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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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From the Editor-in-Chief of "Veterinary Science Today"

Dear readers, authors of our journal, members of the editorial board!

Please accept my heartfelt congratulations on the New Year 2025 and my deep appreciation for your contributions to the development of our journal! The past year has been very fruitful. Thanks to our collaborative efforts, we published four issues of the journal, which included 50 original and review articles containing the results of research conducted by 187 authors from 32 research and educational organizations across 24 cities and three countries.

I would like to express my immense gratitude to the editorial board members for their cooperation, valuable advice, and recommendations; your expertise has contributed to an objective and unbiased editorial process. I also want to extend special thanks to the reviewers for their work in improving the quality of the manuscripts submitted for publication; to the proofreaders and translators for their attentiveness, meticulousness, and diligent work; and to the cohesive team of the journal for their professionalism, prompt communication, high standards of publishing activities, perseverance, and responsible approach to the field of Science! It is only through the coordinated efforts of the entire team that the journal has come a long and challenging way, gained recognition, been included in the "List of peer-reviewed scientific publications, where must be published basic scientific results of theses on competition of a scientific degree of candidate of Sciences, on competition of a scientific degree of the doctor of Sciences", library and scientometric databases, and has acquired the potential for further development.

We hope for continued fruitful collaboration and will strive to expand the geographical scope of the scientific connections of the journal "Veterinary Science Today" not only within Russia but also beyond its borders.

I wish you good health, scientific enthusiasm, strength, creative growth, and the realization of all your ideas and plans!

May the coming year bring everyone the joy of life, peace, and prosperity, and become a happy time for new ideas, significant events, and positive changes!

Sincerely,
Editor-in-Chief of the journal
Doctor of Biological Sciences, Professor
Konstantin N. Gruzdev

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Currently important pathogenic *Listeria* species affecting animals and birds (review)

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ABSTRACT

Listeriosis is one of the most severe gastrointestinal diseases in the world. *Listeria* affect different groups of animals and birds. The pathogen has been detected in meat, milk, fish and fish products. The disease shows spring and autumn seasonality. It has been reliably established that *Listeria monocytogenes* is ubiquitous in the environment. *Listeria monocytogenes* is a facultative intracellular pathogen. Infection with *Listeria monocytogenes* causes an invasive disease in animals and humans, which is transmitted via the fecal-oral route from an animal to a human, from a mother to a fetus. The pathogenesis of *Listeria* infection has been well studied. The gastrointestinal tract is the site of the pathogenic *Listeria* species transit and spread. The infection incubation period is 20–30 days in animals and humans. The clinical course in different animal species, including birds, has a number of specific features. *Listeria* can cross the intestinal, placental and blood-brain barriers. The manifestations of listeriosis include encephalitis, meningitis, gastritis, meningoencephalitis, mastitis, abortions, endometritis, etc. Pathogenic *Listeria* species show hemolytic activity which non-pathogenic species (except *Listeria seeligeri*) lack. The review presents the up-to-date information on the classification of *Listeria*, the pathogenicity factors of *Listeria monocytogenes* as the major pathogen, the mechanisms of *Listeria* infection development in different animal species.

Keywords: review, *Listeria* spp., pathogenicity, biosafety, animals, birds, antibiotic resistance

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Актуальные патогенные виды листерии животных и птиц (обзор)

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РЕЗЮМЕ

Листериоз является одним из наиболее тяжело протекающих заболеваний пищеварительного тракта во всем мире. Листерии поражают различные группы животных и птиц. Возбудитель выявлен в мясе, молоке, рыбе и рыбопродуктах. Отмечается сезонность заболевания в весенний и осенний периоды. Достоверно установлено, что бактерия *Listeria monocytogenes* свободно обитает в окружающей среде. *Listeria monocytogenes* является факультативным внутриклеточным патогеном, заражение которым у животных и людей приводит к инвазивному заболеванию, передающемуся фекально-оральным путем от животного к человеку, от матери к плоду. Патогенез листериозной инфекции хорошо изучен. Желудочно-кишечный тракт является местом транзита и распространения патогенных листерий. Инкубационный период листериозной инфекции длится 20–30 дней у животных и людей. Клиническое течение у различных видов животных, и в том числе птиц, имеет ряд особенностей. Листерии успешно преодолевают кишечный, плацентарный, гематоэнцефалический барьеры. При листериозе отмечается энцефалит, менингит, гастрит, менингоэнцефалит, маститы, аборты, эндометриты и др. Патогенные виды листерий обладают гемолитической активностью, которая отсутствует у непатогенных видов (исключение – *Listeria seeligeri*). В обзоре представлена актуальная информация о классификации листерий, факторах патогенности *Listeria monocytogenes* как основного патогена, механизмах протекания листериозной инфекции у различных видов животных.

Ключевые слова: обзор, *Listeria* spp., патогенность, биобезопасность, животные, птицы, антибиотикорезистентность

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INTRODUCTION

Microorganisms of the genus *Listeria* belong to the family *Listeriaceae*, the order *Bacillales*, the class *Bacilli* and the phylum *Firmicutes*. *Listeria* is a gram-positive bacterium genetically related to *Clostridium*, *Enterococcus*, *Staphylococcus*, *Streptococcus* and *Bacillus*. *Listeria* spp. are facultative anaerobic rods with a size of 0.4 × 1–1.5 μm, which do not form spores, have no capsule and are motile at 10–25 °C [1]. *Listeria* spp. are isolated from various environmental sources such as soil, water, wastewater, animal and human feces, food products. A number of researchers have found that the natural habitat of the bacterium is a decaying plant substrate. Transmission occurs through the fecal-oral route. In rural areas, ruminants are the main vectors of *Listeria* [2, 3, 4]. The genus *Listeria* is currently represented by the following species: *L. monocytogenes*, *L. innocua*, *L. ivanovii* (formerly known as *L. monocytogenes* serotype 5), *L. farberi*, *L. seeligeri*, *L. welshimeri*, *L. ilorinensis*, *L. rocourtiae*, *L. weihenstephanensis*, *L. marthii*, *L. grandensis*, *L. riparia*, *L. cossartiae*, *L. fleischmannii*, *L. portnoy*, *L. rustica*, *L. immobilis*, *L. booriae*, *L. thailandensis*, *L. goaensis*, *L. costaricensis*, *L. floridensis*, *L. aquatica*, *L. grayi*, *L. valentina*, *L. newyorkensis*, *L. swaminathanii*, *L. cornellensis* [5]. Two species, *L. ivanovii* and *L. monocytogenes*, are pathogenic for humans and animals [6]. The official discovery of *Listeria* was in 1924, when a group of researchers (E. G. D. Murray, R. A. Webb, M. B. R. Swann) in Cambridge, England, isolated *L. monocytogenes* as the etiological agent of a septicemic disease affecting rabbits and guinea pigs [7]. The first case of human listeriosis was reported in Denmark in 1929. *L. ivanovii* was first isolated from lambs in Bulgaria in 1955 [8]. Cases of *L. ivanovii* infection are rare; the bacteria of this species are mainly isolated from abortions and neonatal septicemias in sheep and cattle [9, 10, 11]. Although *L. seeligeri* is a non-pathogenic *Listeria* species, one human case of infection with this bacterium has been reported [12, 13].

Listeria spp. are widespread in nature, they have been isolated from more than 90 animal species, as well as from plants and a broad range of food products. These bacteria can persist in the animal body for a long time. Suppressing the growth of *Listeria* in the ready-to-eat products is still a challenge. The examination of the biological and environmental characteristics of *Listeria* is aimed at the prevention of *Listeria* infection and the control of this important food pathogen.

The novelty of the study is the systematization of the up-to-date data on the biological properties and classification of *Listeria*. The specific features of listeriosis in livestock and poultry are presented.

The work was carried out using analytical research methods and the RSCI, Scopus, Web of Science, Library Genesis, Sci-Hub, Google Scholar, PubMed, Cyberleninka databases.

The purpose of the review was to analyze and systematize current knowledge about pathogenic *Listeria* species, the classification of *Listeria*, the mechanism of the disease development and characteristic features of *Listeria* infection in different animal and bird species.

CLASSIFICATION AND BIOLOGICAL PROPERTIES OF *LISTERIA*

The bacteria of the genus *Listeria* are found on all continents. Soft cheeses and milk products, sausages, pastes, salads, smoked fish and, as a rule, ready-to-eat chilled industrially-made products are most often contaminated [14, 15, 16, 17]. *Listeria* is well adapted to survive on the surfaces of the food processing equipment. For example, *Listeria* tolerates high salt concentrations (> 10%) and relatively low pH values (< 5.0) and is able to reproduce at low temperatures [18, 19]. Listeriosis shall be differentiated from rabies, influenza, brucellosis, pasteurellosis, toxoplasmosis.

Pathogenic *Listeria* species show hemolytic activity which non-pathogenic species (except *L. seeligeri*) lack. The hemolysin gene (*hly*) has a key role in cell destruction [20]. The biological characteristics of some species of the genus *Listeria* are presented in Table 1 [21].

The classification of *L. monocytogenes* is based on the structure of the somatic (O) and flagellar (H) antigens, and all members of this species are divided into the following serovars: 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4b, 4c, 4d, 4e and 7 [22].

Listeria monocytogenes is a facultative intracellular pathogen. Infection with *L. monocytogenes* causes an invasive disease in animals and humans, which is transmitted via the fecal-oral route from an animal to a human, from a mother to a fetus [23]. The pathogenicity factors of this bacterium species are shown in Table 2 [21].

Most of the information on the pathogenesis of listeriosis has been obtained on the basis of the interpretation of epidemiological, clinical and histopathological data in experimental infections in animals. The gastrointestinal tract is the main site of the entry and spread of pathogenic *Listeria* [17]. It is reported that the increased acidity of gastric juice can destroy a significant amount of *Listeria*. It is well known that *Listeria* infection incubation period in animals and humans is 20–30 days. *Listeria* spp. penetrating through the intestinal barrier are carried by lymph and blood to the mesenteric lymph nodes, liver, spleen [24].

Table 1
Biological characteristics of some species of the genus *Listeria* [21]

Characteristics		*L. monocytogenes*	*L. innocua*	*L. ivanovii*	*L. seeligeri*	*L. welshimeri*	*L. grayi*
β-hemolysis		+	–	+	+	–	–
CAMP test (*Staphylococcus aureus*)		+	–	–	+	–	–
CAMP test (*Rhodococcus equi*)		±	–	+	–	–	–
Production of acid from	mannitol	–	–	–	–	–	+
	α-methyl-D-mannoside	+	+	–	–	+	+
	L-rhamnose	+	d	–	–	d	±
	soluble starch	–	–	–			+
	D-xylose	–	–	+	+	+	–
Hippurate hydrolysis		+	+	+			–
Nitrate reduction		–	–	–			±
Pathogenicity for mice		+	–	+	–	–	–

"+" – 90% or more of the strains are positive; "–" – 90% or more of the strains are negative; d – 11–89% of the strains are positive;
"±" – a variable variant; the absence of a result means that no tests for this parameter have been carried out.

An experimental study in mice (intravenous administration) has revealed that *L. monocytogenes* is rapidly removed from the bloodstream by resident macrophages of the spleen and the liver [25]. The major bacterial load is located in the liver, in which Kupffer cells are active. These macrophages destroy most of the engulfed bacteria. A number of authors believe that Kupffer cells initiate the activation of anti-listerial immunity by inducing the antigen-dependent proliferation of T lymphocytes and cytokine secretion [26]. Also, various scientific papers note the partial survival of *Listeria* cells after attack by macrophages, which actively grow over the next 2–5 days [27, 28, 29]. *Listeria* cells move by direct passage from hepatocyte to hepatocyte and disseminate in the liver parenchyma without coming into contact with the humoral immune system. The authors conclude that this explains the levelling of the role of antibodies in the antibody-*Listeria* interaction [30]. *L. monocytogenes* is a multi-system pathogen that affects a wide variety of animal and human tissues.

Table 2
Pathogenicity factors of *Listeria* monocytogenes [21]

Protein	Molecular weight, kDa	Gene	Function
prfA	27	*prfA*	Regulation of virulence gene transcription
Listeriolysin O	58	*hly*	Lysis of primary and secondary phagosomes
PICA (phosphatidylinositol-specific phospholipase C)	36	*plcA*	Phagosome lysis
Lecithinase	33	*plcB*	Lysis of secondary phagosome
Metalloprotease	57	*mpl*	Post-translational modification of lecithinase
ActA	67	*actA*	Polymerization of actin
Internalin, *inlB*	88.65	*inlA, inlB*	Induction of phagocytosis

BOVINE LISTERIOSIS

Cattle account for a greater number of pathogenic *Listeria* detections reported worldwide [31]. Bovine listeriosis most commonly manifests itself as encephalitis; miscarriages, septicemia with miliary abscesses are also noted. Foodborne transmission is the main mode of infection in cattle, particularly as a result of low-quality silage feeding, drinking of contaminated water. After ingestion, *Listeria* cells are disseminated via hematogenous spread to the viscera, nervous system and reproductive organs of pregnant cows. Another route of infection is through the abrasions of the skin or the conjunctiva during grazing or via the teats. When the central nervous system is affected, the pathological process localizes in the medulla oblongata and the pons [32, 33]. Damage to the respective nerve underlies the characteristic presentation: damage to the fifth cranial nerve (CN V) and the mandibular nerve leads to the inability to drink or eat and, consequently, to further disorders [34]; the sign of damage to CN IX and CN X is excessive salivation due to the swallowing difficulty; CN XII – the protrusion of the tongue; CN VIII – ataxia and circling, facial paralysis, including unilateral drooping of the lip, eyelid, ear; CN VI – strabismus. In advanced stages of the disease, the animal lapses into a coma and dies within a few days. Postmortem cerebral cortex lesions consist of the foci of necrosis infiltrated with neutrophils, macrophages, bacterial cells [35, 36]. Listeriosis shall be differentiated from rabies.

The genital form of listeriosis in cows presents as abortions in the last trimester of gestation. In case of fetal infection, newborn animals develop meningitis and subsequently die [37].

The authors also note the seasonality of bovine listeriosis, with the number of cases increasing in winter and spring and decreasing in summer [32].

PORCINE LISTERIOSIS

In pigs (the young ones), listeriosis most often manifests itself as septicemia and is less common than in other animal species. Encephalitis and miscarriages are rarely reported [38]. Hepatic necrosis is a characteristic morphological feature of listerial septicemia in newborn piglets [39, 40]. The first case of listeriosis in piglets was reported in Russia in 1936 by T. P. Slabospitsky, who named the pathogen *L. suis* [41]. In sows, *L. monocytogenes* localizes in the tonsils, from where it then enters the gastrointestinal tract. Porcine listeriosis cases are more frequently detected in winter and spring. The symptoms of central nervous system disturbance in young animals with listerial encephalitis are incoordination, weakness and apathy, followed by death. Meningoencephalitis in pigs is characterized by a sudden decrease in appetite, neurological disorders (trembling, partial paralysis, urinary incontinence, seizures), elevated body temperature [42]. Histopathological examination reveals severe monocytic infiltration, many blood vessels show perivascular constriction [43, 44]. The largest outbreak of listerial meningoencephalitis in pigs was reported in India, in which 27 of 75 pigs died [45].

OVINE LISTERIOSIS

Ovine listeriosis is caused by *L. monocytogenes* serotypes 1/2 and 3, as well as by *L. ivanovii*. Ovine listeriosis (circling disease) was first reported in New Zealand in 1929. The frequency of infection in sheep is higher (up to 30%) than in cattle (up to 15%). Ovine listeriosis manifests itself as encephalitis, gastrointestinal septicemia with hepatitis, splenitis, pneumonitis and abortions more commonly occurring in the last trimester of gestation. Encephalitis is the most common form of listeriosis diagnosed in sheep [46]. Lambs aged 5 weeks may develop septicemia, older lambs develop encephalitis. *Listeria* infection in adult sheep presents as a central nervous system disorder (meningoencephalitis), refusal to eat or drink, elevated temperature, teeth grinding, paralysis of the muscles of mastication, excessive salivation caused by the inability to swallow due to damage to the cranial nerve, circling movements (circling disease). In advanced stages, muscular incoordination develops, which is followed by the animal's inability to walk; death occurs within 2–3 days after the onset of the first symptoms. Histological examination reveals microgranulomas and microabscesses in the brainstem. Listerial encephalitis in sheep is most common in late autumn, winter and early spring. After hematogenous spread to the uterus, *L. monocytogenes* is detected within 48 hours in the fetus and the amniotic fluid. Initially, pregnant ewes develop purulent metritis. The clinical symptoms resolve after the abortion, and the ewes begin to feel much better. Morbidity in ewes ranges from 1 to 20%, with mortality of lambs being high. Septicemia is most frequent in newborn lambs and follows 2–3 days after oral infection. It is characterized by high temperature, loss of appetite, diarrhea. Death may occur as a result of extensive liver damage, pneumonia; the mortality rate is much lower for the septicemic form of listeriosis than for the encephalitic one. The factors contributing to the pathogenicity of *Listeria* are overcrowding, stress and feed quality [47, 48].

Listeria ivanovii is a recognized etiological agent of abortions in sheep [48]. Ovine listeriosis cases caused by *L. ivanovii* and characterized by abortions make up 8%. Factors predisposing to infection with *L. ivanovii* are the same as for *L. monocytogenes* and include stress, decreased immunity, feeding of poor-quality feed, contact with infected animals, etc. [49, 50].

As a preventive measure, vaccination with live attenuated strains of *L. monocytogenes* takes place. For example, in Germany and Greece, immunization of sheep with an attenuated vaccine based on serovars 1/2a and 4b reduced the incidence of listeriosis and abortions as compared with the control group. The results of field tests showed that the immunized ewes delivered more lambs free from *Listeria* (92.4 vs 69.7%) and of higher birth weight (2.2 vs 1.8 kg) than lambs from the control unvaccinated ewes. Besides, *L. monocytogenes* was not isolated from the milk samples from the vaccinated ewes, in contrast to the control group, in which *Listeria* was detected in 31.9% of milk samples [47].

