 5.78*	57.33 ± 4.37**
Average cytochemical coefficient, conditional units	1.23 ± 0.02	2.03 ± 0.11**	2.02 ± 0.12**
Day 28 after inoculation of *Mycobacterium* (day 14 after administration of the preparation)			
1st degree, %	3.33 ± 0.67	8.33 ± 2.85	3.66 ± 0.88
2nd degree, %	14.00 ± 0.58	12.66 ± 2.40	9.66 ± 0.33**
3rd degree, %	29.33 ± 2.18	45.33 ± 1.33**	57.00 ± 4.04**
Average cytochemical coefficient, conditional units	1.19 ± 0.06	1.70 ± 0.05**	1.94 ± 0.11**
Day 42 after inoculation of *Mycobacterium* (day 28 after administration of the preparation)			
1st degree, %	5.33 ± 2.33	5.00 ± 0.58	2.66 ± 1.76
2nd degree, %	11.00 ± 0.58	11.66 ± 0.88	7.00 ± 1.73
3rd degree, %	30.00 ± 4.58	33.33 ± 2.73	71.66 ± 2.03***
Average cytochemical coefficient, conditional units	1.17 ± 0.12	1.28 ± 0.08	2.31 ± 0.08**

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Table 2
Enzyme activity of neutrophil myeloperoxidase in animals at different moments post inoculation of *Mycobacterium scrofulaceum*, M ± m

Cytochemical parameters	Group of animals		
	Control	Experimental group 1	Experimental group 2
Day 14 after inoculation of *Mycobacterium*			
1st degree, %	9.33 ± 0.67	9.66 ± 0.88	10.33 ± 3.18
2nd degree, %	12.33 ± 1.85	18.66 ± 1.67	19.33 ± 2.33
3rd degree, %	21.33 ± 3.53	42.66 ± 1.33**	43.00 ± 3.21*
Average cytochemical coefficient, conditional units	0.98 ± 0.08	1.75 ± 0.06**	1.78 ± 0.02**
Day 28 after inoculation of *Mycobacterium* (day 14 after administration of the preparation)			
1st degree, %	5.66 ± 0.67	15.00 ± 1.53	10.00 ± 0.58**
2nd degree, %	7.33 ± 2.60	14.66 ± 2.33	13.00 ± 2.08
3rd degree, %	23.33 ± 0.88	36.00 ± 5.68	44.66 ± 4.98*
Average cytochemical coefficient, conditional units	0.90 ± 0.06	1.52 ± 0.13*	1.70 ± 0.18*
Day 42 after inoculation of *Mycobacterium* (day 28 after administration of the preparation)			
1st degree, %	7.66 ± 1.33	7.33 ± 0.33	5.66 ± 1.85
2nd degree, %	8.66 ± 2.33	13.66 ± 3.18	12.66 ± 1.45
3rd degree, %	26.00 ± 1.00	23.33 ± 2.03	59.33 ± 0.88***
Average cytochemical coefficient, conditional units	1.03 ± 0.03	1.05 ± 0.04	2.09 ± 0.01***

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

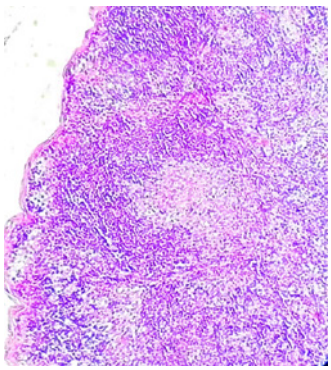

Fig. 1. A lymphoid follicle with a large germinal center. Regional lymph node of a guinea pig (group 1). Staining with hematoxylin and eosin, magnification 50×

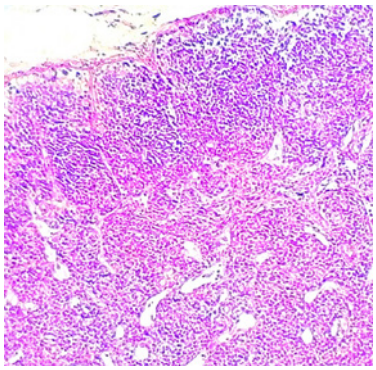

Fig. 2. Reduction of cortical substance volume and size of lymphatic follicles without germinal centres. Regional lymph node of a guinea pig (group 2). Staining with hematoxylin and eosin, magnification 50×

On day 28 following sensitization of guinea pigs with non-tuberculous mycobacteria type II (according to the Runyon classification) the same trend persisted, i.e. a significant increase in concentration of neutrophil cationic proteins in the experimental groups compared to the control group. The activity of neutrophil antimicrobial peptides was higher in the group that had been treated with the experimental preparation on day 14 after inoculation of scotochromogenic mycobacteria (group 2), and was at the same level that had been observed in the test two weeks before. In contrast, the metabolic processes in group 1 were less intensive compared to the previous testing.

After another 14 days, concentration of cationic proteins in guinea pigs of group 1 dropped to the control levels. Thus, the average cytochemical coefficient within the group was 1.28 ± 0.08 c. u., and 1.17 ± 0.12 c. u. in the control. In contrast, neutrophil oxygen-dependent metabolism in the animals immunized with the experimental conjugate was more active due to an increase in the number of highly active phagocytes by 2.39 times ($p < 0.001$), thus, leading to a 1.97-fold increase in the average cytochemical coefficient ($p < 0.01$).

The administration of *Mycobacterium scrofulaceum* to guinea pigs also stimulated neutrophil oxygen-dependent metabolism (Table 2). Thus, the level of the average cytochemical coefficient of myeloperoxidase increased with a high degree of confidence ($p < 0.01$) by 1.79 and 1.82 times in both experimental groups, respectively, due to a 2-fold increase in the number of highly active phagocytes as compared to the control group.

Later, significantly increased myeloperoxidase enzyme activity was observed in guinea pigs of experimental group 2. Thus, the average cytochemical coefficients in the group after administration of the preparation were:

– on day 14, 1.70 ± 0.18 c. u. versus 0.90 ± 0.06 c. u. ($p < 0.05$) in the control;

– on day 28, 2.09 ± 0.01 c. u. versus 1.03 ± 0.03 c. u. ($p < 0.001$) in the control.