In the event of an outbreak, several strains of the same or different *Listeria* serotypes may be isolated. DNA fingerprinting, phage typing, pyrolysis mass spectrometry are used as additional diagnostic methods to confirm the diagnosis [51].

CAPRINE LISTERIOSIS

Clinical listeriosis in goats is similar to that in sheep and manifests itself as septicemia, abortion, encephalitis [52]. The mechanism of the disease development is as follows: pathogenic *Listeria* penetrates into the goat body through the gastrointestinal tract, which leads to a transient bacteremia and further spread to the central nervous system, viscera, as well as to the placenta. Depression, decreased appetite and milk yield, diarrhea, elevated body temperature (41 °C) are the first signs of septicemia. In pregnant does, *L. monocytogenes* penetrates through the placenta into the fetus and subsequently causes late miscarriages [53].

A number of researchers note that meningoencephalitis is the most frequently reported form of listeriosis in goats. Early signs of listerial encephalitis in goats are excessive salivation, ear droop, tongue protrusion, absence of rumination [54]. Some researchers also point out that goats are more susceptible to listeriosis than sheep [52]. For example, during an outbreak of listeriosis in Iraq, the morbidity (30.0 vs 16.7%) and mortality (21.2 vs 14.9%) were higher in goats than in sheep [55]. *Listeria* isolations from goats are more frequent in winter and spring.

AVIAN LISTERIOSIS

Avian listeriosis was first reported in 1935 [56]. Wild ducks, turkeys, pheasants, geese are asymptomatic carriers of *Listeria*. Birds become *Listeria*-infected orally by pecking feces, soil, carcasses. Listeriosis is much less common in birds than in sheep, goats and cattle [57, 58, 59]. Listeriosis in birds can also develop as a secondary infection associated with viral diseases, as well as with salmonellosis, coccidiosis, worm infections, tick-borne encephalitis, lymphocytosis, enteritis and others that contribute to a decrease in immunity [60]. One of the distinguishing features of avian listeriosis is septicemia characterized by focal necrosis of viscera, particularly the liver and the spleen. At the same time, a number of authors note cardiac lesions such as vascular occlusion, pericarditis and increased amounts of pericardial fluid. The septicemic form of listeriosis produces the following conditions: splenomegaly, peritonitis, nephritis, ulcers in the ileum and caecum, necrosis of

the oviduct, generalized pulmonary edema, conjunctivitis, enteritis. In acute cases of the septicemic form of listeriosis, all internal organs are affected. Birds show practically no overt clinical signs other than emaciation and die on days 5–9 of the disease [59, 61].

Listerial meningoencephalitis is far less common in birds. It is characterized by the disturbances of the central nervous system such as incoordination, tremors, torticollis, dropped wings, unilateral or bilateral toe paralysis [62], which later lead to death. Necropsy reveals congestion and necrotic foci in the brain [63, 64].

Chicken embryos and young chickens are the most susceptible to listeriosis [61, 65, 66]. In day-old chicks, *Listeria* is most frequently detected in the caecum, liver, spleen and cloacal swab samples [63].

Thus, the incubation period of listeriosis depends on the overall clinical picture. In animals and birds, the infection occurs in the septicemic, encephalitic, abortive forms. Pathogenic *Listeria* species are important etiological agents of animal and bird diseases that lead to severe consequences and economic damage [67]. To date, listeriosis remains an urgent issue and does not receive sufficient coverage in the public space.

CONCLUSION

Listeria spp. are distributed globally in a wide variety of domestic and wild animals, as well as in humans, and possess a zoonotic potential.

The analysis of scientific publications has made it possible to summarize data on the mechanisms and routes of transmission, pathogenicity factors of *Listeria*, their serological diversity, localization in the body of susceptible animals, the forms of listeriosis in cattle, pigs, sheep, goats and birds.

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Using fecal microbiota transplantation for animal health (review)

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ABSTRACT

Fecal microbiota transplantation is a procedure when fecal matter from a healthy donor is administered into the intestinal tract of a recipient in order to restore microbial balance and strengthen immune responses. Mainly, fecal microbiota transplantation increases bacterial diversity and facilitates a growth in beneficial microorganisms. Thus, the procedure makes it possible to stabilize and maintain a healthy gut microbiome that inhibits the pathogen growth. In veterinary medicine, fecal microbiota transplantation is considered as a potential alternative to traditional antibiotics amid rising antibiotic resistance. Despite the lack of commonly accepted procedures, studies show that the fecal microbiota transplantation for the purposes of veterinary medicine can be used for a wide range of tasks: starting from disease prevention to immunomodulation. This review is devoted to the use of fecal microbiota transplantation for different animal species. An analysis of scientific literature suggests that most researches into the topic describe the use of fecal microbiota transplantation as a method to treat diarrhea, which is a common disorder in animals. Interestingly, the technique has been successfully used to treat canine atopic dermatitis and monitor age-related changes in fish, thus, confirming the universal nature of this procedure. There are research projects when fecal microbiota transplantation demonstrated only partial effectiveness or no effectiveness at all. Scientific evidence suggests that the effectiveness of fecal microbiota transplantation depends on the delivery route and the donor, and the first factor may have a different impact on the therapy effectiveness depending on the animal species under study. The impact of the second factor on the success of transplantation has been most widely studied for calves. Further research is needed into effects of fecal microbiota transplantation on different animals and standards need to be developed to support large-scale and safe use of the technique for animals.

Keywords: review, fecal microbiota transplantation, microbiota, donor, recipient

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Использование процедуры трансплантации фекальной микробиоты в ветеринарии (обзор)

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РЕЗЮМЕ

Трансплантация фекальной микробиоты представляет собой процедуру, при которой фекалии здорового донора вводятся в кишечник реципиента для восстановления микробного баланса и укрепления иммунной защиты. Главным образом трансплантация фекальной микробиоты обеспечивает увеличение бактериального разнообразия и повышение численности полезных микроорганизмов, что позволяет стабилизировать и поддерживать здоровый микробиом, ингибирующий рост патогенов. В ветеринарии трансплантация фекальной микробиоты рассматривается как потенциальная альтернатива традиционным антибиотикам в условиях нарастающей антибиотикорезистентности. Несмотря на отсутствие единых протоколов, исследования показывают, что процедура трансплантации фекальной микробиоты в ветеринарии может применяться для широкого спектра задач: от профилактики заболеваний до иммуномодуляции. Данная обзорная статья посвящена аспектам применения трансплантации фекальной микробиоты на разных видах животных. Согласно анализу научной литературы, большинство работ по данной теме описывают использование трансплантации фекальной микробиоты в качестве терапевтического средства против такого распространенного патологического состояния в ветеринарии, как диарея. Также интересно, что методика успешно применялась для лечения атопического дерматита у собак и мониторинга

возрастных изменений у рыб, что подтверждает универсальность данной процедуры. Есть исследования, в которых трансплантация фекальной микробиоты проявляет эффективность частично или не проявляет вовсе. Научные данные свидетельствуют о том, что результативность трансплантации фекальной микробиоты зависит от таких факторов, как способ введения фекального материала и выбор донора, причем первый аспект может по-разному влиять на эффективность терапии в зависимости от вида исследуемого животного. Влияние второго фактора на успешность проведения трансплантации наиболее полно изучено для телят. Необходимы дальнейшие исследования механизмов воздействия трансплантации фекальной микробиоты на разных животных и разработка стандартов, которые могли бы обеспечить широкое и безопасное применение методики в ветеринарии.

Ключевые слова: обзор, трансплантация фекальной микробиоты, микробиота, донор, реципиент

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INTRODUCTION

In 1954, M. Bohnhoff et al. first described the well-known fact that the gut microbiota plays a fundamental role in immune defense as revealed by the study conducted on mice. Streptomycin-fed mice were much more susceptible to experimentally induced *Salmonella* infections than animals that were not administered antibiotics. This observation is explained by the fact that the antibiotic makes the mouse "vulnerable to the introduction of contaminating microorganisms by suppressing or eliminating some of its normal inhabitants" [1]. Today, scientists, particularly veterinarians, are facing a new challenge of antibiotic resistance, so the search is underway for alternative therapies that could replace antimicrobials. Fecal microbiota transplantation (FMT) may be among such new solutions.

There is no universally accepted definition of FMT [2]. The FMT technique involves introduction of fecal matter from a healthy donor into the intestine of a sick recipient to modulate or replace the intestinal microbiota [3]. FMT's history dates back to the 4th century, and since 2013, it has gained recognition, starting from the moment when the United States Food and Drug Administration approved it for treatment of recurrent and refractory *Clostridium difficile* infection in humans [4, 5].

In veterinary medicine, the transfer of gastrointestinal contents for therapeutic purposes has been used for centuries, for example in cattle (rumen transfaunation) [6]. There are reports on regurgitated cuds used for microbial transplantation and this technique was long used in Sweden to treat ruminal indigestion, and the cud beneficial effect even made it possible to refer to it as a "living thing" [7]. An important difference between FMT and transfaunation is the site where microbiota is collected from the gastrointestinal tract (i. e., rectum and rumen); however, conceptually and functionally both techniques are similar [8]. In small animal gastroenterology, intensive use of FMT procedure has only recently begun [8, 9].

The mechanisms underlying FMT therapy are not fully studied [8], however, it is assumed that FMT may increase bacterial diversity, supply bacteriocins and bacteriophages, and stimulate nutrient metabolism, including conversion of primary bile acids. Restoring eubiosis can enhance intestinal barrier mechanisms and bolster immunity [10]. In humane medicine, FMT is still being studied as an option to deal with various conditions such as chronic enteropathies (inflammatory bowel disease, irritable bowel syndrome), liver disease, obesity, metabolic syndrome, and neuropsychiatric disorders. However, most widely FMT is used to treat recurrent infection caused by *Clostridium difficile* resistant to standard therapy [10, 11].

Nevertheless, despite the extensive research into FMT, there are many issues to be clarified and for which there is no generally accepted opinion. For example, the specific FMT mechanism is not unique, but may have different efficacy depending on the disease and animal species [2].

There is still no universal consensus about another key point, i. e. how FMT-based therapies should be considered and legally regulated. Depending on the country, FMT can be considered, for example, as a biological agent (USA), a medicinal product (UK) or as a cell/tissue transplant (Italy) [2]. Finally, although this procedure is considered generally safe, the potential short-term and especially medium- and long-term risks that may be associated with FMT still need to be carefully studied [2, 12, 13].

GENERAL FMT PROCEDURE

The FMT procedure is used in veterinary medicine to achieve the following goals: to reduce pathogens, restore healthy microbiome and ultimately improve overall animal health condition. According to M. C. Niederwerder, the key effect of FMT is associated with an increase in bacterial diversity and an increase in the number of beneficial microorganisms, thus, stabilizing and maintaining a healthy gastrointestinal microbiocenosis and inhibiting the growth of pathogens [8].

It is generally accepted that commensal bacteria in stool are the key component that ensures FMT efficacy. However, other fecal components such as viruses, fungi, immunoglobulins and bacterial metabolites also play an important role in FMT. Preserving these components is crucial during feces preparation [14].

Fecal microbiota transplantation includes several mandatory steps.

1. Donor selection. The donor must be healthy, free of gastroenterological or infectious conditions and shall not have be exposed to antibiotics within the previous 6 months. Hui Y. et al. emphasized the key role of the donor for successful FMT outcome, when recipients from one particular donor were cured of necrotizing enterocolitis and had higher relative lactobacilli counts [15]. Some researchers also refer to the key importance of the thorough pre-FMT examination with the purpose to detect pathogens in feces [2, 3, 12, 16]. Typically, the animal donor is selected based on its medical history and following tests for a wide range of infectious pathogens. In humane medicine, the universal stool bank model is often applied, since it allows using pre-selected and frozen preparations for FMT [2]. This approach reduces costs due its large scales and improves safety due to standardized procedures and monitoring.

2. Preparing fecal matter solution. Donor material is usually mixed with saline or water (sometimes glycerol is added) and then filtered to remove large particles. This material can be stored frozen, but freshly prepared suspensions are more preferable for tests in animals because they preserve microbial diversity and microbiota much better. In addition, some preparations are commercially available: either for self-filling capsules and ingestion (mainly for humans) or as microbiome tablets for small animals, which may contain fresh or freeze-dried preparations derived from intestinal microbiota [9].

3. Transplantation procedure. Administering the on-site prepared suspension via enema [17, 18, 19], endoscopy [20, 21], nasogastric tube [3, 22, 23], or orally [24, 25, 26, 27].

There is still no strictly established and approved FMT procedure; therefore, it needs to be adapted to specific cases and conditions.

FMT FOR ANIMALS

Nowadays FMT studies in humans are more specific and detailed than in animals, particularly in dogs. Nevertheless, the gut microbiota of dogs closely resembles the human gut microbiota [28]. Accordingly, bacteria, viruses, bacterial fragments, fungi, mucin, immunoglobulin A (IgA) and bacterial metabolites may be important components of FMT both in dogs [14] and in humans [29].

Currently, there are three main directions of FMT use in animals. FMT in animals is currently applied in three main directions: therapeutic, prophylactic and stimulation of pathogen-specific immunity [8].

The FMT therapeutic use is necessary when the goal is to treat clinical signs or eliminate chronic diseases. FMT-based prophylaxis may be a useful part of preventive medicine since it boosts beneficial bacteria before the body is exposed to pathogens. Finally, FMT can be used as an immunostimulant just like vaccination, where transplant material stimulates pathogen-specific immunity to enhance immunoglobulin transfer.

Most scientific sources on FMT are devoted to its therapeutic effect on the evident clinical signs after the diagnosis is made, although its preventive effect and immunogenetic use were primarily tested in pigs and poultry.

As publicly available sources suggest, FMT has been tested in different animal species: fish, mice, chickens, cats, dogs, monkeys, pigs, calves, horses. It can be used as an independent procedure, as well as in combination with other therapeutic tools. The number of FMT iterations also varies across different experiments, ranging from single injections to dozens, which confirms the lack of a unified procedure or of a standardized technique for animals.

Systematized and generalized information and literature sources within the review scope [13, 15–19, 21–27, 30–44] are presented in Table in the Additional Files section at: https://doi.org/10.29326/2304-196X-2024-13-4-314-321

FMT in pets. FMT effects have been best studied in dogs (see Table) to mainly treat gastroenterological disorders together with other diseases, such as atopic dermatitis [25]. It is noteworthy that FMT has proven to be an effective method that makes it possible to achieve complete cure or significantly improve the recipients condition in all mentioned cases. The FMT procedure was mainly performed independently, however, there were a number of exceptions [17, 21, 24, 44], when the tool was applied together with other methods. The successful use of this independent procedure in most cases indicates the possibility of simple FMT standardization which will require no extra costs. Notably, most pet experiments involved a single FMT procedure with successful outcomes, however, during the experiment held by C. A. Rojas et al. the observed cats received totally 50 capsules [31]. As for the route of FMT administration to pets, faecal material is inserted rectally and orally, via a tube.

The key gastrointestinal metabolites that regulate host immunity and maintain immune homeostasis are short-chain fatty acids (SCFAs), which affect lipid metabolism [37]. It is worth noting that when FMT is done orally, SCFAs are rapidly absorbed and oxidized, therefore, enemas or colonoscopy are more preferable. At the same time, oral administration of fecal matter may allow bacteria to colonize the small intestine and ileum, and may allow metabolites that are produced by enteric bacteria (secondary bile acids), to penetrate into the small intestine and ileum. Therefore, combining both FMT administration routes (oral and enema/colonoscopy) is the most reasonable approach [14].

FMT in pig farming. Tests in pigs provide extensive data on FMT effectiveness from different perspectives which include prevention of intestinal diseases, improved feed conversion ratio and boosted immunity [15, 32, 33, 34, 35]. In most experiments, fecal suspension was administered to pigs via nasogastric or rectal tubes. At the same time, A. Brunse et al. (2019) studied the combined administration of the matter to preterm piglets, which showed that this administration route was associated with a higher risk of gut colonization with pathogenic bacteria ultimately resulting in a mortality increase [32]. Whereas purely rectal FMT proved to be effective in reducing the frequency of necrotizing enterocolitis without any negative consequences. This observation contrasts with the conclusion made by K. Li et al. regarding dogs. Perhaps combined administration is not the most effective transplantation route for all animal species [14].

In 2021, A. Brunse et al. conducted a research, where piglets received antibacterial drugs together with FTM. It partially restored microbial diversity and reduced the number of antibiotic-resistant bacteria such as *Enterobacter cloacae* and *Pseudomonas aeruginosa*. The FMT procedure alone (without any prior antibiotic therapy)

proved to be more effective to restore healthy microbiota in piglets' large intestines. Concentrations of such cytokines as IL-6 and CXCL-8 were higher in the group that received just FMT treatment ($p < 0.05$), unlike the other group that was treated using a combined approach. Thus, the combined use of antimicrobials and FMT turned out to be less effective, which suggests there are antagonistic interactions between them [33].

As mentioned above, the correct choice of a donor is one of the key factors behind the FMT success. Thus, piglet experiments show that only the matter from a particular donor reduces the risks of necrotic enterocolitis. PERMANOVA tests (Multivariate Analysis of Variance) conducted between groups at the level of microbiota genera and species ($R^2 = 0.45$ for 16S rRNA; $p = 0.001$), revealed that the microbiome exposed to the fecal matter from this donor differed significantly from other groups. There was a decrease in the concentration of *Enterobacter cloacae*, *Staphylococcus aureus* and other pathogens, and a relative increase in *Limosilactobacillus reuteri* and *Lactobacillus crispatus* counts [15]. Evidence suggests that FMT is effective in treating porcine viral diseases. Thus, M. C. Niederwerder et al. describe how the procedure was successfully used to control circovirus disease and porcine reproductive respiratory syndrome. It contributed to changes in the intestinal microbiocenosis, reducing the count of opportunistic bacteria such as *Vibrionaceae* and *Spirochaetaceae*, as well as increasing the level of antibodies in infected piglets [35].

Feed conversion ratio is a critically important economical parameter in pig farming. As demonstrated by the related research, FMT in sows increases the efficiency of their piglets in converting food into body mass [34]. These animals showed better feed conversion ratios, i.e. reduced residual feed intake and increased microbial diversity. It is due to an increase in bacteria involved in fiber fermentation which account for improved feed digestibility. The inulin addition also contributed to an increase in beneficial bacteria counts and decreased levels of certain pathogens (for example, *Chlamydia*), although this did not lead to significant weight gain. Thus, FMT combined with prebiotics can be an effective strategy to increase productivity in pig farming.

FMT in poultry farming. There are a number of studies on successful FMT use in chickens. Such disorders as intestinal infections [39], changes in circadian rhythms [36] were studied, as well as FMT effect on growth, immune balance [38] and lipid metabolism in birds [37]. Pang J. et al. studied FMT effectiveness for chickens infected with *Campylobacter jejuni*. They got infected either by direct introduction of bacterial suspension into the body or as a results of housing healthy chickens together with the infected ones. FMT turned out to be effective in reducing *C. jejuni* colonization during direct infection. *C. jejuni* counts in this group were reduced by 2.5; 1.2 and 1.7 times compared to the control group on day 5, 10 and 15, respectively ($p < 0.0001$). The number of *Butyricimonas*, *Parabacteroides* and *Parasutterella* colonies grew, thus, enhancing resistance to pathogen colonization. On the contrary, FMT did not have a significant effect in chickens infected via a contact with the sick poultry [39]. This result suggests that the procedure lacks flexibility, and deeper research is required, since the second variant of infection (through contact with the infected poultry) is the most real in poultry farming.

As in pig farming, FMT can help improve economic development of poultry farming. As shown in research conducted by Z. Ma et al., the weight of chickens in the group that received FMT was 10.6% higher than in the control group (627.4 g vs 567.3 g; $p < 0.0001$). The FMT importance for the recipient's immune system was also demonstrated: lactobacilli found in the intestine enhanced tryptophan metabolism, which stimulates Treg cells and suppresses Th17, thus, boosting immune response, reducing inflammation, and, therefore, promoting chicken growth [38]. Excessive fat accumulation in broilers adversely affects poultry farming economics. Impact of fecal microbiota transplantation on lipid metabolism has also been studied [37]. The FMT stimulated growth of *Oscillospira* and *Streptococcus* bacteria, which are known for their ability to produce SCFAs associated with a decrease in fat mass. Thus, FMT contributed to reduction of abdominal fat deposits, confirming the importance of gastrointestinal microbiocenosis in lipid metabolism. Another research was focused on using FMT to correct negative effects coming from the disrupted circadian rhythms in chickens [36]. At the same time, FMT significantly improved the level of mitochondrial DNA and decreased oxidative stress, normalizing the expression of genes associated with the cell cycle. Changes were observed in hormone- and inflammation-associated genes when circadian rhythms were disrupted, but they returned to normal after transplantation.

FMT in cattle. Researches on calves showed that FMT is more effective than antibiotics to restore beneficial intestinal microbiota (*Bacteroides* and *Firmicutes*) which increases the SCFAs levels and reduces diarrhea symptoms [40]. FMT led to an active growth in calves, thus, confirming FMT potential to increase livestock productivity. Islam J. et al. conducted a large-scale analysis (metagenomic, metabolomic and biochemical) to identify factors contributing to FMT effects and to improve the donor and recipient selection procedure. The procedure was successful in 70% of cases and the success was proved to be dependant a lot on amino acids and SCFAs [16]. Representatives of the *Veillonellaceae* family and the *Selenomonas* genus in donors and recipients were considered as key microorganisms behind the FMT effectiveness, whereas sporobacteria (*Sporobacter*) have been proposed as a marker of an optimal donor. The research conducted by Y. Li et al. was not focused on the FMT procedure itself, but it was devoted to studying the effect of two strains of *Lactobacillus reuteri* L81 and *Lactobacillus johnsonii* L29 isolated from cow feces after FMT on growth, immunity and intestinal barrier function of weaned calves. As a result, *L. reuteri* L81 and *L. johnsonii* L29 increased calve growth rates, reduced the frequency of diarrhea, boosted immunity and reduced markers of intestinal permeability [45].

FMT in horses. Research conducted by D. P. M. Dias et al. showed that FMT is a highly effective tool to treat acute colitis in horses, since just within one day after a single procedure clinical symptoms completely disappeared in all the recipients. This method turned out to be faster and cheaper than traditional antibiotic therapy, moreover, no side effects such as dysbiosis or antibiotic resistance were observed [22]. In another research conducted by Y. Kinoshita et al., the FMT application did not lead to a successful outcome in horses with metronidazole-induced intestinal dysbiosis [23].

FMT in other animal species. The overall effect of FMT on the recipient's gut microbiota has been studied in a number of experiments. One of them, conducted by C. N. Ross and K. R. Reveles, showed that FMT is safe for common marmoset (*Callithrix jacchus*), which is confirmed by the absence of side effects, although microflora changes directly depended on the basic intestinal condition of the recipients than on the microbiota of donors. Variations in relative abundances of bacterial taxa demonstrate FMT potential to ensure stable changes in the intestinal microbiome of common marmosets [43]. For experiments in mice, domestic and wild pigs were selected as donors. The best effect on intestinal microbiocenosis was demonstrated in the mice who had FMT from wild pigs and sticked to a diet rich in dietary fiber. There was also an increase in concentration of beneficial fatty acids (nicotinic) [44].

Research on fish was also conducted. Thus, Z. Han et al. focused on FMT ability to accelerate restoration of intestinal microbiota in koi carp with florfenicol-induced dysbiosis [41]. The researchers demonstrated effectiveness of this procedure, accompanied by restored levels of beneficial bacteria such as *Lactobacillus*, *Bifidobacterium*, *Bacteroides* and *Faecalibacterium*. It was also determined that such metabolites as aromatic amino acids and glutathione compounds play a key role in normalizing intestinal metabolism after dysbiosis. Other experiments were devoted to the FMT effect on the life cycle and health status of the middle-aged African fish *Nothobranchius furzeri* who received FMT from the young donors [42]. As a result, the life span of the FMT fish recipients increased by 37% as compared to the control group (Logrank test, $p < 0.001$). Such bacterial genera as *Exiguobacterium*, *Planococcus*, *Propionigenium* and *Psychrobacter*, that are typical for young fish, remained in the aging fish who received FMT. The average distance covered by the FMT-treated aging fish within 20 minutes was 15% longer compared to the control group, thus, suggesting that they were physically as active as the young stock.

USING FMT: CHALLENGES AND PROSPECTS

Despite promising results, many aspects of FMT effectiveness and safety remain understudied, especially in the veterinary field, where there are still no standardized FMT procedures [9]. The lack of variety among strains included into the fecal matter used for FMT does not allow to classify FMT as a probiotic [2, 46]. Therefore, further FMT development is primarily associated with the possibility to develop targeted microbial communities that will allow to produce "clean" products without potentially hazardous microorganisms, which result in standardization and will increase safety of the method [8].

Fecal microbiota transplantation has a number of undeniable advantages (support of the mucosal immune system, mucosal barrier and homeostasis, colonization resistance) [3], but now there are not so many peer-reviewed scientific papers that reveal the true value of FMT in treatment of gastrointestinal diseases. Although the valuable data on FMT design, disease, choice of donor and recipient, FMT procedure, and following observations are publicly available, they are limited, thus, more research is needed [24]. In addition, the choice of donor and recipient in the veterinary field is likely to vary greatly depending on geographical differences in infectious and non-communicable gastrointestinal diseases, as well as other factors [8, 9].

Fecal microbiota transplantation has great prospects in livestock sector, but there are still some challenges in place. First, it is critically important to chose the administration route and the donor since these factor directly impact the procedure outcome [15, 32]. Although the FMT use for treatment of viral infections demonstrates positive results [35], it also requires additional testing on large samples and under different conditions. FMT may not always be considered as a highly effective method in real conditions. For example, as the study conducted by J. Pang et al. shows FMT is effective only in case of direct administration to recipients, and when recipients get infected via a contact with sick individuals, clinical indicators do not improve after FMT from the donor [39]. In another case, the FMT in horses did not lead to a successful outcome either [23]. This requires a detailed search for the underlying reasons.

The good potential of further FMT use in livestock sector is confirmed by the research that demonstrates how effectively the procedure increases livestock productivity [34, 37, 38]. Experiments in fish are also interesting, where FMT has proven to be a promising tool to treat intestinal diseases [41] and as an approach that promotes the rejuvenation of aging individuals [42].

Thus, although FMT, as an independent tool, has many times proven its high effectiveness in treating a number of gastrointestinal diseases and other indirectly related disorders, further research is needed to understand the exact mechanisms of transplantation and to develop standard operational procedures that should both increase FMT effects and reduce risks for recipients.

CONCLUSION

Research on FMT use for animals demonstrated its potential as an effective preventive, therapeutic and immunomodulatory intervention. The results show that FMT is capable of restoring healthy intestinal microbiota of the recipient, which is especially important under conditions of antibiotic resistance and the increasing need for alternative approaches to treat animal diseases. Although the procedure has already demonstrated positive results for some animal species, it is still required to standardize protocols and to study more precisely its effect on the body, so that in future it will be possible to classify FMT as a probiotic approach in the veterinary medicine.

The FMT success primarily depends on the folowing key factors: a pathogen-free donor shall be carefully selected; and the fecal material shall be properly prepared and an adequate administration route shall be chosen depending on the animal species. In most successful cases of FMT use, it was a procedure applied alone, however, its combination with, for example, prebiotics, also proved to be highly effective.

Positive FMT results are observed in treating both bacterial and viral animal infections. The FMT in farm animals confirms its potential for improving feed conversion rate and weight gain, which is of economic significance in livestock sector. The tests in fish have demonstrated the FTM "anti-ageing" potential.

The published data provide confirming evidence that FMT can be considered as a potential alternative to antibiotic therapy for animals, however, more extensive research is required taking into account unique features of different animal species.

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Astrovirus infection in animals (literature review)

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ABSTRACT

Viral agents are a major cause of mass gastroenteritides in newborn calves in the countries around the world. Early postnatal diarrhea as the main reason of morbidity and mortality in young animals leads to serious problems in the commercial livestock farming and causes a considerable economic damage. The most common viral gastroenteritis agents in calves are rotaviruses, coronaviruses and pestiviruses, and, along with these, astroviruses are increasingly being detected. The members of the family *Astroviridae* can cause various pathologies in animals: enteritis, hepatitis and nephritis in birds, gastroenteritis, neurological syndromes and encephalitis in mammals. The role of these viruses in the etiology of respiratory pathology in animals has been demonstrated. The following animals are the natural hosts of astrovirus: cattle, small ruminants, camels, deer, yaks, roe deer, buffaloes, alpacas, pigs, wild boars. The virus has been detected in bats, rodents and marine mammals, as well as in mollusks. Presently, the list of animals susceptible to astrovirus infection has expanded to over 80 species from 22 families, including domestic, synanthropic and wild animals, birds and mammals living in the terrestrial and aquatic environments. In recent times, there has been a lot of evidence of occurrence of recombinant astrovirus isolates, which contributes to the emergence of new genetic variants of the pathogen. A wide variety of infected animal species, the genetic diversity of the virus and the recombination events are indicative either of the cross-species transmission and subsequent adaptation of the virus to new hosts, or of the coinfection of the same host with different virus genotypes, which may lead to the emergence of novel astroviruses that are capable of infecting animals or possess a zoonotic potential. Astrovirus infection has no specific clinical features that allow for its differentiation from other intestinal infections. The presented data highlight the necessity for taking into account astrovirus infection when testing pathological material samples from diarrhea-affected newborn calves, lambs, goat kids and piglets on the commercial farms of the country.

Keywords: review, *Astroviridae, Avastrovirus, Mamastrovirus*, diarrhea, gastroenteritis, respiratory pathology, encephalitis, cattle, small ruminants

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РЕЗЮМЕ

Во всех странах мира основной причиной массовых гастроэнтеритов новорожденных телят являются возбудители вирусной природы. Диарея в ранний постнатальный период, как основная причина заболеваемости и смертности молодняка, вызывает серьезные проблемы в промышленном скотоводстве и причиняет значительный экономический ущерб. Наиболее распространенными возбудителями вирусного гастроэнтерита у телят являются ротавирусы, коронавирусы и пестивирусы, наряду с которыми все чаще выявляют астровирусы. Представители семейства *Astroviridae* способны вызывать у животных различные патологии: у птиц – энтерит, гепатит и нефрит, у млекопитающих – гастроэнтерит, неврологические синдромы и энцефалит. Доказана роль данных вирусов в этиологии респираторной патологии животных. Естественными хозяевами астровируса являются: крупный и мелкий рогатый скот, верблюды, олени, яки, косули, буйволы, альпаки, свиньи, дикие кабаны. Возбудитель был выявлен у летучих мышей, грызунов и морских млекопитающих, а также в моллюсках. В настоящее время список животных, восприимчивых к астровирусной инфекции, расширился более чем до 80 видов из 22 семейств, включая домашних, синантропных и диких животных, птиц и млекопитающих, обитающих в наземной и водной среде. В последнее время увеличивается количество свидетельств о появлении изолятов астровируса с рекомбинациями, что способствует возникновению новых генетических

вариантов возбудителя. Большое разнообразие видов инфицированных животных, генетическое разнообразие вируса и случаи рекомбинации указывают либо на межвидовую передачу и последующую адаптацию вируса к новым хозяевам, либо на коинфекцию одного и того же хозяина разными генотипами возбудителя, что может приводить к появлению новых астровирусов, способных заражать животных или имеющих зоонозный потенциал. Астровирусная инфекция не обладает клиническими особенностями, позволяющими дифференцировать ее от других кишечных инфекций. Представленные данные указывают на необходимость учета астровирусной инфекции при исследовании проб патологического материала от больных диареей новорожденных телят, ягнят, козлят и поросят в промышленных хозяйствах страны.

Ключевые слова: обзор, *Astroviridae, Avastrovirus, Mamastrovirus*, диарея, гастроэнтериты, респираторная патология, энцефалиты, крупный рогатый скот, мелкий рогатый скот

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INTRODUCTION

Gastrointestinal diseases of newborn calves and young cattle are widespread all over the world and second only to respiratory pathology in the extent of the economic damage caused [1, 2, 3, 4, 5]. Newborn calf diarrhea is the main cause of morbidity, mortality and economic losses in livestock farming [3, 6, 7, 8, 9, 10, 11, 12]. Vaccines have been developed to prevent rotavirus, coronavirus infections and bovine viral diarrhea/mucosal disease (BVD-MD) [1, 2, 3, 13, 14]. However, diarrhea is sometimes reported in newborn calves born to vaccinated cows. Diagnosis in mass viral diarrhea cases is based on the detection of rotavirus, coronavirus and pestivirus (the causative agent of viral diarrhea) or postinfection antibodies. There is no difference in the clinical signs demonstrated by diseased newborn calves with diarrheas caused by bovine viral diarrhea/mucosal disease agents, rotaviruses, coronaviruses, kobuviruses, toroviruses, parvoviruses, enteroviruses, noboviruses, noroviruses, bopiviruses [3, 8, 11, 14, 15, 16, 17, 18]. Also, no differences in the postmortem lesions were detected by the necropsy of calves that had died of rotavirus, coronavirus, parvovirus and enterovirus infections.

The negative results of laboratory tests aimed at the detection of the said pathogens prompted additional tests of pathological material samples using other diagnostic methods. Reports on the detection of astroviruses in the faecal samples from diarrhea-affected calves were published in 1977–1978 [19]. The astrovirus isolated from the faecal samples from calves with diarrhea in England was found to be antigenically related to the pathogen recovered from a diseased animal from Florida (USA). Then the targeted testing of faecal samples for astroviruses revealed the wide occurrence (46%) of the virus on livestock farms. On 88% of the tested farms, other pathogens (rotaviruses, coronaviruses, parvoviruses, noroviruses and enteroviruses) were detected along with astrovirus. In 8% of cases, only astroviruses were detected [5, 19, 20, 21]. The members of the family *Astroviridae* can cause diseases in various vertebrates, with the isolates recovered from birds and mammals being the most well studied.

MAIN PART

Until recently, human pathology was thought to be associated with 8 serotypes of astroviruses (*Human astrovirus*, HAstV). However, in the last few years, the wide use of molecular biological test methods allowed for the detection of some more groups of the pathogen (MLB and VA) differing from conventional human astroviruses in patients with acute diarrhea manifestations. These groups of astroviruses are detected quite rarely, but can cause a group disease [22, 23, 24]. Astrovirus serotypes (genotypes) 1 and 2 are most common in children, and serotype 4 – in older persons. Astrovirus infection has no specific clinical features that allow for its differentiation from other intestinal infections. The contribution of this infection to the sporadic morbidity in different regions of the world varies widely (4–17%) [25, 26].

Spherical non-enveloped virions with a size of about 28–30 nm were detected in the faecal samples from diarrhea-affected newborn calves born to cows immunized with vaccines against rotavirus, coronavirus infections and bovine viral diarrhea/mucosal disease. The surface of the virion resembled five- or six-pointed stars. Such virions were first detected using electron microscopy in 1975 during the tests of faecal samples from children with diarrhea; subsequently, similar viruses were found in faecal samples taken from diarrhea-affected animals of different species [27, 28]. The name of the detected virus comes from the Greek word "astron", meaning "star", which the virions resemble in electron micrographs (Fig. 1) [13, 26, 29].