In contrast, as the time after inoculation with mycobacteria passed by, experimental group 1 demonstrated a decrease in the oxygen-dependent metabolism of neutrophils to the level of the control group (by day 42 from the beginning of the experiment).

Indirect immunofluorescence of blood samples tested on day 42 after inoculation of *Mycobacterium scrofulaceum,* demonstrated mycobacterial antigen only in 2 guinea pigs from experimental group 1.

Thus, administration of the immunobiological product enhances functional activity of aerobic and anaerobic neutrophil bactericidal systems resulting in accelerated elimination of non-tuberculous mycobacteria from the experimental animals.

Histopathological tests conducted on day 45 from the start of the experiment also demonstrate reduced antigen load on the guinea pigs treated with the experimental conjugate. Thus, an increase in the number of lymphatic follicles with a large proliferation center was observed in the regional inguinal lymph nodes of the animals from experimental group 1 (Fig. 1), where macrophage hyperplasia was recorded. Macrophage proliferation was also found in the cortex. The medullary cords housed mainly lymphocytes and an insignificant number of plasmocytes.

In contrast, inguinal lymph node cortex in experimental group 2 was significantly thinner. The lymphoid follicles were also smaller; moreover, they lacked proliferation centers (Fig. 2), even if they had such centers, there were only dendritic reticulocytes in them.

As for the inguinal lymph nodes adjacent to the site of *Mycobacterium scrofulaceum* inoculation, significantly fewer lymph follicles were observed there compared with the regional lymph nodes in the same group. Fewer proliferation centres were observed in them, and fewer macrophages were found in the proliferation centres and stroma. The animals treated with the preparation (group 2) had even fewer lymph follicles in the cortex of the lymph nodes located opposite to the regional ones.

CONCLUSION

The performed experiments demonstrate that sensitization of guinea pigs with *Mycobacterium scrofulaceum* induces hyper-reactivity of neutrophil intracellular bactericidal components lasting up to 28 days. Further on, there is a drop in their activity to the level recorded in animals of the control group. Administration of the experimental preparation accelerates withdrawal of mycobacteria from the guinea pigs (on day 7 post administration) owing to stimulation of phagocytes, which is confirmed by immunofluorescence and histological tests.

REFERENCES

1. Parte A. C., Sardà Carbasse J., Meier-Kolthoff J. P., Reimer L. C., Göker M. List of prokaryotic names with standing in nomenclature (LPSN) moves to the DSMZ. *International Journal of Systematic and Evolutionary Microbiology.* 2020; 70 (11): 5607–5612. https://doi.org/10.1099/ijsem.0.004332

2. Ghielmetti G., Friedel U., Scherrer S., Sarno E., Landolt P., Dietz O., et al. Non-tuberculous *Mycobacteria* isolated from lymph nodes and faecal samples of healthy slaughtered cattle and the abattoir environment. *Transboundary and Emerging Diseases.* 2018; 65 (3): 711–718. https://doi.org/10.1111/tbed.12793

3. Naimanov A. H., Gulukin M. I., Tolstenko N. G., Vangeli E. P., Kalmykov V. M. Organization of the fight against animal tuberculosis in Russia. *Veterinariya*. 2019; (4): 3–7. https://doi.org/10.30896/0042-4846.2019.22.4.03-07 (in Russ.)

4. Baratov M. O., Sakidibirov O. P., Abduragimova R. M., Dzhabarova G. A. Immune and protective properties of non-tuberculosis acid-resistant mycobacteria. *Problems of Development of the Agro-Industrial Complex of the Region.* 2022; (1): 73–79. https://doi.org/10.52671/20790996_2022_1_73 (in Russ.)

5. Nuru A., Zewude A., Mohammed T., Wondale B., Teshome L., Getahun M., et al. Nontuberculosis mycobacteria are the major causes of tuberculosis like lesions in cattle slaughtered at Bahir Dar Abattoir, northwestern Ethiopia. *BMC Veterinary Research.* 2017; 13 (1):237. https://doi.org/10.1186/s12917-017-1168-3

6. Hernández-Jarguín A. M., Martínez-Burnes J., Molina-Salinas G. M., de la Cruz-Hernández N. I., Palomares-Rangel J. L., López Mayagoitia A., Barrios-García H. B. Isolation and histopathological changes associated with non-tuberculous mycobacteria in lymph nodes condemned at a bovine slaughterhouse. *Veterinary Sciences.* 2020; 7 (4):172. https://doi.org/10.3390/vetsci7040172

7. Gomez-Buendia A., Alvarez J., Bezos J., Mourelo J., Amado J., Saez J. L., et al. Non-tuberculous mycobacteria: occurrence in skin test cattle reactors from official tuberculosis-free herds. *Frontiers in Veterinary Science.* 2024; 11:1361788. https://doi.org/10.3389/fvets.2024.1361788

8. Kamalieva Yu. R., Mingaleev D. N., Ravilov R. Kh. Identification of non-tuberculosis mycobacteria isolated from cattle in the Republic of Tatarstan. *Agrarian Science*. 2021; 354 (11–12): 32–35. https://doi.org/10.32634/0869-8155-2021-354-11-12-32-35 (in Russ.)

9. Baratov M. O., Huseynova P. S. Actual bovine tuberculosis situation in the Republic of Dagestan. *Veterinary Science Today.* 2022; 11 (3): 222–228. https://doi.org/10.29326/2304-196X-2022-11-3-222-228

10. Biet F., Boschiroli M. L. Non-tuberculous mycobacterial infections of veterinary relevance. *Research in Veterinary Science.* 2014; 97 (Suppl.): S69–S77. https://doi.org/10.1016/j.rvsc.2014.08.007

11. Lara G. H. B., Ribeiro M. G., Leite C. Q. F., Paes A. C., Guazzelli A., da Silva A. V., et al. Occurrence of *Mycobacterium* spp. and other pathogens in lymph nodes of slaughtered swine and wild boars (*Sus scrofa*). *Research in Veterinary Science.* 2011; 90 (2): 185–188. https://doi.org/10.1016/j.rvsc.2010.06.009