In 1995, the International Committee on Taxonomy of Viruses assigned all astroviruses to the new family *Astroviridae* [30]. This family includes two genera: *Mamastrovirus* (from the Latin word "mamma", meaning "mammary gland") and *Avastrovirus* (from the Latin word "avis", meaning "bird") [25, 31, 32]. Viruses belonging to the genus *Mamastrovirus* cause pathology in humans and mammalian animals. The representatives of the genus *Avastrovirus* cause the disease in birds (Fig. 2). Methods such as electron microscopy, polymerase chain reaction and metagenomic analysis allowed for the detection of astroviruses

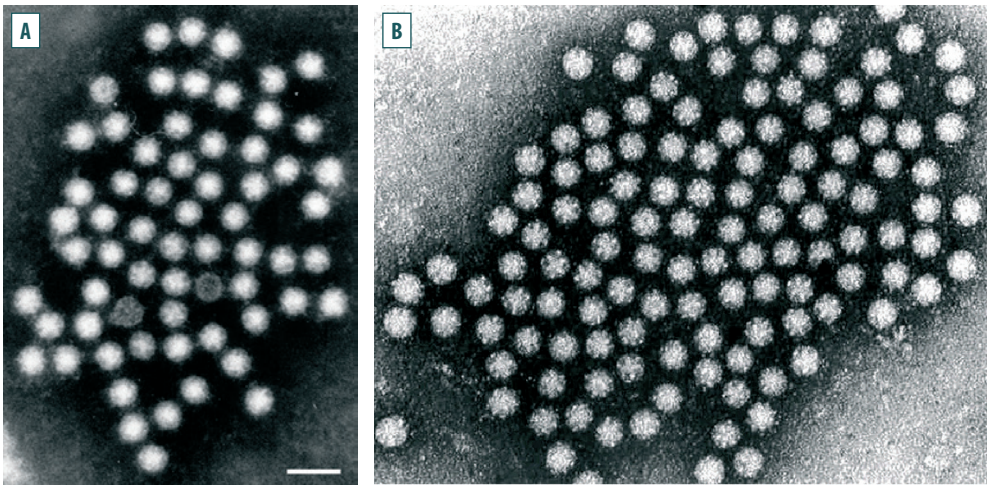

Fig. 1. An electron micrograph of an astrovirus: A – [26]; B – [29]

in pathological material samples from domestic and wild animals, including cattle (*Bovine astrovirus*, BoAstV), camels (*Dromedary camel astrovirus*, DcAstV), sheep (*Ovine astrovirus*, OAstV), goats (*Caprine astrovirus*, CapAstV), pigs (*Porcine astrovirus*, PoAstV), dogs (*Canine astrovirus*, CaAstV), cats (*Feline astrovirus*, FeAstV), minks (*Mink astrovirus*, MiAstV), mice (*Murine astrovirus*, MuAstV), rats (*Rat astrovirus*, RatAstV), dolphins (*Bottlenose dolphin astrovirus*, BdAstV), pinnipeds (*California sea lion astroviruses*, CslAstV; *Steller sea lion astroviruses*, SslAstV), chickens (*Chicken astrovirus*, CAstV), turkeys (*Turkey astrovirus*, TAstV), ducks (*Duck astrovirus*, DAstV), geese (*Goose astrovirus*, GAstV) and other animal species [25, 28, 33].

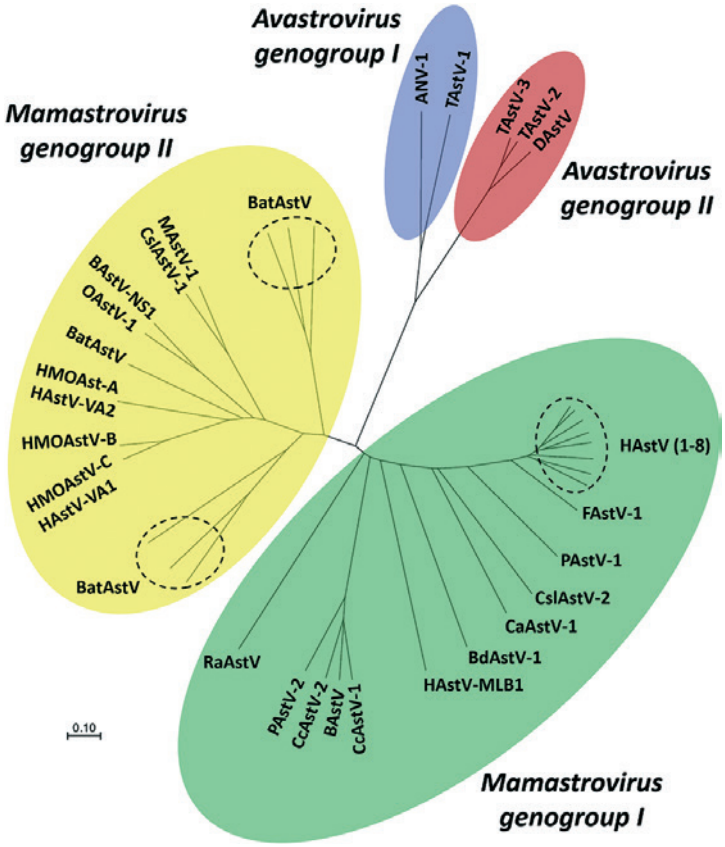

Fig. 2. Phylogenetic relationships within the family Astroviridae [28]

Astroviruses are represented by non-enveloped virions of icosahedral symmetry with a diameter of 28–30 nm. The nucleocapsid consists of three main capsid proteins. The virion capsid is covered with 30 dimeric spikes protruding above the virion surface at 3–8 nm. The molecular weight of the virion is 8 MDa. The astrovirus genome is represented by a single-stranded infectious RNA (6.4–7.9) × 10^3 nucleobases long, containing three open reading frames. The genomic sequence of bovine astroviruses was determined in 2011 [27, 34, 35, 36]. The buoyant density of astrovirus in caesium chloride is 1.36–1.39 g/cm^3. The sedimentation constant is 140S.

Astroviruses are inactivated at 50 °C for 60 minutes or at 60 °C for 5 minutes. The virus is stable at pH 3.0, as well as resistant to various detergents and fat solvents. In faecal samples from animals with astrovirus diarrhea, the pathogen was detected at a concentration of up to 10^{10} particles/mL. The natural hosts of astrovirus are cattle, small ruminants, camels, deer, yaks, roe deer, buffaloes, alpacas, pigs, wild boars. The pathogen has been detected in bats, rodents and marine mammals, as well as in mollusks. Presently, the list of animals susceptible to astrovirus infection has expanded to over 80 species from 22 families, including domestic, synanthropic and wild animals, birds and mammals living in the terrestrial and aquatic environments [25, 37, 38, 39, 40, 41, 42, 43].

Astroviruses are transmitted via the faecal-oral route. The RNA of this pathogen is infectious, and after the virion enters the target cell, it serves as an mRNA for the translation of two non-structural proteins. Astroviruses replicate in the cytoplasm of sensitive cells, they destroy intestinal cells and release during lysis [2, 13, 27, 44].

Astrovirus infection is geographically widespread throughout the world [6]. Genetic variability has been described for almost all sufficiently studied species of astroviruses infecting mammals and birds; however, antigenic variability has been demonstrated in human astrovirus, but is much less studied in animal viruses. In recent times, there has been a lot of evidence of occurrence of recombinant astrovirus isolates, which contributes to the higher genetic variation in this group of viruses. A wide variety of infected animal species, the evident genetic diversity of the viruses and the recombination events are indicative either of the cross-species transmission and subsequent adaptation of the virus to new hosts, or of the coinfection

of the same host with different astroviruses. It is believed that the coinfection may lead to the emergence of novel astroviruses that are capable of infecting animals or possess a zoonotic potential [25, 45, 46].

It was found that an astrovirus can be cultivated in a primarily trypsinized human embryo cell culture when the serum-free maintenance medium is supplemented with 10 μg/mL of trypsin to activate the replication. In the absence of trypsin, the astrovirus entry into the target cells and their infection, as well as the release of the infectious pathogen do not occur. All this indicates that astrovirus replication is trypsin-dependent [1, 47, 48, 49]. Astroviruses of cattle and small ruminants replicate in the primarily trypsinized cultures of calf embryo kidney cells, as well as in continuous cell lines (MDBK, BT, EBK, GBK). The astrovirus infection incubation period is 4.5 days [50]. The studies of the disease pathogenesis in newborn lambs showed that two-day-old animals developed diarrhea 48 hours after experimental infection [12, 51]. In the body of newborn calves and lambs, astrovirus replicates in the enterocytes of the apical surface of the small intestine villi. After entering the intestines of newborn animals, the virus infects the enterocytes of the ileum and epithelial M cells of the epithelium of the dome of Peyer's patches [34, 44, 52, 53]. Despite astrovirus detection in faeces, in some cases calves had no clinical signs of the disease.

Bovine astroviruses were detected in 60% of faecal samples taken from newborn calves with diarrhea on Brazilian farms. According to the phylogenetic analysis data, the detected isolates demonstrated a 74.3–96.5% similarity based on the amino acid sequence [47].

Chinese scientists conducted polymerase chain reaction tests of 211 rectal swab samples from cattle and water buffaloes with the signs of diarrhea living in the same ecocluster. Astrovirus RNA was detected in 46.10% of the samples from cattle and in 36.84% of the samples from buffaloes. The phylogenetic analysis results indicate that the pathogens had a common ancestor [34]. In the pastures of Tibet, diarrhea of newborn cattle and yak calves is the most common disease causing significant economic damage. The tests of the faecal samples collected from young yaks revealed the presence of the following viruses: rotavirus, parvovirus, astrovirus, nebovirus, enterovirus, influenza A virus, hepatitis E virus, kobuvirus and bovine viral diarrhea/mucosal disease virus. The astrovirus isolated from the faecal samples from yaks was 46.4–66.2% identical to the virus isolated from the faeces of diseased cattle. The test results showed that yak astroviruses belong to the cluster of bovine astroviruses. However, yak astroviruses demonstrate a more close genetic relatedness to deer astroviruses. The researchers suggested that interspecies recombination had occurred in the astrovirus ORF2. All this indicates that the pathogen isolated from the faeces of newborn yak calves with diarrhea is a novel astrovirus [9]. In South Korea, the studies of diarrhea etiology in 115 newborn calves from different farms revealed the presence of astroviruses in 7.83% of samples [36]. Zhu J. et al. detected astrovirus both in the faecal samples taken from clinically healthy calves and in the samples from calves with diarrhea [54].

Astroviruses were detected in 3.15% of faecal samples from newborn calves with diarrhea in three provinces of Central Turkey. Based on the phylogenetic analysis data, the detected new strains of astrovirus were 75.8–100.0% identical [55]. In 2012–2013, faecal samples were collected on 36 farms in Scotland to investigate the etiology of mass diarrhea cases in newborn calves. Astroviruses were detected in 80.0% of the samples from diseased calves, and rotaviruses were detected in 77.1% of the samples. Astroviruses (64.4%) and rotaviruses (17.8%) were also detected

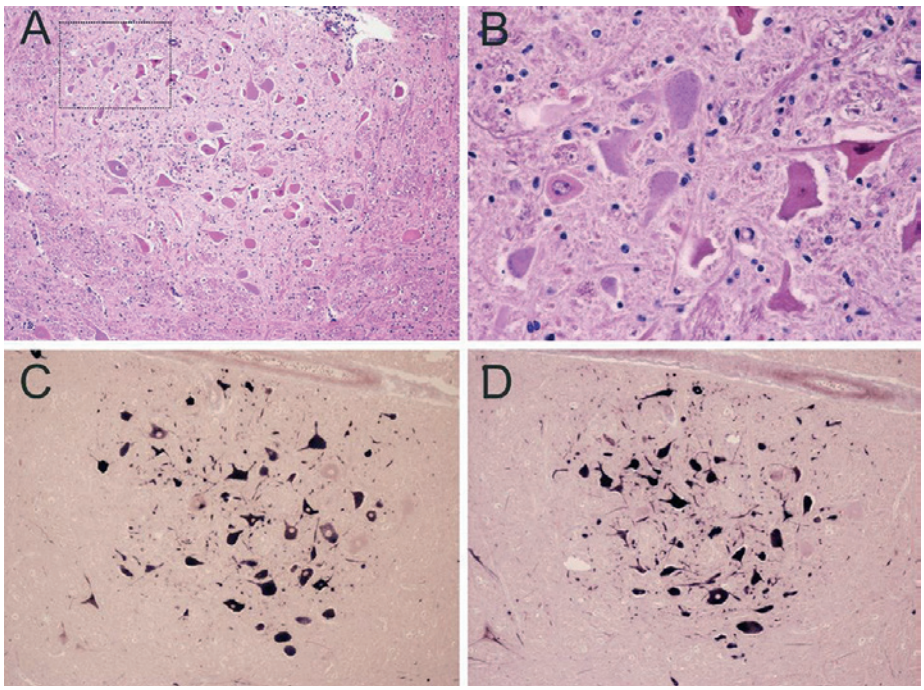

Fig. 3. Histopathological changes and detection of BoAstV RNA in the affected brain tissues of a cow with non-suppurative encephalitis: A – gliosis and neuronal necrosis; B – necrotic neurons (magnification of the marked area in panel A); C and D – dark blue labelling indicates the presence of the viral RNA [59]

in faecal samples from clinically healthy calves. Astroviruses were isolated from 15.0% of the faecal samples taken from adult cattle from the same farms. The detected astroviruses belonged to three genetic lineages [21].

Polymerase chain reaction tests of faecal samples from 25 calves with diarrhea from two farms in Egypt detected rotaviruses in 48% of the samples, noroviruses in 24% of the samples and astroviruses in 32% of the samples. Two pathogens were detected in 37% of the samples. The results of these studies indicate a high degree of similarity in the nucleotide sequences of the Egyptian and Brazilian isolates of bovine astroviruses [56]. Astroviruses were detected in faecal samples from diarrhea-affected sheep from different countries [12, 51, 53]. When studying the etiology of diarrhea in animals, Swiss researchers detected astroviruses in faecal samples from 10.7% of lambs, 14.3% of goat kids, 10.0% of alpacas and 16.7% of fawns. High genetic similarity between ovine and caprine astroviruses is indicative of the multiplicity of the pathogen transmission pathways [53].

Astroviruses detected in the samples collected from marine mammals (a sea lion, dolphins) were found to be related to the viruses detected in the samples taken from terrestrial animals. This diversity of marine mammal astroviruses and their similarity to terrestrial animal astroviruses suggest that the marine environment plays an important role in the ecology of the pathogen [40, 41].

Japanese researchers conducted a metagenomic analysis of 146 faecal samples collected from calves with diarrhea in the period from 2009 to 2015 in three prefectures of the country. Astroviruses were detected in 15 samples. Based on the phylogenetic analysis data, 9 astrovirus isolates were found to be similar to the Chinese isolates and were classified as belonging to lineage 1. Three strains were classified as belonging to the group of American strains isolated from cattle with respiratory pathology (lineage 2). One isolate was classified as belonging to a separate group along with type 5 porcine astrovirus and ovine astrovirus. The results of these studies served as the basis for the assumption of the existence of the interspecies transmission of astroviruses [35]. The investigation of the causes of diarrhea in the European population (Danish population) revealed the presence of astroviruses belonging to types 1 and 2 of the pathogen in faecal samples [21]. Astroviruses were also detected in faecal samples from European roe deer, red and white-tailed deer [42, 43, 57, 58]. The phylogenetic analysis results demonstrate a close genetic relatedness of the strains isolated from roe deer. These pathogens were also related to the astroviruses isolated from cattle, deer, water buffaloes, yaks, two-humped (Bactrian) camels, Sichuan takins, pigs and porcupines [57].

Reverse transcription polymerase chain reaction tests of 215 faecal samples taken from one-humped (dromedary) camels in the United Arab Emirates detected astrovirus (DcAstV) in 4 of them. Camel astroviruses were found to belong to a separate cluster of pathogens, which are 60–66% related to type 2 porcine astroviruses. These data served as the basis for the assumption that one-humped camels are a natural reservoir in which the astrovirus has steadily evolved. Camel astroviruses are a novel species of the genus *Mamastrovirus* of the family *Astroviridae* [33].

Several countries reported cattle cases with central nervous system disorders. Tests of the brain samples (Fig. 3) taken from the corpses of diseased animals detected an astrovirus [59, 60, 61, 62]. The role of astroviruses in the etiology of respiratory pathology in animals was demonstrated [35, 63, 64]. A number of researchers classify astroviruses as pathogens with a zoonotic potential [25, 28, 38, 52, 65]. All this indicates the importance of the timely diagnosis of astrovirus infection. Currently, all modern immunochemical and molecular biological methods are used to diagnose this infection in animals [51, 61, 66].

CONCLUSION

Astrovirus infection is considered to be one of the most common causes of mass gastroenteritides in various animal species in many countries of the world. The role of astroviruses in the etiology of respiratory pathology in cattle has been determined. The disease cases in cattle characterized by central nervous system disorders have been reported. The results of molecular biological tests of astrovirus isolates recovered from pathological material samples indicate the genetic variability of the virus. There are regular reports of recombinations detected in astroviruses. A wide variety of infected animal species and the occurrence of recombinations are indicative of the cross-species transmission and subsequent adaptation of the astrovirus to new hosts or of the coinfection of the same host with different viruses. This can also lead to the emergence of novel astroviruses that infect animals and possess a zoonotic potential. The presented data highlight the necessity for taking into account astrovirus infection when testing pathological material samples from diarrhea-affected newborn calves, lambs, goat kids and piglets on the commercial farms of the country. Astrovirus infection should also be taken into consideration when examining pathological material samples collected from animals with respiratory pathology. Data on recombination events occurring between human astroviruses and animal astroviruses suggest the possibility of formation of new groups of viruses potentially capable of causing the disease in animals. Feed and water, as well as animal care tools contaminated with the pathogen can serve as astrovirus transmission factors. All this indicates the need to take into account astrovirus infection when conducting epizootiological investigations and identifying the etiology of mass cases of gastrointestinal pathology in newborn calves, piglets, lambs, goat kids, foals, dogs and cats.

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BVDV comprehensive studies and species identification in high-yielding livestock populations in the Sverdlovsk Oblast

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ABSTRACT
The paper presents results of comprehensive studies of the bovine viral diarrhea virus circulating in cattle populations in the Sverdlovsk Oblast. In 2018–2024, 113 biological samples were tested using polymerase chain reaction, the viral RNA specific regions were detected in 15.9% of cases. The BVDV RNA was isolated from biological samples collected from aborted cows (61.1%) and calves under one month of age (38.9%). Based on typing results, the virus isolates detected in four samples (nasopharyngeal swabs of calves, suspension prepared from aborted fetus organs and placenta) were classified as BVDV-1 virulent genotypes. The BVDV RNA, *Mycoplasma bovis* and *Chlamydophila pecorum* DNAs were detected simultaneously in 44% of vaginal swab samples from aborted cows and, in single cases, in the placenta and parenchymatous organs of dead calves; BVDV RNA and *Bovine herpesvirus* type 1 DNA were detected in 16% of pathological samples from dead calves. In some cases, the BVDV RNA, *Chlamydophila pecorum* and *Mycoplasma bovigenitalium* DNA were detected in nasopharyngeal swabs of calves. The "Comprehensive Programme for Biosecurity and Bovine Viral Diarrhea Situation Improvement in Agricultural Organizations" implemented in the Sverdlovsk Oblast in 2018 resulted in decreased number of agricultural establishments affected by bovine viral diarrhea. Acute and persistent infection forms among young animals were recorded 4 and 3.5 times less frequently, respectively, but at the same time, a 2.5-fold increase in the diagnosed latent form of the disease was observed in adult livestock, which is associated with an increase in the number of laboratory tests performed.

Keywords: bovine viral diarrhea, BVDV, genotyping, viral and bacterial infections, mixed infections, health improvement measures

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Комплексные исследования и видовая идентификация вируса вирусной диареи крупного рогатого скота в популяциях высокопродуктивных животных на территории Свердловской области

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РЕЗЮМЕ
Представлены результаты комплексных исследований вируса диареи крупного рогатого скота, циркулирующего в популяциях крупного рогатого скота на территории Свердловской области. В период с 2018 по 2024 г. с помощью полимеразной цепной реакции было исследовано 113 проб биологического материала, при этом специфические участки РНК вируса были обнаружены в 15,9% случаев. Геном возбудителя вирусной диареи крупного рогатого скота был выделен из биологических проб, полученных от абортировавших коров (61,1%) и молодняка до 1 месяца (38,9%). В результате типирования обнаруженные в 4 пробах (смывы из носоглотки телят, суспензия из органов абортированных плодов и плаценты) изоляты вируса были отнесены к вирулентным генотипам BVDV-1. В 44% проб влагалищных смывов от абортировавших коров и единично в плаценте и паренхиматозных органах

от павших телят одновременно выявляли РНК BVDV, ДНК *Mycoplasma bovis* и *Chlamydophila pecorum*; в 16% проб патматериала от павших телят – РНК BVDV и ДНК *Bovine herpesvirus* 1-го типа. В единичных случаях в смывах из носоглотки телят обнаруживали РНК BVDV, ДНК *Chlamydophila pecorum* и *Mycoplasma bovigenitalium*. Внедренная в 2018 г. на территории Свердловской области «Комплексная программа биологической защиты и оздоровления сельскохозяйственных организаций от вирусной диареи крупного рогатого скота» привела к снижению количества сельскохозяйственных предприятий, неблагополучных по вирусной диарее крупного рогатого скота. Острая и персистентная форма инфекций среди молодняка регистрировалась в 4 и 3,5 раза реже соответственно, но при этом отмечали увеличение в 2,5 раза диагностируемой латентной формы течения болезни у взрослого поголовья, что связано с увеличением количества проводимых лабораторных исследований.

Ключевые слова: вирусная диарея крупного рогатого скота, BVDV, генотипирование, вирусно-бактериальные инфекции, смешанные инфекции, оздоровительные мероприятия

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INTRODUCTION

Bovine viral diarrhea (BVD) is classified as Class B infectious disease according to the World Organisation for Animal Health (WOAH). Official programmes aimed at BVD epizootological control and cattle population health improvement are implemented in the USA, UK and most European countries [1].

Establishing high-yielding dairy cattle herds is considered one of top priorities in the livestock industry of our country within achieving key objectives of the Strategy for Scientific and Technological Development of the Russian Federation (point 21g) [2]. However, viral infectious diseases negatively impact livestock industry development in the Russian Federation, same as in foreign countries [3, 4, 5].

Bovine viral diarrhea-induced economic losses for commercial and breeding farms include abortions, birth of weak and non-viable progeny, emergency slaughter of calves, reduced milk yields and shorter life expectancy of livestock [6]. The mortality level in BDV-infected farms is high (up to 10%). Asymptomatic occurrence of BVD in herds aggravates the situation at agricultural establishments, leading to total herd infection, decreased productivity and reproduction, and increased costs on treatment measures [6, 7].

According to both Russian and foreign researchers, it is possible to reduce economic losses by regular activities on livestock health improvement and viral infection prevention [8, 9, 10]. The process of improving the BVD situation in livestock establishments takes a very long time and is not always successful due to the pathogenetic properties of the agent [8].

Bovine viral diarrhea virus (BVDV) is classified into the following species: BVDV-1, BVDV-2, HoBi-like (atypical) ruminant pestivirus, which are subdivided into subgenotypes based on phylogenetic analysis [11, 12]. BVDV can be divided into cytopathic (CP) and non-cytopathic (NCP) biotypes [13, 14]. The cytopathic biotype of BVDV can induce different cytopathological effects (CPE) in the host cell systems: cytoplasmic vacuolization and cell death, that are not observed for NCP biotypes of the virus [15]. Since the fetal immune system is immature, NCP BVDVs (BVDV-1, BVDV-2) inhibit the induction of type I interferon. Thus, NCP BVDV-1 or BVDV-2 may cause abortion or stillbirth in cows at early pregnancy stage [13, 14, 15, 16].

Pestivirus type 1 (BVDV-1) is considered predominant among widespread cattle pathogens worldwide, while type 2 (BVDV-2) is more frequently reported in the USA and Canada, less frequently in Japan, India, South America and occasionally in European countries [17]. The emergence of new strains as well as BVDV strains with higher virulence, such as those causing haemorrhagic disease in animals (North America, genotype 2), emphasizes the need for diagnostic tests associated with virus genotyping [18]. The dose and virulence of the pathogen strains, the animal age and immunocompetence are the factors determining the pathogenicity level [19].

Faeces and excreta of animals containing a large number of viral particles are one of the important sources of infection [13]. The intestinal form of BVD is manifested by high fever, anorexia, diarrhea, severe dehydration, and blood in the faeces. The disease is usually sporadic, the incubation period lasts 1–2 weeks, and the mortality rate is extremely high [14]. The typical clinical signs in cows with mucosal disease are haemorrhagic, necrotic and ulcerative lesions. In addition, BVD can be manifested by loss of intestinal crypts, erosions, ulcers and large-scale mucosal necrosis, partially or throughout the gastrointestinal tract [16]. Animals that are immunotolerant to BVDV, once infected, become life-time carriers of the virus constantly shedding it, and a source of infection in the animal population, complicating the eradication of the disease [20, 21].

In addition, BVDV-1 is detected in semen, posing a great threat of vertical transmission and indicating the need for continuous testing of servicing bulls for virus carriage [22, 23, 24].

Since the BVD clinical manifestations are similar to those of other diseases such as mycoplasmosis, chlamydia, paratuberculosis, parainfluenza-3, infectious bovine rhinotracheitis, laboratory tests play a crucial role for diagnosis [1].

Recently, a large number of respiratory and intestinal disease cases detected in cattle reared in the Sverdlovsk Oblast, have occurred in the form of mixed infections, when the pathogenic agents are at the same time viruses, bacteria and fungi [1]. Due to that, the disease is characterized by a pronounced severe course and a clinical picture not typical of monoinfections, and most often bacterial infection predominates, which complicates the diagnostic process. Herpesvirus type 1 (BHV-1), BVDV, as well as chlamydia (*Chlamydophila abortus*, *Chlamydophila pecorum*), pathogenic mycoplasma species (*Mycoplasma bovis*, *Mycoplasma bovigenitalium*, *Mycoplasma* spp.), various bacteria and their associations are recognized as prevailing pathogens in the development of the above-mentioned pathological forms in cattle [5, 25, 26].

The aim of the work was to conduct a comprehensive study of circulating bovine viral diarrhea virus and associated pathogens, and perform BVDV species identification in cattle populations in the Sverdlovsk Oblast.

MATERIALS AND METHODS

The study was carried out in the Department of Monitoring and Forecasting of Infectious Animal Diseases and in the Laboratory for Microbiological and Molecular Genetic Test Methods of the Ural Veterinary Research Institute being the structural subdivision of the Ural Federal Agrarian Research Centre, Ural Branch of the Russian Academy of Sciences within the framework of Federal Assignment No. 0532-2021-0007 "Study of the structure of the antigen pattern of livestock emergent infectious pathogens, and biological features of the mechanisms of their interaction with the macroorganism".

Comprehensive laboratory tests were conducted in 21 livestock breeding establishments in the Sverdlovsk Oblast, where BVD health improvement programmes were implemented in 2018–2024.

Table 1
Primer nucleotide sequences

Target	Sequence 5′-3′	Product length
BVDV_F	CTCGAGATGCCATGTGGAC	172 bp
BVDV_PESTER5	CTCCATGTGCCATGTACAGCA	
BVDV_I	FAM-CAGCCTGATAGGGTGCTGCAGAGGC-BHQ1	
BVDV_II	ROX-CACAGCCTGATAGGGTGTAGCAGAGACCTG-BHQ2	

Table 2
Amplification programme

Temperature	Time	Number of cycles
95 °C	5 min	1
94 °C	20 sec	40 (FAM/ROX)
56 °C	20 sec	
72 °C	20 sec	

The following biological material samples collected from Holstein cattle were used for testing: blood and blood sera; cervical and vaginal swabs of cows; nasopharyngeal swabs of calves; semen of servicing bulls; placenta samples; pieces of internal organs from dead calves (liver, kidneys, lungs) and aborted fetuses (n = 113).

Real-time polymerase chain reaction (PCR) was performed according to the manufacturer's instructions for the use of test-kits. Test-kits for detection of BVDV RNA (Izogen Laboratory LLC, Russia), *Mycoplasma* spp., *Mycoplasma bovis* and *Mycoplasma bovigenitalium* DNAs (Russia), BHV1 DNA (GenPak PCR Test BHV1), *Chlamydophila abortus* and *Chlamydophila pecorum* DNAs (VectorBest LLC, Russia) were used. Amplification was performed using QuantStudio 5 unit (Thermo Fisher Scientific Inc., USA).

Additionally, 16 biological samples tested positive for BVDV RNA were placed for ultra-low temperature storage (–70 °C) for further virus typing with PCR. Sample types: pooled samples of nasopharyngeal swabs of 20-day-old calves, blood sera, aborted fetuses, placenta.

For BVDV genotyping, the following was performed: RNA isolation from samples using the AmpliPrime® RIBO-Prep VET kit (NextBio LLC, Russia), reverse transcription PCR (RT-PCR), real-time amplification and electrophoresis. Synthetic oligonucleotides previously developed by C. Letellier and P. Kerkhofs were used in this study [18] (Table 1).

These primer pairs are specific for highly conserved regions of the 5′ UTR. The sequences of probes labelled with FAM and ROX dyes with a three nucleotide-difference allowed differentiation between BVDV genotypes I and II.

The cDNA was generated using a commercial reagent kit for reverse transcription with MMLV-RH (Diaem LLC, Russia) according to the provided amplification programme in compliance with the instructions for use.

After RT-PCR and cDNA generation, real-time PCR was performed using BioMaster HS-Taq PCR (2×) master mix reagents (LLC Diaem, Russia); 100 mM Tris-HCl, pH 8.5 (at 25 °C), 100 mM KCl, 0.4 mM each deoxynucleoside triphosphate, 4 mM $MgCl_2$, 0.06 active units/μL Taq DNA polymerase, 0.2% Tween 20, HS-Taq DNA polymerase stabilisers. For optimisation, different concentrations of the primers were selected. Amplification was performed using CFX 96 Touch system (Bio-Rad Laboratories, Inc., USA), parameters are shown in Table 2.

The RNAs isolated from the veterinary medicinal product *Bovi-shield* Gold FP5 L5 (Zoetis Inc., USA) that contained attenuated viruses of infectious bovine rhinotracheitis (*Bovine herpesvirus*, type 1), diarrhea (BVDV, types 1 and 2), parainfluenza-3 (PIV-3), respiratory syncytial infection (BRSV) were used as controls.

The PCR product was separated by gel electrophoresis using agarose gel and Mini-Sub Cell GT with visualisation in the ChemiDoc XRS+ camera and interpretation of results using Gel Doc XR+ (Bio-Rad Laboratories, Inc., USA). A 100 bp size standard (SibEnzyme, Russia) was used.

The obtained data were processed using Microsoft Excel within the Microsoft OfficePro 19 software package.

RESULTS AND DISCUSSION

The implementation of the "Comprehensive Programme for Biosecurity and Bovine Viral Diarrhea Situation Improvement in Agricultural Organizations" began in the Sverdlovsk Oblast in 2018. The results of studies on the BVD transmission dynamics in the establishments

of the Sverdlovsk Oblast previously published by O. V. Sokolova [15] have been supplemented with new data and are presented in Figure 1.

During the health improvement programme implementation a trend for decrease in the number of BVD-infected cattle farms was observed. In young animals, acute and persistent forms of infection were registered 4 and 3.5 times less frequently, respectively. It should be noted that there was a 2.5-fold increase in the diagnosed latent form of the disease in adult stock, which is associated with an increase in the level of laboratory diagnostic testing at agricultural establishments. At the same time, there were incompliances with the vaccination requirements detected. Thus, the analysis of the step-by-step implementation of the health improvement programme in farms showed that 47.7% of deficiencies occurred due to the human factor (Fig. 2).

As for the establishments where incompliances with vaccination schedules and inconsistent vaccination of physiological groups were detected, the latent BVD in adult cattle was registered in 67% cases of all tested samples.

Testing of 113 biological samples from cattle with diseases of the reproductive system, respiratory and gastrointestinal tract of unclear etiology received from 21 agricultural organizations in 2018–2024 showed that specific BVDV RNA sites were detected in 15.9% of cases: mixed infection – 10.6%, BVDV – 5.3% (Fig. 3).

Bovine viral diarrhea virus RNA was detected in 61.1% of biological samples from cows aborted at different stages of gestation, as well as in 38.9% of samples from calves.

As for samples of adult cows aborted at different stages of gestation, BVDV genetic material was more frequently detected in the homogenate of organs of aborted

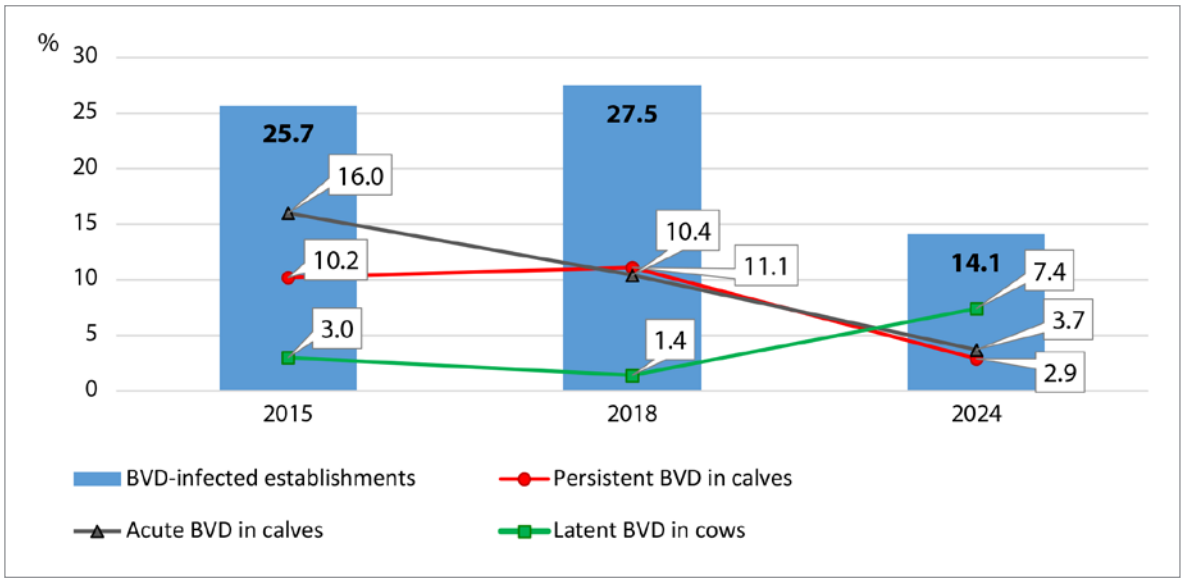

Fig. 1. BVD transmission dynamics in establishments in the Sverdlovsk Oblast (2015–2024)