12. Klanicova-Zalewska B., Slana I. Presence and persistence of *Mycobacterium avium* and other nontuberculous mycobacteria in animal tissues and derived foods: a review. *Meat Science.* 2014; 98 (4): 835–841. https://doi.org/10.1016/j.meatsci.2014.08.001

13. Varela-Castro L., Barral M., Arnal M. C., Fernández de Luco D., Gortázar C., Garrido J. M., Sevilla I. A. Beyond tuberculosis: Diversity and implications of non-tuberculous mycobacteria at the wildlife-livestock interface. *Transboundary and Emerging Diseases.* 2022; 69 (5): e2978–e2993. https://doi.org/10.1111/tbed.14649

14. Muwonge A., Oloya J., Kankya C., Nielsen S., Godfroid J., Skjerve E., et al. Molecular characterization of *Mycobacterium avium* subspecies *hominissuis* isolated from humans, cattle and pigs in the Uganda cattle corridor using VNTR analysis. *Infection, Genetics and Evolution.* 2014; 21: 184–191. https://doi.org/10.1016/j.meegid.2013.11.012

15. Leão C., Canto A., Machado D., Sanches I. S., Couto I., Viveiros M., et al. Relatedness of *Mycobacterium avium* subspecies *hominissuis* clinical isolates of human and porcine origins assessed by MLVA. *Veterinary Microbiology.* 2014; 173 (1–2): 92–100. https://doi.org/10.1016/j.vetmic.2014.06.027

16. Kontturi A., Soini H., Ollgren J., Salo E. Increase in childhood nontuberculous mycobacterial infections

after bacille Calmette-Guérin coverage drop: A nationwide, population-based retrospective study, Finland, 1995–2016. *Clinical Infectious Diseases.* 2018; 67 (8): 1256–1261. https://doi.org/10.1093/cid/ciy241

17. Zimmermann P., Finn A., Curtis N. Does BCG vaccination protect against nontuberculous mycobacterial infection? A systematic review and meta-analysis. *The Journal of Infectious Diseases.* 2018; 218 (5): 679–687. https://doi.org/10.1093/infdis/jiy207

18. Abate G., Hamzabegovic F., Eickhoff C. S., Hoft D. F. BCG vaccination induces *M. avium* and *M. abscessus* cross-protective immunity. *Frontiers in Immunology.* 2019; 10:234. https://doi.org/10.3389/fimmu.2019.00234

19. Fritschi N., Curtis N., Ritz N. Bacille Calmette Guérin (BCG) and new TB vaccines: Specific, cross-mycobacterial and off-target effects. *Paediatric Respiratory Reviews.* 2020; 36: 57–64. https://doi.org/10.1016/j.prrv.2020.08.004

20. Vlasenko V. S., Kosobokov E. A., Dengis N. A., Novikova N. N. Studying immunotherapeutic properties of the immunomodulator KIM-M2 in guinea pigs infected with nontuberculous mycobacteria. *Bulletin of KrasSAU*. 2022; (5): 91–97. https://doi.org/10.36718/1819-4036-2022-5-91-97 (in Russ.)

21. Orme I. M., Collins F. M. Efficacy of *Mycobacterium bovis* BCG vaccination in mice undergoing prior pulmonary infection with atypical mycobacteria. *Infection and Immunity.* 1984; 44 (1): 28–32. https://doi.org/10.1128/iai.44.1.28-32.1984

22. Buddle B. M., Wards B. J., Aldwell F. E., Collins D. M., de Lisle G. W. Influence of sensitisation to environmental mycobacteria on subsequent vaccination against bovine tuberculosis. *Vaccine.* 2002; 20 (7–8): 1126–1133. https://doi.org/10.1016/S0264-410X(01)00436-4

23. Palmer M. V., Thacker T. C. Use of the human vaccine, *Mycobacterium bovis* Bacillus Calmette Guérin in deer. *Frontiers in Veterinary Science.* 2018; 5:244. https://doi.org/10.3389/fvets.2018.00244

24. Shah J. A., Lindestam Arlehamn C. S., Horne D. J., Sette A., Hawn T. R. Nontuberculous mycobacteria and heterologous immunity to tuberculosis. *The Journal of Infectious Diseases.* 2019; 220 (7): 1091–1098. https://doi.org/10.1093/infdis/jiz285

25. Koshkin I. N., Vlasenko V. S., Pleshakova V. I., Alkhimova L. E., Elyshev A. V., Kulakov I. V. Morphology of lymphoid tissue in the lungs of guinea pigs infected with *Mycobacterium bovis* against the background of vaccine immunity and the action of betulin and its derivatives. *Vaccines*. 2022; 10 (12):2084. https://doi.org/10.3390/vaccines10122084

26. Novikova N. N., Baiseitov S. T., Vlasenko V. S., Krasikov A. P. Using indirect immunofluorescence to diagnose bovine leukosis: guidelines. Almaty: NOVA Press; 2020. 17 p. (in Russ.)

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Use of DIABAX feed additive and a biogenic stimulant in calves during their rehabilitation after gastrointestinal infections

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ABSTRACT

The results of the use of DIABAX feed additive alone and in combination with a biogenic stimulant for the correction of biochemical, hematological blood parameters in young cattle after gastrointestinal infections taking into account the disease and survival rates, as well as their performance indicators are presented. Three groups of calves at the age of less than 30 days old, two test groups and one control group, were formed for analogous pairs-based trial. Calves of control group were subcutaneously injected with saline solution at a dose of 8 mL on day 1, 5, 10 of the trial; calves of test group 1 (T-1) were fed with DIABAX feed additive with milk at a dose of 3.0 mL once a day; calves of test group 2 (T-2) were intramuscularly injected with the biogenic stimulant at a dose of 0.5 mL/10 kg of body weight on day 1, 5, 10 of the trial and also received DIABAX at a dose of 3.0 mL once a day during 15 days. The tests showed that co-administration of the biogenic stimulant and DIABAX feed additive (in T-2 group) contributed to 100% survival rate in calves, as well as significant increase in calcium and magnesium levels in animal sera by 14.5–23.8 and 61.2–79.5%, respectively, as compared with the initial levels and the levels in control group; increase in albumin and *α*-globulin protein fraction concentrations by 10.1 and 43.2% ($p \leq 0.05$), respectively, albumin/globulin ratio – by 17.5%, color index – by 1.1%, increase in the total immunoglobulin G amount by 2.7 times as compared to the initial values. Daily administration of DIABAX feed additive to calves of T-1 group for 15 days reduced recurrent disease rate in the calves by 14.4%, resulted in significant increase in calcium and magnesium levels in sera by 10.1 and 75.0% ($p \leq 0.05$), respectively, as well increase in immunoglobulin G level by 2.3 times, erythrocyte level – by 3.8%, hemoglobin level – by 8.0%, leukocyte level – by 21.8%, albumin/globulin ratio – by 35.1% in sera as compared to initial values.