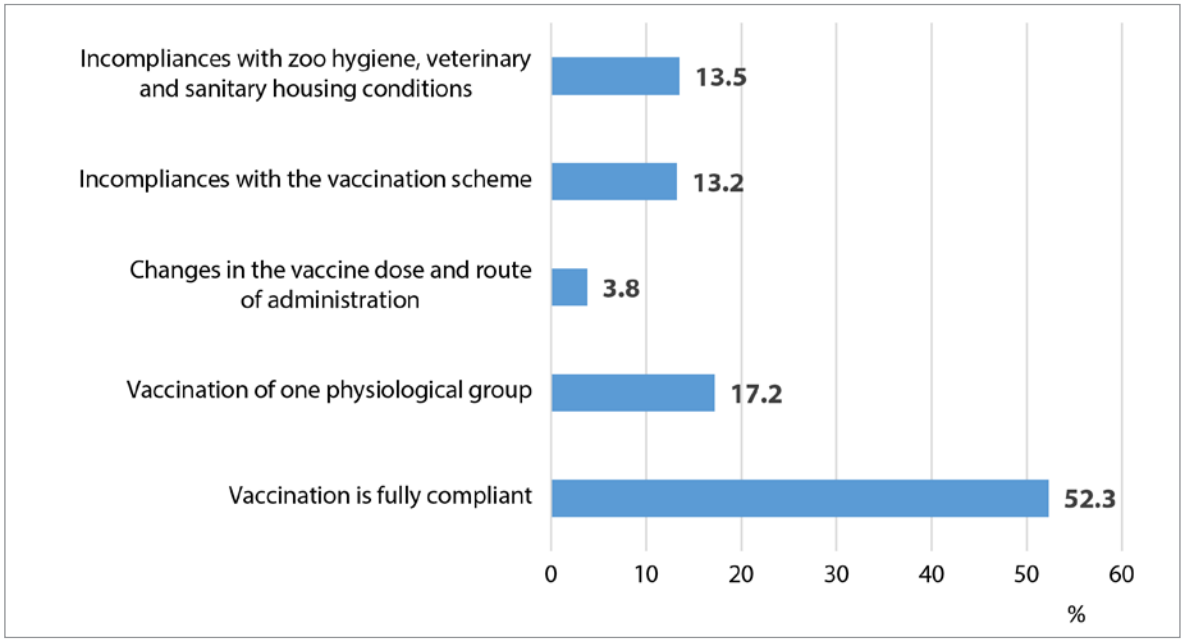

Fig. 2. Assessment of Animal Health Improvement Programme implementation

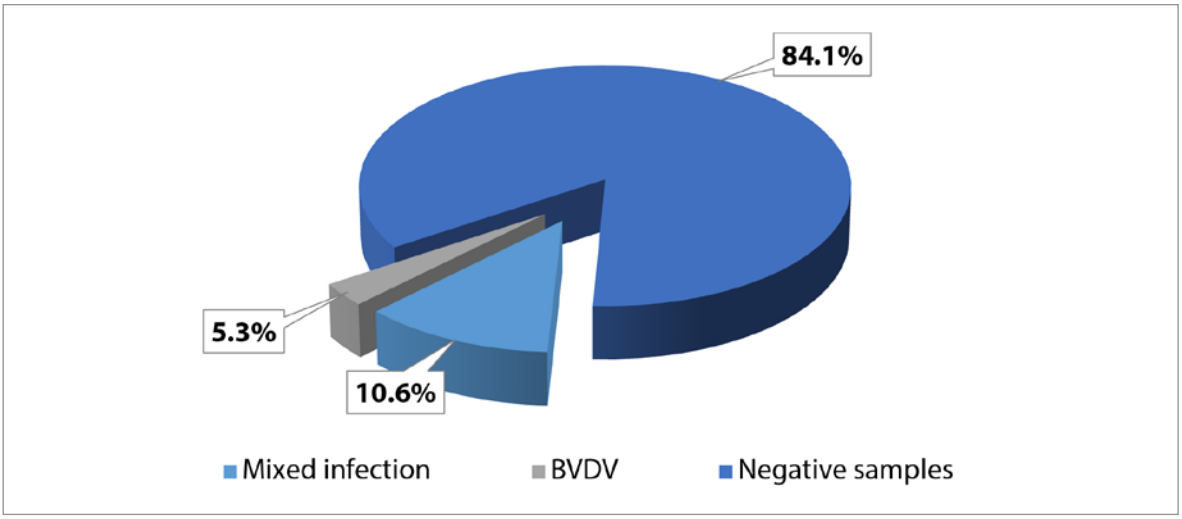

Fig. 3. PCR results for biological samples tested for presence of BVDV RNA and agents of mixed co-infections (2018–2024), n = 113

fetuses (38.8%), in vaginal swabs and in the placenta (11.2%). Also, the BVDV genome was detected in 27.7% cases in nasal swabs of calves with signs of acute respiratory and gastrointestinal diseases of unclear etiology, and in 11.1% of cases – in parenchymatous organs (suspensions of liver, heart, spleen, kidney and small intestine) of dead calves.

Additional BVDV typing tests using a primer system revealed the presence of genotype I virus in 4 out of 16 biological samples (nasopharyngeal swabs from 20-day-old calves, suspension of organs of aborted fetuses and placenta). Genotype II virus was not detected in the tested samples. It can be assumed that the BVDV-1 isolate (nasopharyngeal swabs of calves, aborted fetuses and placentas) is highly virulent, since the mortality of young animals was up to 20% and the number/frequency of abortions in dairy cattle was up to 5%.

The results of BVDV typing with RT-PCR are presented in Figure 4.

In 10.6% of samples, other infectious agents besides BVDV were detected. BVDV RNA, *Mycoplasma bovis* and *Chlamydophila pecorum* DNA were simultaneously detected in 44.0% of vaginal swabs from aborted cows and in single cases in placenta and parenchymatous organs from dead calves. Several pathogens were also detected in 16.0% of the samples from dead calves: BVDV + *Bovine herpesvirus* type 1. In single cases, BVDV RNA, *Chlamydophila pecorum* and *Mycoplasma bovigenitalium* DNA were detected in nasopharyngeal swabs of calves.

Thus, all measures to be implemented, including monitoring tests, diagnostic testing in "sentinel" animal groups, establishment of "closed-type herds", animal vaccination and biosecurity, detection of latent infection carriers and timely treatment measures, are considered

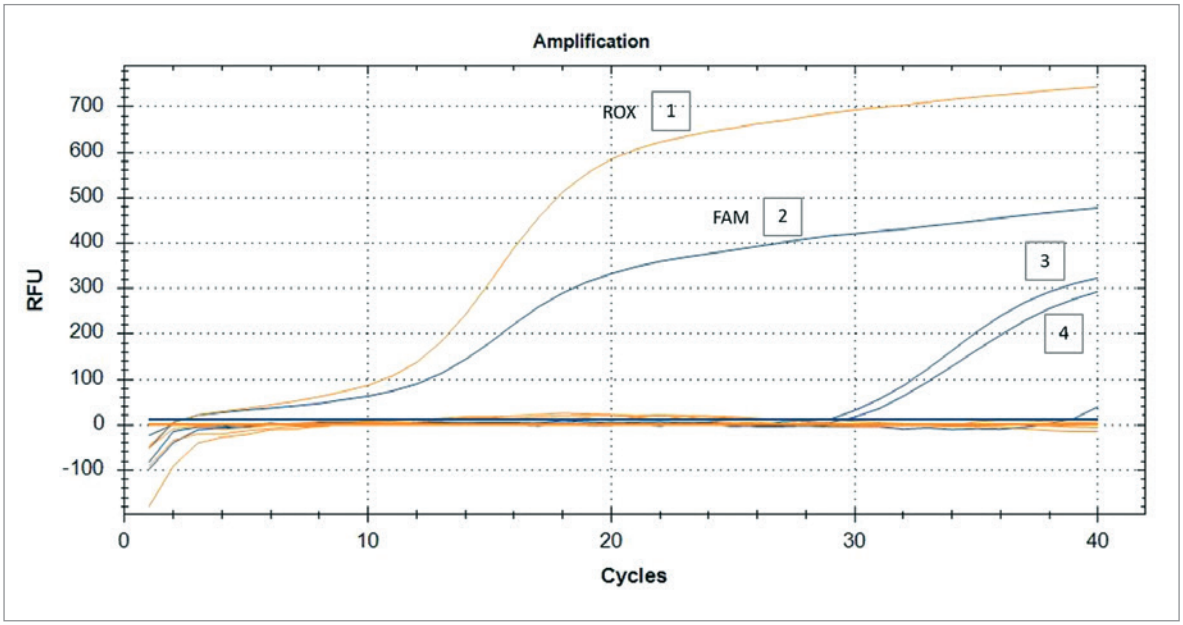

Fig. 4. Typing of BVDV detected in pooled samples of nasopharyngeal swabs from calves using RT-PCR, n = 6. Fluorescence curves: 1, 2 – control plus (FAM channel – BVDV-1; ROX channel – BVDV-2); 3, 4 – positive samples (FAM channel – BVDV-1)

effective, and further monitoring of their implementation quality and removal of deficiencies will ensure BVD health improvement in the conditions of commercial establishments.

CONCLUSION

Polymerase chain reaction tests of 113 biological samples collected from cows and calves in 2018–2024 demonstrated presence of BVDV RNA in 15.9% of cases. The BVDV genome was detected in samples from cows aborted at different gestation stages in 61.1% of cases and from calves in 38.9% of cases. The virus typing showed its belonging to type 1 (in 4 samples: nasopharyngeal swabs from calves, suspension from organs of aborted fetuses and placenta). In addition to BVDV, other infectious agents were detected in 10.6% of samples, indicating the presence of mixed infection. Combinations of BVDV with such pathogens as *Mycoplasma bovis*, *Mycoplasma bovigenitalium*, *Chlamydophila pecorum*, *Bovine herpesvirus* (type 1) were detected.

Monitoring diagnostic tests indicate positive dynamics in reduction of BVD cases in the establishments of the Sverdlovsk Oblast, as well as demonstrate successful implementation of the "Comprehensive Programme for Biosecurity and Bovine Viral Diarrhea Situation Improvement in Agricultural Organizations". Currently, there is a 2-fold decrease in the number of BVD-infected farms, while the share of detected latent infection cases in adult stock increased by 2.5 times, which is due to the extension of the diagnostic test panel, regular monitoring of herds, as well as incompliances with vaccination procedure during this programme implementation.

Further monitoring of the quality of the programme implementation, including clinical and laboratory tests aimed at detecting BVDV circulation and eliminating incompliances with vaccination regulations, will ensure health improvement of herds at commercial establishments.

Work is to be continued on BVDV typing, which will include quantification of the virus genomes and sequencing of BVDV circulating in animal herds in the Sverdlovsk Oblast for subsequent genetic analysis (subtyping, tests for virulence of isolates).

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Вклад авторов: Безбородова Н. А. – формирование идеи, развитие ключевых целей и задач, анализ и интерпретация полученных данных, принятие ответственности за все аспекты работы, целостность всех частей статьи и за ее окончательный вариант; Кожуховская В. В. – проведение исследований, анализ и интерпретация полученных данных; Печура Е. В. – разработка или дизайн методологии, создание моделей; Мартынов Н. А. – проведение экспериментов, сбор данных, анализ и интерпретация полученных данных, участие в научном дизайне; Томских О. Г. – предоставление материалов, лабораторных образцов, проведение исследований; Васильева А. Н. – сбор данных, анализ и интерпретация полученных данных.

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Study of possible intrauterine infection of goat fetus with caprine arthritis-encephalitis virus

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ABSTRACT

Caprine arthritis-encephalitis is a serious challenge for the modern goat breeding both in Russia and abroad. The disease is quite widespread in our country, causing serious economic damage to the backyard and family-operated farm owners. The etiologic agent of caprine arthritis-encephalitis (CAE) is a virus of the *Retroviridae* family, which is part of the group of small ruminant lentiviruses subdivided into five genetic groups. A number of reasons prevent timely disease diagnosis, for example, lack of a legal framework regulating preventive measures, prolonged asymptomatic virus-carrier state, and absence of pathognomonic symptoms. There are two routes of the CAE virus spread: vertical (lactogenic), when colostrum or milk from a seropositive goat serves as a transmission factor; and horizontal – from a diseased animal to a healthy one in case of crowded housing, feeding from common drinkers and feeders, as well as during mating. The published data on the possibility of the intrauterine virus transmission from mother to fetus are diametrically different. The purpose of this study was to explore the possibility of intrauterine infection with the CAE virus. Pregnant goats with ELISA- and PCR-confirmed CAE diagnosis were used in the study. Pathological material was collected from newborn goats obtained by sterile kidding, and the samples were PCR tested for the CAE causative agent. None of the tested samples demonstrated CAE that gives evidence of absence of the fact of intrauterine virus transmission from the diseased mother to the fetus. Results of long-term monitoring of the goat population kept on two backyards located in the Southern Federal District and Novosibirsk Oblast comprise an indirect evidence of that. The owners of these backyards, where 100% of the goat population were infected with CAE virus, decided to carry out a complex of the disease control measures. Sterile kidding followed by kids' feeding with colostrum and milk pasteurized at 60 °C for 30 minutes with the subsequent use of a whole milk substitute allowed to obtain a CAE-free herd in two years avoiding any significant economic losses and purchase of healthy animals from other farms.

Keywords: caprine arthritis-encephalitis (CAE), intrauterine infection, sterile kidding, lentiviruses, prevention

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Изучение возможности внутриутробного заражения козлят вирусом артрита-энцефалита коз

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РЕЗЮМЕ

Вирусный артрит-энцефалит коз является серьезной проблемой современного козоводства как в России, так и за рубежом. Заболевание достаточно широко распространено на территории нашей страны, нанося серьезный экономический ущерб владельцам личных подсобных и крестьянских фермерских хозяйств. Этиологическим фактором артрита-энцефалита коз является вирус семейства *Retroviridae*, входящий в группу лентивирусов мелких жвачных животных, разделенных на пять генетических групп. Своевременной диагностике данного заболевания мешает ряд причин, например, отсутствие нормативно-правовой базы, регулирующей проведение профилактических мероприятий, длительное бессимптомное вирусоносительство возбудителя инфекции, отсутствие патогномоничной симптоматики. Выделяют два пути распространения вируса артрита-энцефалита коз: вертикальный (лактогенный), при котором фактором передачи служит молозиво или молоко от серопозитивной козы, и горизонтальный – от больного животного здоровому при скученном содержании, кормлении из общих поилок и кормушек, а также при половом контакте. В отношении возможности внутриутробной передачи вируса от матери плоду информация, приведенная в литературных источниках, диаметрально отличается. Целью данного исследования было изучить возможность внутриутробного заражения вирусом артрита-энцефалита коз. В опыте использовали сукозных коз с подтвержденным методами иммуноферментного анализа и полимеразной цепной реакции диагнозом «вирусный артрит-энцефалит коз». От новорожденных козлят, полученных методом стерильных родов, брали патологический материал и с помощью полимеразной цепной реакции исследовали на наличие возбудителя артрита-энцефалита коз. Ни в одном из исследованных образцов вирус артрита-энцефалита коз не обнаружен,

что доказывает отсутствие факта внутриутробной передачи вируса от больной матери плоду. Косвенным доказательством этого служат результаты многолетнего наблюдения за поголовьем коз, содержащихся в двух личных подсобных хозяйствах, расположенных в Южном федеральном округе и Новосибирской области. Владельцы данных хозяйств, где охват поголовья вирусным артритом-энцефалитом составлял 100%, приняли решение провести комплекс оздоровительных мероприятий. Применение технологии стерильных окотов с последующим выкармливанием козлят пастеризованным при температуре 60 °С в течение 30 мин молозивом и молоком с дальнейшим использованием заменителя цельного молока позволило в течение двух лет получить свободное от артрита-энцефалита коз поголовье без существенных экономических потерь и покупки здоровых животных в других хозяйствах.

Ключевые слова: артрит-энцефалит коз, внутриутробное заражение, стерильные окоты, лентивирусы, профилактика

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INTRODUCTION

Caprine arthritis-encephalitis (CAE) is one of the burning issues of the modern goat breeding in Russia and abroad. According to the published data, the carriers of the disease causative agent are more than 45–60% of the entire population of the goats kept on the backyards and family-operated farms located in all federal districts of the Russian Federation [1].

The CAE etiological factor is a virus of the *Retroviridae* family, which is part of the group of small ruminant lentiviruses (SRLVs), divided into five genetic groups [2]. The disease belongs to the group of lentiviral infections and it is characterized by prolonged asymptomatic virus carrying with the subsequent development of a complex of symptoms involving lesions of the musculoskeletal system (arthritis), respiratory system and mammary gland tissue. Young animals of 2–3 months of age demonstrate disorders of the central nervous system, manifested by loss of orientation, tilting of the head and incoordination [3, 4, 5, 6].

There are vertical and horizontal routes of the infectious agent transmission. The animals are infected lactogenically when newborn kids are fed with the colostrum or milk from CAE-virus carrier goats [7, 8, 9], as well as by airborne route in case of crowded housing, and less often sexually [10, 11, 12].

The published opinions regarding the intrauterine route of the virus transmission differ. Volkova I. Yu. [13] indicates that this route of infection is possible. However, in a number of publications the authors say that presence of syndesmochorial type of placenta in goats prevents the virus transmission from mother to fetus [3, 8, 14, 15]. Nevertheless, some foreign publications provide data on the virus detection during the examination of newborn kids using polymerase chain reaction (PCR) [16, 17].

Despite this, the World Organisation for Animal Health's (WOAH) Terrestrial Animal Health Code does not provide information on a possible intrauterine route of CAE virus transmission [18].

Lack of specific therapy and disease prevention tools hinders the implementation of highly effective mass measures to prevent the spread of this pathology in the goat population. The main method of the disease prevention and control on the farms involves continuous monitoring of the entire population, and in case of detection of seropositive animals – taking a set of measures to replace the diseased herd with the healthy one. One of such measures involves introduction of sterile kidding, i.e. a set of measures to prevent direct contact of a seropositive goat with a newborn kid, followed by feeding it with disinfected colostrum and milk.

In view of the diametrically opposite information in the published literature, as well as taking into account the fact that currently CAE is included in the "List of contagious animal diseases, including highly dangerous ones, for which quarantine can be imposed"[1], the purpose of the study was formulated as follows: to study the possibility of intrauterine infection of newborn kids with CAE virus.

MATERIALS AND METHODS

The study was carried out in 2023–2024 in the Laboratory of Diseases of Young Animals of the Experimental Veterinary Medicine Institute for Siberia and Far East of the Siberian Federal Scientific Centre of Agro-BioTechnologies of the Russian Academy of Sciences and on a family-operated farm located in one of the regions of the Ural Federal District. Eighteen pregnant CAE-seropositive Saanen goats were used in the study. The animals were diagnosed by testing blood samples for the proviral DNA using PCR, as well as by double testing of the sera by the enzyme-linked immunosorbent assay (ELISA) for the antibodies to CAE virus a week before insemination and on day 60 of pregnancy.

The blood samples from female goats were collected in Bodywin vacuum tubes (China) with coagulation activator and ethylenediaminetetraacetic acid.