Keywords: calves, disease rate, survival rate, morphological, biochemical blood parameters, biogenic stimulant

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Применение кормовой добавки «Диабакс» и биогенного препарата телятам, переболевшим желудочно-кишечными инфекциями, в восстановительный период

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РЕЗЮМЕ

Представлены результаты применения кормовой добавки «Диабакс» в отдельности и в сочетании с биогенным препаратом для коррекции биохимических, гематологических показателей крови молодняка крупного рогатого скота, переболевшего желудочно-кишечными инфекциями, с учетом

заболеваемости, сохранности, продуктивности животных. Для проведения опыта по принципу пар-аналогов были сформированы 3 группы телят до 30-суточного возраста: две опытные и одна контрольная. Телятам контрольной группы подкожно вводили физиологический раствор в дозе 8 мл в 1, 5, 10-й дни опыта; животным 1-й опытной группы (Т-1) в течение 15 дней с молоком задавали кормовую добавку «Диабакс» в дозе 3,0 мл 1 раз в сутки; телятам 2-й опытной группы (Т-2) внутримышечно инъецировали биогенный препарат в дозе 0,5 мл на 10 кг массы тела в 1, 5, 10-й дни опыта и выпаивали 15 дней подряд «Диабакс» в дозе 3,0 мл 1 раз в сутки. На основании проведенных исследований установлено, что совместное применение биогенного препарата и добавки «Диабакс» (в группе Т-2) способствует 100%-й сохранности телят, достоверному увеличению кальция и магния в сыворотке крови животных на 14,5–23,8 и 61,2–79,5% соответственно по сравнению с исходными показателями и показателями контрольной группы, повышению в сравнении с контрольной группой альбуминовой и *α*-глобулиновой фракций белка на 10,1 и 43,2% ($p \leq 0,05$) соответственно, альбумин-глобулинового коэффициента – на 17,5%, цветного показателя – на 1,1%, увеличению общего количества иммуноглобулина класса G в 2,7 раза по сравнению с исходными данными. Ежедневное выпаивание добавки «Диабакс» в течение 15 дней (в группе Т-1) приводит к снижению количества повторных заболеваний телят на 14,4%, достоверному увеличению в сыворотке крови кальция и магния на 10,1 и 75,0% ($p \leq 0,05$) соответственно, повышению уровня иммуноглобулина класса G в 2,3 раза, эритроцитов – на 3,8%, гемоглобина – на 8,0%, лейкоцитов – на 21,8%, альбумин-глобулинового коэффициента – на 35,1% относительно исходных значений.

Ключевые слова: телята, заболеваемость, сохранность, морфологические, биохимические показатели крови, биогенный препарат

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INTRODUCTION

Livestock survivability and performance improvement is one of the important challenges facing the Russian Federation agro-industrial complex [1, 2]. Calf rearing are challenging since the newborns' bodies are poorly adapted to adverse environmental conditions due to the morphofunctional immaturity of immune system and gastrointestinal tract during their first days of life [3]. This, in turn, results in the various gastrointestinal diseases in calves and their deaths [4, 5, 6], as well as the disease recurrence [7, 8].

Therewith, complex rehabilitation procedures including use of various biological stimulants enabling vital component deficiency filling and contributing to the metabolic normalization and full-calf body resistance enhancement are required to maintain the metabolic status and homeostasis of the calves during their recovery [9, 10, 11, 12, 13].

The mechanism of biogenic stimulant action includes changes in activity of some enzymes due to the biogenic stimulant attachment to the enzyme protein. Changes in the enzyme activity results in endocrine restructuring, an increase in tropic hormones secreted by pituitary gland that stimulate adrenal gland, thyroid and pancreas functions, etc. They have a favorable impact on trophic function of the nervous system, enhance thyroid tissue and adrenal gland functions, stimulate corticosteroid hormone secretion and pancreatic gland function, regulate gastrointestinal tract secretory and motor functions, gas and phosphorus metabolism, intermediary metabolism, reticuloendothelial system and regenerative processes, improve body state, appetite, assimilation processes that contribute to higher weight gains [14, 15].

The study was aimed at testing of DIABAX feed additive and a biogenic stimulant for their effectiveness for correction of biochemical, hematological blood parameters in young cattle after gastrointestinal infections.

Test tasks:

1. To test used feed additive and biogenic stimulant for their effects on morphological and biochemical blood parameters in calves during their rehabilitation after gastrointestinal infections.

2. To test the feed additive and biogenic stimulant for their effects on disease rate and survival rate in calves, calf performance during their rehabilitation period.

MATERIALS AND METHODS

Bacteriological tests of calf feces were carried out for the purpose of diagnosis. Isolated microorganisms were tested for their sensitivity to antimicrobials with disk diffusion test [16].

A pilot batch of biogenic stimulant was prepared from raw materials (slaughter waste, category II offal) kept in a refrigerator at temperature of +2 up to +4 °C for 5–7 days, ground and then mixed with an extraction agent at the Department of the All-Russian Institute of Antler Reindeer Breeding, Altai Scientific Center for Agrobiotechnologies. The ultrasonic-assisted extraction was carried out; the final product was filtered, packed, and sterilized in an autoclave [17]. Administration of the biogenic stimulant enhances metabolism, body's resistance and stimulates body's functions.

DIABAX, a new feed additive, developed by the GK KONSTANTA (Saransk) [18] is a viscous fluid of light-brown color with slight odor, has a bactericidal,

Table 1
Scheme of tested product administration to calves of test and control groups

Group of animals	Number of animals	Administered product
C	6	Saline solution administered at a dose of 8.0 mL subcutaneously on day 1, 5, 10 of the trial
T-1	7	DIABAX administered daily at a dose of 3.0 mL once a day for 15 days
T-2	5	Biogenic stimulant administered intramuscularly at a dose of 0.5 mL/10 kg of body weight on day 1, 5, 10 of the trial + DIABAX administered daily at a dose of 3.0 mL once a day for 15 days

bacteriostatic effect on a wide range of microorganisms and pathogenic fungi owing to potassium iodide and electrostatically charged polyelectrolyte polydimethyldiallylammonium chloride contained in it. DIABAX feed additive is highly soluble in water, does not change medium pH level, remains active in acidic and alkaline media, as well as in protein and fatty media. The principle feature of the additive is inhibition of pathogenic microflora based on its physical, but not chemical effect, while its components has no adverse effect on normal gastrointestinal cells of animals. Polyelectrolyte has an electrostatic charge opposite to the charge of cells of pathogenic bacteria, fungi and other microorganisms. When Diabax feed additive interacts with the pathogen surface. This interaction leads to microorganism cell membrane is encapsulation that slows down and then completely inhibits microorganism breathing, nutrition and reproducibility.

For the trial aimed at testing of the restorative treatment for its effectiveness in calves during their rehabilitation after gastrointestinal infections, 3 groups of 10–30 day-old calves were formed according to the analogous pairs principle: two test (T-1, T-2) groups and one control (C) group. Tested products were administered to the calves according to the scheme presented in Table 1.

All procedures involving animals were carried out in accordance with ethical standards laid down by European Convention ETS No. 123.

The feed additive and biogenic stimulant were tested for their effectiveness based on the following: morphological tests of blood (total erythrocyte counts, total leukocyte counts, hemoglobin level) using conventional methods [19]; biochemical tests of sera: refractometric determination (IRF-22, Russia) of serum total protein, nephelometric determination of protein fractions [20]; ELISA determination of total immunoglobulin G using appropriate ELISA test-kit; determination of serum mineral content by unified method using Vital Diagnostics SPb kits (Russia) and Stat Fax® 1904+ biochemical photometer (Awareness Technology, Inc., USA); bacteriological tests of biological material samples in accordance with the Methodological Guidelines[1, 2].

[1] MG 4.2.2723-10 Laboratory diagnosis of salmonellosis, *Salmonella* detection in food products and environmental samples: methodical guidelines (approved by Chief Medical Officer of the Russian Federation on 13 August 2010). https://docs.cntd.ru/document/1200083950?ysclid=lvgmjzwhv062935169

[2] Methodical Guidelines for laboratory diagnosis of animal and avian pasteurellosis: approved by the Veterinary Department of the Ministry of Agriculture of the USSR No. 22-7/82 on 20 August 1992. https://docs.cntd.ru/document/456071306?ysclid=lvgn11uqfc818248150

Blood samples were collected before the trial and 10 days after the trial completion. Mean values was assessed for their reliability of using Student's – Fisher's test.

RESULTS AND DISCUSSION

The gastrointestinal disease incidence in calves kept on the farm periodically rises due to violation of zootechnical and veterinary rules for animal keeping, feeding and handling during mass calving. The etiological causes are: diseased animals, convalescent animals, dams – pathogenic microorganism strain carriers, infected environment. The following bacteria were detected in feces from diseased calves subjected to bacteriological tests: *Salmonella Dublin, Mannheimia haemolytica*, all isolated bacteria strains were pathogenic for white mice. Levofloxacin, marfloxin, enrofloxacin, norfloxacin, ofloxacin, polymyxin, kanamycin were found to be effective antimicrobials. At the final stage of the trial, no pathogenic microorganism strains were detected during the bacteriological tests of fecal samples from calves of test and control groups.

Tests of the blood samples collected before the trial aimed at testing DIABAX feed additive and biogenic stimulant for their effectiveness for correction of biochemical, hematological blood parameters in calves during their rehabilitation after gastrointestinal infections showed decrease in phosphorous level by 19.1%, calcium level by 9.2%, magnesium level by 46.3% as compared with the physiological norm and simultaneous increase in zinc level by 3.5% as compared to the physiological norm (Table 2).

Calves of both test groups demonstrated normalization of serum phosphorus level, a significant increase in serum calcium level by 19.0% in the T-1 group and by 23.8% in the T-2 group ($p < 0.05$) as compared to that ones in sera from control group calves, in serum magnesium level – by 57.1% in T-1 group and by 61.2% in T-2 group ($p < 0.05$), serum potassium level – by 2.2% in T-1 group and by 4.3% in T-2 group 10 days after completion of tested feed additive and biogenic stimulant administration. There was a positive dynamics of zinc level decrease by 0.8% in sera from control group calves, by 5.5% in sera from T-1 test group calves, by 6.3% in sera from T-2 test group calves as compared to initial values during the rehabilitation period.

Biochemical tests of calf sera collected before the trial showed slight decrease in albumin protein fraction by 4.7%, and α-globulin protein fraction by 23.3%. Decrease in albumin-globulin ratio by 31.3% is indicative of imbalance between the protein fractions, 14.0% decrease in the amount of immunoglobulins G mainly responsible for humoral immunity is indicative of body protective function suppression in animals used for the trial (Table 3).

Table 2
Micro- and macroelement levels in sera from calves used for the trial

Mean value for group	P, mmol/L	Ca, mmol/L	Mg, mmol/L	K, mmol/L	Zn, μmol/L
Normal range	1.78–2.42	2.50–3.00	0.82–1.23	4.10–4.86	15.40–23.00
I	1.44 ± 0.17	2.27 ± 0.442	0.44 ± 0.14	4.90 ± 1.99	23.80 ± 1.56
C	2.10 ± 0.161	2.10 ± 0.111	0.49 ± 0.09	4.60 ± 0.38	23.60 ± 1.08
± to/from I, %	+ 45.80	– 7.50	+ 11.40	– 6.10	– 0.80
T-1	1.78 ± 0.121	2.50 ± 0.120*	0.77 ± 0.02*	4.70 ± 0.18	22.50 ± 1.52
± to/from I, %	+ 23.60	+ 10.10	+ 75.00	– 4.10	– 5.50
± to/from C, %	– 15.20	+ 19.00	+ 57.10	+ 2.20	– 4.70
T-2	1.79 ± 0.152	2.60 ± 0.110*	0.79 ± 0.03*	4.8 ± 0.67	22.3 ± 1.27
± to/from I, %	+ 24.30	+ 14.50	+ 79.50	– 2.00	– 6.30
± to/from C, %	– 14.80	+ 23.80	+ 61.20	+ 4.30	– 5.50

* $p < 0.05$; I – initial values, C – values in control group.