Presence of CAE virus antibodies in the sera was determined using the ID Screen® MVV/CAEV Indirect Screening test kits for indirect ELISA (IDVet, France). The results were

[1] The list of contagious animal diseases, including highly dangerous ones, for which quarantine can be imposed: approved by Order of the Ministry of Agriculture of the Russian Federation No. 476 of 19 December 2011 (as amended on 25 September 2020). https://docs.cntd.ru/document/902324591

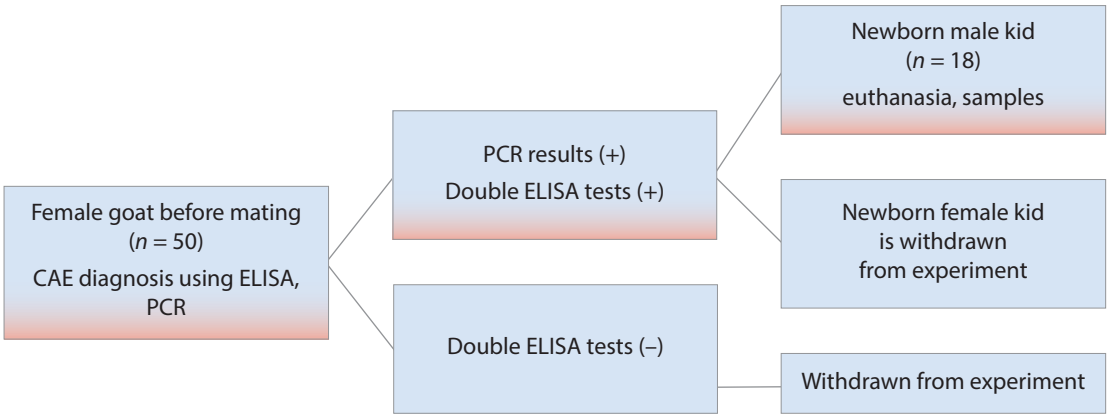

Fig. Scheme of selecting newborn animals for the experiment

recorded on a semi-automatic microplate ELISA reader TECAN Infinite F50 (Austria).

Euthanasia of newborn animals was carried out in accordance with the requirements of the European Convention for the Protection of Pet Animals (Chapter 2, Article 11)[2]. Stunning method was used for euthanasia [19].

Autopsy of the newborn kids was carried out using the generally accepted method of G. V. Shor [20]. Blood and internal organ samples were aseptically collected in sterile test tubes using sterile disposable sample collecting probes (VetGenomics, Russia), as well as biological sample collection and storage cards "DNA Archive" (Russia).

For primary isolation of nucleic acids from the biological material, "RealBest extraction 100" kit (Vector-Best, Russia) was used.

Caprine arthritis-encephalitis virus was detected in the biological samples using recording amplifier manufactured by Bio-Rad Laboratories, Inc. (USA) and a "Kit of reagents for detection of proviral DNA of caprine arthritis encephalitis virus (CAE) using real-time polymerase chain reaction" (Vector Best JSC, Russia). Fifty amplification cycles were performed. Samples with $Ct < 40$ were considered positive.

RESULTS AND DISCUSSION

Before the study, all the goats ($n = 50$) housed on one of the family-operated farms were tested for CAE virus antibodies using ELISA before the insemination. Polymerase chain reaction for proviral DNA was used as a confirmation test. Goats with a seropositivity coefficient of Cs ≥ 100 (according to ELISA results) and positive PCR test results ($n = 29$) were selected for further study. Then, on day 60 of the pregnancy, a second ELISA was performed to confirm the CAE diagnosis in the goats.

During the kidding, the newborn kids were aseptically taken and removed, completely excluding their postpartum contact with the mother. Only males were used in the experiment, since they do not have any commercial value. A total of 18 goats were selected for the study (Fig.).

After euthanasia, an autopsy and biological material collection were aseptically performed. The following samples were collected: blood samples, heart tissue, liver, lungs. The biological materials were stored and transported at 4 °C.

[2] European Convention for the Protection of Pet Animals. Strasbourg, 13.XI.1987. https://rm.coe.int/168007a67d

Further work with the biological samples was carried out in the PCR laboratory of the Experimental Veterinary Medicine Institute for Siberia and Far East of the Siberian Federal Scientific Centre of Agro-BioTechnologies of the Russian Academy of Sciences

The test results are demonstrated in Table 1.

The CAE virus (CAEV) was not detected in any of the biological samples tested using real-time PCR. This fact confirmed that intrauterine infection of newborn kids by the virus-carrying mothers is impossible.

The data obtained during the experiment differ from the information cited in a number of foreign publications. Thus, J. Furtado Araújo et al. [17] indicate that when testing 73 newborn kids by PCR, the CAEV was detected in 46.57% of the samples. One of the explanations for the discrepancy between our results and the cited published data can be a comment from the article by O. L. Kolbasova et al. [2] that the quality of PCR directly depends on the primers, which should match the genetic variant of the virus circulating in the animals housed on the farm.

During the experiment, all goats were tested using a combination of ELISA and PCR diagnostic tests, and the animals that demonstrated double positive results were selected for the experiment. Therefore, the complex of primers included in the PCR system used in the experiment matched the genetic variant of the virus circulating on the farm.

The data confirm the relevance of using the sterile kidding (complete exclusion of any contact of the mother and newborn kid immediately after parturition) as one of the main approaches to the prevention of CAE spread inside the farm and to the formation of the disease-free herd.

The following examples confirm the effectiveness of this method and, as a result, the absence of an intrauterine route of CAEV transmission.

Table 1
Results of biological material tests for CAE virus

Type of biological material	Amount	CAEV test results	
		positive, %	negative, %
Blood samples	18	–	100
Internal organ samples	54	–	100

Example 1. The experiment was conducted on the backyard located in the Southern Federal District. The number of goats at the beginning of the experiment was 18 (Table 2).

The presented data demonstrate that in 2022, during the initial examination of the population consisting of 18 sexually mature animals, the antibodies to the CAE virus were detected in the sera of 11 goats. The backyard owners decided to improve the health of the herd, therefore, they put into practice mandatory sterile kidding for all pregnant goats and feeding newborn kids with colostrum pasteurized at 60 °C for 30 minutes, with further use of the whole milk substitute. Four goats with obvious CAE clinical signs were isolated from the herd, the rest were mated by the available seropositive male goats.

In 2023, when examining the repair population formed of the kids born by sterile kidding (13 kids), all animals demonstrated negative results. The only seropositive animal turned out to be a female goat, which the owners wanted to use for mating again, but as soon as they received data that all the goats were seronegative, it was decided to send it to slaughter.

In 2024, upon repeated double ELISA testing of the newly formed goat population, all animals demonstrated negative results for CAE virus antibodies.

Example 2. The experiment was carried out on the backyard located in the Novosibirsk Oblast. At the beginning of the monitoring, the herd consisted of 24 Nubian dairy goats (Table 3).

During the initial ELISA testing of the animals in 2020, it was found that the entire goat herd was infected with the CAE virus.

The owners of the farm decided to carry out a set of measures to improve the health of the herd. To do this, all subsequent kiddings were carried out using sterile kidding technology, and the resulting kids were fed with the colostrum pasteurized at 60 °C for 30 min and with milk derived from mother goats.

Testing of the young animals obtained using this technology in 2021 demonstrated that 12 out of 20 animals were the CAE virus carriers. When interviewing the owners, it was found that after the end of the period of feeding with milk, the animals were placed in the common herd at the age of 3 months, which resulted in their infection with the CAE virus from the diseased mother goats.

Given this fact, the backyard owners housed all kids derived from seropositive goats in 2022 in a separate room, completely excluding any contact with infected animals and items of their care. As a result, during the ELISA testing of the newly formed herd (6–7 months of age), all animals (23 goats) demonstrated no CAEV antibodies in their sera. The owners decided to cull all seropositive animals, disinfect the premises and form a new herd of seronegative young animals.

The repeated double tests in 2023 confirmed the absence of CAE virus circulation in the entire goat herd on this backyard. In 2024, the owners ELISA tested all animals again at a six-month interval and the results were negative.

The data obtained during the experiment, as well as the results of the monitoring tests given in the examples, confirm the effectiveness of sterile kidding as the main method of preventing the CAE spread on the goat farms. Strict compliance with all procedures makes it possible to obtain a healthy offspring with high breeding and economic value from virus-carrying animals.

Table 2
Changes in the number of seropositive animals in the goat population on backyard No. 1

Year	Number of tested animals	CAEV antibody test results, animals	
		seropositive	seronegative
2022	18	11	7
2023	14	1	13
2024	13	–	13

Table 3
Changes in the number of seropositive animals in the goat population on backyard No. 2

Year	Number of tested animals	CAEV antibody test results, animals	
		seropositive	seronegative
2020	24	24	–
2021	20	12	8
2022	23	–	22
2023	23	–	23
2024	23	–	23

CONCLUSION

Currently, the scientific literature provides contradictory data on the possibility of intrauterine infection of goats with the caprine arthritis-encephalitis virus. A number of foreign authors cite the research results indicating CAE agent isolation from newborn kids derived from seropositive animals. At the same time, there is no data in the domestic literature, as well as in the WOAH Terrestrial Animal Health Code on possible intrauterine route of CAE infection.

The results of our tests of biological materials collected from newborn kids derived from CAE seropositive goats indicate that the syndesmochorial type of placenta characteristic of small ruminants is a natural barrier to small ruminant lentiviruses, which excludes intrauterine transmission of the caprine arthritis-encephalitis causative agent. Given this fact, as well as the fact that currently there are no means of specific prevention and therapy of caprine arthritis-encephalitis, the only way to prevent the disease are technological methods, in particular sterile kidding, which excludes the virus transmission from the mother to the newborn kid.

This fact has been confirmed by long-term monitoring of the number of goats kept on two farms located in the Southern Federal District and in the Novosibirsk Oblast for the presence of CAE seropositive animals in the herd. In both cases, the owners' transition to the use of sterile kidding, which excludes any contact of the newborn kids with the seropositive mothers, within two years allowed for complete removal of the infected animals from the herd and replacement of all population with seronegative animals thus avoiding economic losses and purchase of healthy animals from other farms.

The presented data confirm that the use of sterile kidding followed by feeding the offspring with colostrum pasteurized at 60 °C for 30 minutes and milk is currently the only way to prevent the CAE spread inside the goat herd kept on the same farm.

Nevertheless, given the small sample of animals used in the experiment, as well as the fact that published opinions on the possibility of intrauterine infection differ diametrically, further research is needed, which should cover more animals.

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African swine fever clinical scoring system

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ABSTRACT
African swine fever (ASF) is a hemorrhagic viral disease that brings serious implications for animal health and economy due to high mortality rate, quarantine measures and restrictions on international trade in pig products. Only domestic and wild species of the *Suidae* family of all breeds and ages are susceptible to infection with ASF virus. To date, no safe and effective ASF vaccines have been developed, but in recent years some progress has been made in development of ASF modified live virus first-generation vaccine candidates, which have been used only in some countries of Southeast Asia. The expansion of their use is hindered, among other things, due to the lack of international and state recommendations (requirements) for the evaluation of purity, activity, safety and effectiveness of ASF vaccine candidates. Clinical signs of the disease are one of the main indicators of safety and effectiveness of ASF modified live virus vaccine candidates. The purpose of this work was to develop a clinical symptom-based scoring system to be used for characterizing of newly recovered ASFV isolates causing various forms of the disease, as well as for the determination of safety and effectiveness of ASF modified live virus vaccine candidates. It is proposed to take into account 7 major clinical manifestations: an increase in body temperature, reduced liveliness, loss of appetite, skin lesions, joint swelling, laboured breathing, neurological disorders, each scored from 0 to 3 or 4. The study of twelve ASFV strains of various virulence revealed that acute and subacute ASF produce the maximum clinical scores ranged from 13 to 22, chronic form gives 6–18 points, subclinical form is scored 0–8.

Keywords: African swine fever, clinical signs, vaccine candidates

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Балльная система оценки клинических признаков при африканской чуме свиней

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РЕЗЮМЕ
Африканская чума свиней (АЧС) – это геморрагическая вирусная болезнь, которая вызывает серьезные санитарные и экономические последствия из-за высокого уровня смертности животных, карантинных мероприятий и ограничений международной торговли продукцией свиноводства. Вирус АЧС поражает исключительно домашних и диких свиней семейства *Suidae* всех пород и возрастных групп. До настоящего времени безопасные и эффективные средства специфической защиты против АЧС не разработаны, но в последние годы достигнут определенный прогресс в исследованиях по разработке вакцин первого поколения на основе модифицированного живого вируса, которые ограниченно использовали в некоторых странах Юго-Восточной Азии. Расширение их применения сдерживается в том числе из-за отсутствия международных и государственных рекомендаций (требований) по оценке чистоты, активности, безопасности и эффективности кандидатных вакцин против АЧС. Клинические признаки болезни являются одним из основных показателей безопасности и эффективности кандидатных вакцин против АЧС на основе модифицированного живого вируса. Целью данного исследования являлась разработка системы балльной оценки клинических признаков, пригодной для использования при характеристике вновь выделенных изолятов вируса АЧС, вызывающих различные формы течения болезни, а также при определении безопасности и эффективности кандидатных вакцин, изготовленных на основе модифицированного живого вируса. Предложено учитывать 7 преобладающих клинических признаков: повышение температуры тела, снижение активности, снижение аппетита, поражение кожных покровов, поражение суставов, нарушение дыхания, поражение центральной нервной системы, – каждый из которых оценивается от 0 до 3 или 4 баллов. В результате исследования двенадцати штаммов вируса АЧС различной вирулентности установлено, что при острой и подострой формах АЧС максимальные суммы баллов клинических признаков составляли от 13 до 22, при хронической форме – от 6 до 18, при субклинической – от 0 до 8.

Ключевые слова: африканская чума свиней, клинические признаки, кандидатные вакцины

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INTRODUCTION

African swine fever (ASF) is an infectious disease of domestic and wild pigs of all breeds and ages caused by a virus of *Asfivirus* genus, *Asfarviridae* family. Since its introduction in 2007 to Georgia ASF outbreaks have been reported in more than 50 countries of Europe, Asia, Africa and the Caribbean Basin region [1, 2, 3].

As regards the degree of virulence, ASFV strains can be divided into high, moderate, low virulence or non-virulence. Depending on the strain /isolate virulence the clinical course of ASF can be hyperacute, acute (highly virulent isolates/strains), subacute (highly and moderately virulent strains), chronic (moderate and low virulent strains), subclinical and asymptomatic (non-virulent strains) [4]. Superacute ASF kills pigs on days 3–5 post infection, either without clinical manifestations, or with short-term hyperthermia (> 41.0 °C), without loss of activity and appetite. Acute ASF is manifested by an early increase in body temperature (> 41.0 °C), loss of appetite, dullness (animals are recumbent most of time), rapid breathing, cyanosis of ear, underbelly, hind limbs and perineum skin, laboured breathing, leg paresis and paralysis, sometimes constipation or bloody diarrhea may be present. Death occurs on days 6–14 post infection. Hyperacute and acute forms of ASF result in 100% mortality rate. Pigs infected with subacute forms of ASF demonstrate clinical signs similar to the acute form, but they are less pronounced. Recurrent hyperthermia, depression, loss of appetite are reported, joint swelling and severe respiratory disorders occur at later stages of the disease. Most animals (about 70%) die within 15–30 days post infection [3, 5, 6]. Chronic form lasts for more than 30 days with periodic relapses. Animals demonstrate intermittent hyperthermia, exhaustion, stunting, arthritis of varying severity, respiratory disorders, and necrotic skin ulcers. The mortality rate is about 30% [7, 8]. Subclinical and asymptomatic forms of ASF are observed in wild indigenous pigs (warthogs, giant forest hogs, bushpig) in African endemic countries, in wild boars in Sardinia, as well as in domestic pigs and wild boars experimentally infected with some ASFV attenuated strains [9, 10, 11].

In recent years, some progress has been made in the research and development of ASF modified live virus (MLV) first-generation vaccine candidates, which are used in some Southeast Asian countries (Vietnam, Philippines) [12]. A serious obstacle to the possible use of already developed and future ASF MLV vaccine candidates is the lack of internationally and nationally agreed parameters (requirements) for the evaluation of their purity, activity, safety and efficacy. In the world's leading laboratories, various methods are used to evaluate the safety and efficacy of the vaccine candidates, which hinders the scientific community from side-by-side comparison and assessment of the results obtained, and government agencies and institutions authorized to issue permits for their use do not have such grounds.

Currently, the Biological Standards Commission of the World Organization for Animal Health is approving the updated Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, which includes harmonized standards and recommendations for ASF MLV vaccine candidates. Similar work is being done in the Russian Federation.

The main parameters of the safety and efficacy of ASF MLV vaccine candidates are: clinical signs, viremia, survival rate after immunization and virulent 'homologous' challenge [13]. To assess the clinical signs in pigs infected with ASF virus of various pathogenicity, a number of authors proposed using clinical scoring systems that cover thirteen signs (Table 1). The least number of clinical signs (4) was covered by the scoring system proposed by E. B. Howey et al. [14]; the most extensive scoring system (11) was created by A. S. Olesen et al. [15], which totaled 12 and 42 points, respectively.

The purpose of this work was to develop a clinical symptom-based scoring system to be used for characterizing of newly recovered ASFV isolates causing various forms of the disease, as well as for the determination of safety and efficacy of ASF MLV vaccine candidates.

MATERIALS AND METHODS

The results of our own experiments conducted with ASF virus strains of various virulence were used in the study:

– highly virulent strains: Stavropol 01/08, Vladimir-Vyazniki/2017, Bryansk-21 (VIII serotype, II genotype), Lisbon-57 (I, I), Mozambique-78 (III, V), France-32 (IV, I);

– moderately virulent: Novgorod-2019 (VIII, II), PSA-1-NH (IV immunotype, I);

– non-virulent (attenuated): Katanga-350 (I, I), MK-200 (III, V), FC-32/135 (IV, I), Stavropol 71/2017 (VIII, II) [22, 23, 24, 25, 26, 27, 28, 29].

Twenty-five to thirty kilograms large white pigs were received from the Unit of experimental animal

preparation of the Federal Research Center for Virology and Microbiology. Experiments on pigs were performed in accordance with the "Guidelines for keeping and use of laboratory animals" [30]. The observed ASF clinical signs were recorded daily. Body temperature was measured rectally using mercury thermometers, other signs were determined visually.