Table 3
Concentrations of total protein, protein fractions in sera from the calves used for the trial

Group of animals	Total protein, g/L	Albumins, %	Globulins, %			A/G ratio, units	Immunoglobulin G, mg/mL
			α	*β*	*γ*		
Normal range	56.9–65.0	38–50	12–20	10–16	25–40	0.83–1.19	> 10
I	66.4 ± 8.42	36.2 ± 8.91	9.2 ± 4.34	15.8 ± 7.57	39.0 ± 5.09	0.57 ± 0.22	8.6 ± 3.39
C	60.0 ± 1.71	38.5 ± 1.21	12.5 ± 1.53	21.2 ± 1.72	27.8 ± 1.92	0.63 ± 0.114	14.8 ± 3.45
± to/from I, %	– 9.6	+ 6.4	+ 35.9	+ 34.2	– 28.7	+ 10.5	+ 72.1
T-1	56.9 ± 2.88	43.5 ± 0.82*	12.7 ± 0.76	13.6 ± 1.15*	30.2 ± 1.28	0.77 ± 0.010	19.7 ± 1.78
± to/from I, %	– 14.3	+ 20.2	+ 38.0	– 13.9	– 22.6	+ 35.1	2.3-fold increase
± to/from C, %	– 5.2	+ 13.0	+ 1.6	– 35.8	+ 8.6	+ 22.2	+ 33.1
T-2	60.1 ± 3.66	42.4 ± 1.06*	17.9 ± 1.27*	15.0 ± 1.18*	24.7 ± 2.05	0.74 ± 0.052	23.0 ± 2.16
± to/from I, %	– 9.5	+ 17.1	+ 94.6	– 5.1	– 36.7	+ 29.8	2.7-fold increase
± to/from C, %	0	+ 10.1	+ 43.2	– 29.2	– 11.2	+ 17.5	+ 55.4

* $p < 0,05$; A/G – albumin/globulin ratio, I – initial values, C – values in control group.

At the final stage of the trial, albumin and *α*-globulin protein fractions in the calf sera were found to normalize to the physiological norm with a significant increase ($p \leq 0.05$) in albumin levels in T-1 and T-2 groups and *α*-globulin fraction in T-2 group. In calves of control group, serum *β*-globulin level increased by 34.2% as compared to the initial values and significantly differed ($p \leq 0.05$) from the test group values. Albumin-globulin ratio in animals of control group and test groups increased by 10.5% and 29.8–35.1% during the period of rehabilitation of the calves after gastrointestinal infections.

Immunoglobulin G amount reached its physiological norm in sera from test group calves. Immunoglobulin G amount increased by 72.1% in calves of control group and by 2.3–2.7 times in calves of test groups as compared to the initial values.

Analysis of hematological blood parameters in calves at the beginning and at the end of the trial showed no significant differences between the control and test groups. There was a positive trend towards an increase in the tested blood parameters within the physiological norm at the final stage of rehabilitation of calves in test groups as compared to the initial values and blood parameters of calves in control group (Table 4).

In test groups all calves survived (100% survival rate) during rehabilitation period when the tested feed additive and biogenic stimulant were administered to the calves and then during 60-day clinical observation period (Table 5). Disease rate was 66,7% in control group calves that was higher by 14.4 and 40.0% than that one in T-1 and T-2 groups, respectively.

Increase in average daily weight gains by 33.5% in T-1 group and by 27.9% in T-2 group as compared

Table 4
Hematological blood parameters in the calves used for the trial

Group of animals	Erythrocytes, 10^{12}/L	Hemoglobin, g/L	Leukocytes, 10^{9}/L	Color index, units
Normal range	7.40–8.60	99.00–128.00	4.50–12.00	0.70–1.10
I	8.00 ± 1.36	98.00 ± 17.40	5.50 ± 1.16	0.87 ± 0.14
C	8.20 ± 0.33	104.00 ± 4.67	6.00 ± 0.50	0.90 ± 0.04
± to/from I, %	+ 2.5	+ 6.1	+ 9.1	+ 3.4
T-1	8.30 ± 0.60	105.80 ± 4.33	6.70 ± 0.63	0.90 ± 0.04
± to/from I, %	+ 3.8	+ 8.0	+ 21.8	+ 3.4
± to/from C, %	+ 1.2	+ 1.7	+ 11.7	0
T-2	8.40 ± 0.42	108.00 ± 4.84	6.90 ± 0.59	0.91 ± 0.06
± to/from I, %	+ 5.0	+ 10.2	+ 25.5	+ 4.6
± to/from C, %	+ 2.4	+ 3.8	+ 15.0	+ 1.1

I – initial values, C – values in control group.

Table 5
Disease and survival rates in the calves used for the trial

Group of animals	Number of animals	Diseased		Died		Decrease in proportion of diseased animals in test group as compared to control group, %
		Number of animals	%	Number of animals	%	
C	6	4	66.7	1	16.7	–
T-1	7	4	57.1	–	–	14.4
T-2	5	2	40.0	–	–	40.0

to daily weight gains in control group was recorded during the first control weighing of the calves used for the trail (30 days after the trial start). During the second control weighing of the calves preformed 60 days after the trial start, 55.5–67.7% increase was recorded. Average daily weigh gain in T-1 group calves and in T-2 group calves was by 10.6% and 4.3% higher than that one in control group calves during the whole calf raising period (Table 6).

The test results show that administration of the biogenic stimulant of animal origin by injections and DIABAX feed additive (in T-2 group) with milk contribute to 100% survival rate in calves, significant increase in calcium and magnesium levels in the animal sera by 14.5–23.8% and 61.2–79.5%, respectively, as compared to the initial levels and levels in control group, an increase in albumin and α-globulin protein fractions by 10.1 and 43.2% ($p \leq 0.05$), respectively, as compared to that ones in control group, increase in albumin/globulin ratio by 17.5%, color index – by 1.1% as compared to that ones in control group, the total immunoglobulin G amount was 2.7 times higher than the initial value. Daily administration of DIABAX feed additive to calves of T-1 group for 15 days reduced recurrent disease rate in the calves by 14.4%, resulted in significant increase in serum calcium and magnesium levels by 10.1 and 75.0% ($p \leq 0.05$), respectively, as well as in increase in immunoglobulin G level by 2.3 times, erythrocyte level – by 3.8%, hemoglobin level – by 8.0%, leukocyte level – by 21.8%, albumin/globulin ratio – by 35.1% as compared to the initial values.

CONCLUSIONS

1. Daily administration of DIABAX feed additive to calves for 15 days contributed to significant increase

Table 6
Average body weight of the calves by group

Group of animals	Birth weight	Control weighing, kg			Observation period, days	Average daily weight gain, g	
		at the beginning of the trial	30 days after the trial start	60 days after the trial start		during 30/60 days of observation	during the whole raising period
C	39.00 ± 0.61	89.20 ± 10.34	105.30 ± 5.59	126.50 ± 12.05	113	537/622	774 ± 87.6
T-1	38.60 ± 0.46	85.00 ± 5.23	106.50 ± 8.89	143.00 ± 8.78	122	717/967	856 ± 67.7
T-2	37.20 ± 0.27	73.80 ± 3.84	94.40 ± 8.40	136.40 ± 8.59	123	687/1,043	807 ± 70.0

in serum calcium, magnesium, albumin, β-globulin levels ($p \leq 0.05$) in T-1 group calves, decrease in number of diseased animals by 14.4% and 100% survivability in T-2 group calves as compared to control group calves, increase in average daily weigh gain by 55.5% as compared to that ones in control group calves during 60-day observation period.

2. Combination of the biogenic stimulant injections to calves on day 1, 5, 10 of the trial and adding DIABAX feed additive to the calf diet for 15 consecutive days contributed to a significant increase in calcium, magnesium, albumin levels, α-, β-globulin protein fractions ($p \leq 0.05$), decrease in disease rates by 40.0 and 29.9%, as well as an increase in average daily weight gain by 67.7 and 7.9% as compared to that ones in control and T-1 groups, respectively, during 60-day observation period with 100.0% survival rate in T-2 test group calves.

REFERENCES

1. Chernitskiy A. E., Shabunin S. V. Prophylaxis of respiratory diseases in neonatal calves with low viability. *Veterinariya*. 2017; (9): 10–16. https://elibrary.ru/zmmitx (in Russ.)

2. Gerceva K. A., Nikulova L. V., Kiseleva E. V. An effective strategy for the treatment of toxic dyspepsia in calves. *International Journal of Veterinary Medicine*. 2023; (1): 307–317. https://doi.org/10.52419/issn2072-2419.2023.1.307 (in Russ.)

3. Vakhrusheva T. I. Analiz zabolevaemosti molodnyaka krupnogo skota vnutrennimi nezaraznymi patologiyami v AO PZ «Krasnoturanskii» Krasnoyarskogo kraya = Analysis of the occurrence of non-contagious internal organ pathologies in calves on the AO PZ "Krasnoturansky" breeding farm in the Krasnoyarsk Krai. *Rol' agrarnoi nauki v ustoichivom razvitii sel'skikh territorii: sbornik IV Vserossiiskoi (natsional'noi) nauchnoi konferentsii (Novosibirsk, 20 dekabrya 2019 g.) = The role of agricultural science in the sustainable development of rural areas: proceedings of the IV All-Russia (National) Scientific Conference (Novosibirsk, 20 December 2019).* Novosibirsk: ITs NSAU "Zolotoi Kolos"; 2019; 194–197. https://elibrary.ru/quvwdb (in Russ.)

4. Vakhrusheva T. I. Dispepsiya telyat – opyt lecheniya i profilaktiki v usloviyakh khozyaistv Krasnoyarskogo kraya = Calf dyspepsia – experience of treatment and prevention on farms in the Krasnoyarsk Krai. *Nauchnoe obespechenie zhivotnovodstva Sibiri: materialy IV Mezhdunarodnoi nauchnoi prakticheskoi konferentsii (Krasnoyarsk, 14–15 maya 2020 g.) = Scientific support of Siberian animal farm industry: proceedings of the IV International Scientific and Practical Conference (Krasnoyarsk, 14–15 May 2020).* Krasnoyarsk: Krasnoyarsk Scientific-Research Institute of Animal Husbandry – Division of Federal Research Center "Krasnoyarsk Scientific Center of the Siberian Branch of the RAS"; 2020; 417–421. https://elibrary.ru/gniozf (in Russ.)

5. Novikova T. V., Mekhanikova M. V., Bilkov V. A. The reaction of hematological parameters of calves to transferred dyspepsia. *Journal of Biochemical Technology*. 2023; 14 (2): 1–5. https://doi.org/10.51847/dSUMJIfip2

6. Köse S., Şehu A. Effects of a commercial feed additive used for prophylactic purposes on health and blood parameters in neonatal calves. *Tierärztliche Praxis Ausgabe G: Grosstiere/Nutztiere.* 2024; 52 (1): 16–24. https://doi.org/10.1055/a-2220-1645

7. Shadskaya A. V. The effective treatment setting for calves with dyspepsia under production conditions. *Bulletin of Agrarian Science*. 2022; (5): 65–69. https://elibrary.ru/ftaywv (in Russ.)

8. Poryvaeva A. P., Krasnoperov A. S., Tomskikh O. G., Lysova Ya. Yu. Model of estimation of risk of development of complications when dyspepsia in calves. *Agrarian Bulletin of the Urals.* 2019; (1): 31–37. https://doi.org/10.32417/article_5ca4e6ff8e24c2.87308778 (in Russ.)

9. Burova O., Blokhin A., Isaev V. Prevention of gastrointestinal diseases of calves with application of biologically active substances. *Agricultural Science Euro-North-East*. 2014; (3): 36–39. https://elibrary.ru/scjkqt (in Russ.)

10. Smolentsev S. Yu. Imroving the preseration of calves by using immunostimulants together with a mineral fodder additive. *Scientific Life.* 2017; (2): 49–55. https://elibrary.ru/yhfazn (in Russ.)

11. Sanin A. V., Narovlyanskiy A. N., Pronin A. V., Kozhevnikova T. N. Increase of the innate immunity and hemostasis correction in calves using medications with immunomodulating and biostimulating activity. *Russian Veterinary Journal*. 2020; (2): 31–38. https://doi.org/10.32416/2500-4379-2020-2-31-38 (in Russ.)

12. Bazekin G. V. Immunobiochemical and clinical-morphological assessment of the glycyrrhizic acid and nucleostim effect on animals: Author's abstract of thesis for degree of Dr. Sci. (Veterinary Medicine). Ufa; 2022. 363 p. (in Russ.)

13. Sanin A. V., Savoyskaya S. L., Kozhevnikova T. N., Sanina V. Yu., Sosnovskaya O. Yu. Safety, growth, development and non-specific resistance increasing of calves with help of modern immunomodulatory preparations. *Veterinaria Kubani*. 2019; (2): 11–14. https://doi.org/10.33861/2071-8020-2019-2-11-14 (in Russ.)

14. Mashkovsky M. D. State Pharmacopeia of the Russian Federation. 16th edition, revised, corrected and supplemented. Moscow: Novaya Volna; 2012. 1216 p. (in Russ.)

15. Daricheva N. N., Ermolaev V. A. Tissue therapy in veterinary medicine: monograph. Ulyanovsk: Ulyanovsk State Agricultural Academy; 2011. 168 p. (in Russ.)

16. Gosmanov R. G., Ravilov R. Kh., Galiullin A. K., Nurgaliyev F. M., Idrisov G. G. Laboratory diagnosis of infectious diseases: study guide. Saint Petersburg: Lan'; 2022. 196 p. (in Russ.)

17. Shanshin N. V., Evseeva T. P. Biogenic preparations production method. Patent No. 2698707 Russian Federation. Int. Cl. A61K 35/12, B01D 11/02, B01J 19/10. FGBNU FANTSA. No. 2019113424. Date of filing: 29.04.2019. Date of publication: 29.08.2019. Bull. No. 25. (in Russ.)

18. DIABAX™ feed additive; DIABAX: declaration of conformity РОСС RU Д-RU.PA02.B.14405/21. https://reestrinform.ru/reestr-declaratcii-sootvetstviia/reg_number-РОСС_RU_Д-RU.PA02.B.14405--21.html

19. Kudryavtsev A. A., Kudryavtseva L. A. Clinical hematology of animals. Moscow: Kolos; 1974. 399 p. (in Russ.)

20. Methods for veterinary clinical laboratory diagnosis: guide. Ed. by I. P. Kondrakhin. Moscow: KolosS; 2004. 520 p. (in Russ.)

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Вклад автора: Шаньшин Н. В. – лично автором произведен отбор биоматериала и изготовлена опытная партия биогенного препарата, осуществлен забор крови у животных, проведены морфо-биохимические исследования, анализ, систематизация и статистическая обработка полученных данных, подготовка статьи к публикации.

On 75th anniversary of Valery V. Mikhalishin

Valery V. Mikhalishin was born on 1 May 1949 in Ozerany village, Rahachow District, Gomel Region.

After graduating from the Vitebsk Veterinary Institute in 1972, Valery V. Mikhalishin worked for one year as an epizootologist at the Ivatsevichi Animal Disease Control Station, Brest Region, and then as a senior laboratory assistant and junior researcher in All-Union Foot-and-Mouth Disease Research Institute (Vladimir). From 1975 to 1978, he studied at the full-time postgraduate school; in 1982 he defended his Candidate of Science thesis. From 1984, he worked as a Senior Researcher, from 1989 as Acting Head of the Laboratory for Inactivated Vaccines; from 1991 to 2007 he headed the Laboratory of Biotechnology.

Dr. Mikhalishin's scientific developments were summarized in his doctoral dissertation, which he successfully defended in 1997. In 2005, he was awarded the academic title of Professor. From 2007 to 2009, he headed the Department of Monitoring of Highly Dangerous and Exotic Animal Diseases. Currently, he is the Chief Researcher of the Information Analysis Center of the Federal Centre for Animal Health.

The main area of Valery V. Mikhalishin's scientific activity was cultivation of foot-and-mouth disease virus in newborn animals for the creation of inactivated foot-and-mouth disease vaccines, development of the methods for purification, concentration and inactivation of lapinized virus of all seven types. As a result of the experimental work carried out by Valery V. Mikhalishin and laboratory staff, a technology for manufacture of polyvalent inactivated culture emulsion and universal FMD vaccines was developed and introduced into practice.

In recent years, under the leadership of Valery V. Mikhalishin, technologies for the manufacture of the inactivated vaccines against rabies and Aujeszky's disease based on the viruses reproduced in a suspension BHK-21/2-17 cell culture as well as of the viral vaccines for oral vaccination of wild carnivores against rabies were developed. Technology for manufacture of inactivated culture vaccines against all seven types of foot-and-mouth disease virus was also improved. Still relevant remains the research on the creation of adjuvant-free vaccines that form early immunity.

The research results were published in more than 200 scientific papers, the novelty of the research was confirmed by 30 certificates of authorship and patents, and awarded medals of the USSR VDNH. Ten Candidate of Science theses were defended under the scientific supervision of Dr. Mikhalishin. Valery V. Mikhalishin is a member of the Academic and Dissertation Councils of the Federal Centre for Animal Health.

He transfers his extensive experience in the manufacture of FMD vaccines to young employees of the Laboratory for FMD Prevention, participates in the development of regulatory and technical documentation, prepares recommendations for veterinary practitioners on FMD outbreak eradication using effective FMD vaccines with due account for the regional specifics.

We are sincerely grateful to you, Dr. Mikhalishin, for your dedication to veterinary science, patience, kind words in support of young researchers, for your faith, attention and inspiration, for your willingness to help, advise, guide and cheer. You are, undoubtedly, a worthy example to follow!

Please accept our sincere wishes for health, well-being, strength and energy for further fruitful work!

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