A detailed description of porcine leukocyte (PL) cell culture preparation and determination of ASFV infectivity were performed as described earlier [22, 31]. The results were evaluated by hemadsorption or cytopathogenic effect during 7 days. Virus titers were calculated using Kärber method, modified by I. P. Ashmarin, and expressed as 50% hemadsorbing units (HAE_{50}/cm^3) or tissue culture infectious doses ($TCID_{50}/cm^3$) [32].

RESULTS AND DISCUSSION

Based on our tests using 144 animals and the published criteria for evaluation of ASF clinical signs, seven major clinical signs that were observed in infected pigs were selected: increased temperature, dullness, loss of appetite, skin lesions, joint involvement, respiratory disorders, neurological disorders. Each symptom, depending on the severity, was scored 3 or 4 points (Table 2). Such ASF clinical signs as eye discharges (conjunctivitis), diarrhea, bloody feces, bloody urine, vomiting, were not demonstrated by most infected animals, and they were not included in the scoring system. The system is based on the following principles: a) inclusion of the most characteristic clinical signs; b) increasing score representing a greater severity of clinical manifestation; c) the characteristic of a specific score does not exclude the simultaneous presence of those rated with lower scores.

Hyperthermia, loss of activity and appetite were observed in all pigs with acute, subacute and chronic forms of ASF, and redness and cyanotic skin were noted in 80–95% of the animals. These signs were scored from 0 to 4 points (Table 2). Less often (60–70%) the above mentioned forms of ASF induced incoordination and breathing problems. Joint involvement was observed in 35–50% of pigs with subacute and chronic disease. These three signs were scored from 0 to 3 points. Constipation, diarrhea, and bloody feces were reported only in some animals with acute and sub-acute ASF.

The developed ASF clinical scoring system was evaluated by the experiments on pigs infected with 12 strains of various virulence (Table 3) [22, 23, 24, 25, 26, 27, 28, 29].

It should be noted that the minimum and maximum total scores given in Table 3 are not the sum of the minimum and maximum points for each of the seven clinical

Table 1
Published ASF clinical sign scoring systems

Clinical signs	Corresponding clinical scores							
	[14]	[15]	[16]	[17]	[18]	[19]	[20]	[21]
Temperature	0–4	0–5	–*	0–5	1–3	–	0–5	–
Appetite/anorexia	–	0–6	0–3	1–6	1–3	1–3	1–6	0–3
Behaviour/liveliness	0–4	0–6	0–3	1–6	1–3	0–3	1–6	0–3
Skin lesions, cyanosis	–	0–3	0–3	0–3	1–3	1–3	1–3	0–3
Joint swelling	–	0–4	0–3	1–4	–	–	1–4	0–3
Respiration	–	0–3	0–3	1–3	1–3	1–3	1–3	0–3
Ocular discharges	–	0–2	0–3	1–2	–	1–3	1	0–3
Defecation	0–2	0–4	0–3	1–3	1–3	1–3	1–4	0–3
Urination	–		–	4	–	–	4	–
Vomiting	–	–	–	1–3	–	1–3	4	–
Neurology	0–2	–	–	0–6	–	1–3	–	0–3
Posture	–	0–6	–	–	–	1–3	–	0–3
Body condition	–	–	–	–	1–3	1–3	–	
Maximum score	12	42	21	42	18	30	40	27

*(–) – the parameter was not taken into account;
clinical signs in the colored cells are considered to be one parameter.

Table 2
African swine fever clinical sign scoring

Clinical signs	Points				
	0	1	2	3	4
Increase in body temperature, °C	38.0–40.0; no	40.1–40.5	40.6–41.0	41.1–41.5	41.6–42.0
Decreased activity	no	slight dullness	recumbent, if touched rises quickly	after a few touches rises with difficulty	recumbent, can't rise
Loss of appetite (feed consumption rate, %)	no	about 10% of the feed remains in the feeder	about 50% of the feed remains in the feeder	comes up to the feeder, but does not eat	loss of interest in food (complete refusal)
Skin lesions	no	erythema, redness the ear tip skin	cyanosis of the ear tips and tail	limited cyanosis in various parts of the body with sporadic necrotic lesions	extensive cyanosis of the skin, multiple necrotic skin lesions
Joint involvement	no	joint swelling	joint swelling and slight lameness	joint swelling and evident painful lameness	–
Respiratory disorders	no	Shortness of breath	laboured breathing, nasal discharge	painful breathing, wheezing, coughing	–
Neurology	no	unsteady walk	paresis of one or more limbs	convulsions, paralysis, muscle tremor	–

signs considered; they correspond to individual scores of the test animals in each of these groups.

Highly virulent strains (Stavropol 01/08, Vladimir-Vyazniki/2017, Lisbon-57, Mozambique-78, France-32, Bryansk-21) caused acute and less often subacute disease regardless of the infection routes and doses used. Acute ASF produced hyperthermia (41.0–42.0) °C, partial or complete loss of activity and appetite, redness or cyanosis of the ear, abdominal wall, tail and perineum, severe painful breathing with nasal discharge, leg paresis and paralysis, convulsions were recorded. Bloody diarrhea was observed in only a few animals. Before death, on days 6–14 post infection, the body temperature in pigs decreased to 38.0 °C. The subacute ASF produced the following clinical signs: hyperthermia, loss of activity and appetite, minor redness or cyanosis (tips of the ears, tail). Acute and subacute ASF, regardless of the infection routes or doses, gave the clinical scores in different individuals ranging from 13 to 22.

Chronic ASF was observed in pigs infected intramuscularly with Novgorod-2019 or PSA-1-NH strains. The disease was characterized by intermittent hyperthermia (40.5–41.5) °C, loss of activity and appetite. In 10–14 days, the animals demonstrated cachexia, stunting, different respiratory disorders and joint problems (arthritis). Some animals demonstrated spotted skin redness, which subsequently could become cyanotic and sporadically necrotic (PSA-1-NH strain). The duration of the disease could be different and lasted for more than 30 days. "Clinical recovery" was recorded in some animals with subsequent recurrence, manifested by an in increase in body temperature. The ASF clinical scores in such animals ranged from 6 to 18.

Less pronounced signs of chronic ASF were recorded in 40–60% of pigs after intranasal inoculation of PSA-1-NH strain, as well as in 7% of pigs inoculated with attenuated Katanga-350 strain. Recurrent hyperthermia was observed (from 40.1 to 40.7 °C), a slight decrease in activity (got up quickly when touched) and appetite (50–90% of feed consumed) during 1–4 days, in some cases shortness of breath and slight joint swelling were observed. The maximum ASF clinical scores in these animals reached 6–10.

Most pigs infected intramuscularly with attenuated Katanga-350 strain, attenuated but not protective Stavropol 71/2017 strain, as well as intranasally with a low dose (10^3 $TCID_{50}$) of naturally attenuated PSA-1-NH strain demonstrated subclinical ASF: body temperature in 40–60% of animals not exceeded 40.5 °C during 1–4 days, 90–100% of the feed was consumed. No other changes were recorded. The total clinical score ranged from 0 to 5.

The asymptomatic ASF was recorded in 100% of pigs inoculated intramuscularly with strain FK-32/135, in 75–90% of pigs inoculated with strain MK-200, in 40–60% with strain Katanga-350. ASF clinical signs in such animals were not recorded, the total scores were 0.

It should be noted that the ASF clinical scoring system is not applicable for isolates causing peracute disease, since the death of pigs occurs 3–5 days after infection without most clinical signs manifested due to intramuscular administration of virulent virus isolates in large doses.

CONCLUSION

Over the past 5–7 years, circulation of ASF genotype II virus isolates of various virulence, from highly virulent

Table 3
Clinical sign scores of pigs infected with various ASFV strains

Name of the strain	Infection route	Dose*	Survivors/total	Clinical signs and their scores							Total score
				Increased temperature	Decreased activity	Loss of appetite	Skin lesions	Joint involvement	Respiratory disorders	Neurology	
Stavropol 01/08	i/m	$10^{2–3}$	0/15	4	4	4	3–4	0	2–3	2–3	19–22
	nas.	$10^{2–3}$	0/4	4	3–4	4	3–4	0	2–3	1–3	17–22
	oral	10^{7}	0/3	3–4	3	3–4	2–3	0	2	2	15–18
Vladimir-Vyazniki/2017	i/m	$10^{2–3}$	0/6	3–4	3–4	3	2–3	0	1–2	1–3	13–19
	nas.	$10^{2–3}$	0/6	3–4	3–4	3–4	2–3	0	1–2	1–3	13–20
	oral	$10^{4–7}$	0/6	3	3–4	3–4	1–2	0	1–3	1–2	12–18
	cont.	–	0/4	3–4	3	3–4	1–3	0	1–2	1–2	14–17
Lisbon-57	i/m	10^{3}	0/5	4	4	3–4	3–4	0	2–3	2–3	18–22
Mozambique-78	i/m	10^{3}	0/5	4	4	4	2–4	0	2–3	2–3	18–22
France-32	i/m	10^{3}	0/5	3–4	3–4	3–4	2–3	0	1–2	1–3	13–17
Bryansk-21	i/m	10^{3}	0/4	3–4	3	3	2–3	0	1–2	1–2	13–15
Novgorod-2019	i/m	10^{3}	1/5	3	3	3–4	1–3	0	1–2	1–3	13–18
				2–3	2	1–2	0	1–2	1–2	0	7–11
PSA-1-NH	i/m	10^{3}	4/5	1–4	1–4	1–4	0	0	1–3	0	1–15
	i/m	10^{5}	7/15	2–4	3–4	2–4	1–4	1–3	1–3	1–2	12–18
	nas.	10^{3}	5/5	1–3	1–2	1–2	0	0	1–2	0	1–8
	nas.	10^{5}	4/5	3–4	2–3	2–3	1	1–2	2–3	1–2	8–15
Katanga-350	i/m	10^{6}	15/15	0–2	0–2	0–2	0	1	1	0	0–8
MK-200	i/m	10^{6}	16/16	0–3	0–2	0–2	0	0	1	0	0–8
FK-32/135	i/m	$10^{6–7}$	16/16	0	0	0	0	0	0	0	0
Stavropol 71/2017	i/m	10^{5}	9/9	1	0–1	0–1	0	0	0	0	1–3

i/m – intramuscular, nas. – nasal, cont. – contact;
* $TCID_{50}$ for PSA-1-NH strain, HAU_{50} for other strains.

to naturally attenuated, have been evidenced in Europe and Asia [2, 3, 6, 9, 11, 33].

According to our observations, the dynamics of ASF clinical signs varies depending on the virulence of the isolate/strain, to a lesser extent on the dose and method of the virus inoculation [26, 27, 28]. The developed and tested ASF clinical scoring system is suitable both for the characterization of newly recovered isolates and for the evaluation of MLVs and MLV vaccine candidates. Based on our experience, for candidate strains, the total clinical score should not exceed 4–5 points. Ideally, when the score is 0, as for example after vaccination of pigs with ASFV FK-32/135 strain [26].

It should be noted that for the evaluation of MLV vaccine safety, in addition to clinical signs, it is proposed to study the levels and duration of viremia, virus shedding, post-mortem changes, persistence of the vaccine and virulent (infecting) viruses in tissues, potential transmission and reversion to virulence, safety in the field and some others [13]. However, if, after the inoculation of a candidate vaccine strain to pigs, the total clinical score exceeds 5, it is not reasonable to conduct other studies.

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Canine adenovirus serotype 2 isolation and determination of its cultivation parameters

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ABSTRACT

Adenovirus infection in dogs caused by canine adenovirus serotype 2 predominantly results in respiratory disease typically manifested by respiratory tract lesions. Infectious laryngotracheitis is the most often recorded in dogs in the central part of the Russian Federation and its incidence tends to increase. Therefore, preventive immunization against this disease remains important. Primarily, the virus strains currently important and circulating in the particular territory shall be used for vaccine production to induce long-term and strong immunity in animals. The study was aimed at isolation of canine adenovirus type 2 remaining stable during five or more passages from the biological samples collected from animals with adenovirus infection signs as well as at determination of its cultivation parameters. As a result, five virus isolates were recovered, one of the recovered virus isolates had optimal properties for its use for vaccine production. Comparative analysis of continuous Vero, MDCK (NBL-2 and NBL-9 line) cell cultures as well as primarily trypsinized cell cultures (baby dog kidney, baby dog spleen, baby cat kidney, baby cat spleen) for their susceptibility to the recovered virus showed that MDCK (NBL-2 line) was the most susceptible. The virus cultivation parameters in this cell culture was determined at the next step. The following optimal conditions under which the virus accumulated to the maximum titres were determined: cell culture monolayer age for inoculation – 48 hours, multiplicity of infection – 0.01 $TCID_{50}$/cell, preliminary holding time – 60 min, temperature – (37.0 ± 0.5) °C, cultivation period – 120 hours.

Keywords: canine adenovirus serotype 2, recovery of isolate, adenovirus infection, virus infectivity, cultivation parameters

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Выделение аденовируса собак 2-го серотипа и определение параметров культивирования

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РЕЗЮМЕ

Аденовирусная инфекция собак, вызванная аденовирусом 2-го серотипа, является преимущественно респираторным заболеванием, которое классически проявляется поражением органов дыхательной системы. Заболеваемость собак инфекционным ларинготрахеитом наиболее часто регистрируется в центральной части Российской Федерации и имеет тенденцию к росту. В связи с этим сохраняется актуальность профилактической иммунизации против данного заболевания. В первую очередь для формирования длительного и напряженного иммунитета у животных при изготовлении вакцины необходимо использовать штаммы вируса, распространенные на конкретной территории и актуальные на данный отрезок времени. Целью данного исследования являлось выделение возбудителя аденовироза собак 2-го типа из биологического материала от животных с признаками аденовирусной инфекции, обладающего устойчивостью на протяжении пяти и более пассажей, а также определение параметров его культивирования. В результате эксперимента было выделено пять изолятов вируса, один из которых обладал оптимальными свойствами для использования при производстве вакцинных препаратов. При проведении сравнительного анализа чувствительности к выделенному вирусу перевиваемых культур клеток Vero, MDCK линий NBL-2 и NBL-9, а также первично трипсинизированных культур клеток (почка щенка, селезенка щенка, почка котенка, селезенка котенка) было установлено, что наиболее чувствительной является клеточная культура MDCK линии NBL-2. Следующим этапом было определение параметров культивирования вируса в данной культуре клеток. В результате установлены оптимальные условия, при которых происходит накопление вируса в максимальных титрах: возраст монослоя культуры клеток для заражения – 48 ч, множественность заражения – 0,01 $ТЦД_{50}$/кл, время предварительного контакта – 60 мин, температура – (37,0 ± 0,5) °С, срок культивирования – 120 ч.

Ключевые слова: аденовирус собак 2-го серотипа, выделение изолята, аденовирусная инфекция, инфекционная активность вируса, параметры культивирования

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INTRODUCTION

Adenovirus infection in dogs (infectious laryngotracheitis) caused by adenovirus serotype 2 (CAV-2) predominantly results in respiratory disease typically manifested by respiratory tract lesions [1, 2, 3]. The infection agent is widespread in the central part of the Russian Federation due to the dense population of dogs and regular events characterized by crowded animal keeping. Despite regular preventive vaccination of animals, infectious laryngotracheitis incidence tends to increase.

The canine adenovirus serotype 2 belongs to *Varidnaviria* realm, *Bamfordvirae* kingdom, *Preplasmiviricota* phylum, *Tectiliviricetes* class, *Rowavirales* order, *Adenoviridae* family, *Mastadenovirus* genus, *Mastadenovirus canidae* species. It was included in the register of the International Committee on Taxonomy of Viruses (ICTV) in 1976 [4]. The virus was first isolated in Canada in 1961 [5].

Currently, two serotypes of the causative agent of adenovirus infection in dogs are known: the 1st serotype of the virus (Canine adenovirus 1, CAV-1) is characterized by its systemic effect on the animal's body, affects most of the main organs and causes infectious hepatitis; the 2nd serotype of the virus (Canine adenovirus 2, CAV-2), like many representatives of the *Mastadenovirus* genus, is characterized by local effect and causes mainly respiratory tract lesions, less often gastrointestinal tract lesions [2, 6, 7, 8, 9].

Canine adenovirus serotype 1 (CAV-1) is more virulent than CAV-2. Canine adenovirus serotype 2 (CAV-2) is a non-enveloped DNA virus, weakened variant of CAV-1, sharing approximately 75% nucleotide sequence identity [10]. Coinfection with other viruses increases the pathogenicity of adenoviruses [11].

Canine adenovirus serotype 2 has been reported in dogs, raccoons, horses, cattle, cats and wolves. It shows subclinical circulation in the population of wild carnivores [11, 12, 13, 14].

It should be noted that canine infectious respiratory disease complex called infectious laryngotracheitis in dogs, or kennel cough, is caused by several different microorganisms, that, besides adenovirus serotype 2, include the following pathogens: canine distemper virus, canine herpesvirus, canine parainfluenza virus, canine influenza virus, canine respiratory coronavirus, canine pneumovirus, as well as the following bacteria: *Mycoplasma cynos*, *Bordetella bronchiseptica* and *Streptococcus equi* subspecies [3, 15, 16, 17, 18]. Bocavirus and canine hepacivirus rarely cause respiratory disease symptoms and are not considered during differentiation [19].

Various data on CAV-2 cultivation are available. Adenoviruses are known to demonstrate the best growth in the cells of their natural animal hosts [6]. Dog kidney cell cultures are the most susceptible to the virus but other types of canine cell cultures are not susceptible to the virus. Primary or continuous other mammalian cell cultures, such as humans, sheep, and monkeys, are also not susceptible to the virus. In world practice, Madin – Darby canine kidney (MDCK) cell culture is considered optimal for CAV-2 cultivation [11, 20, 21, 22]. According to other studies, CAV-2 can be also cultivated in Vero cell culture (continuous African green monkey kidney cell culture) [7].

The study was aimed at isolation of canine adenovirus serotype 2 remaining stable during five or more passages, as well as at determination of its cultivation parameters.

Cell cultures for CAV-2 virus reproduction were selected by serial passaging from available cell lines mainly used for cultivation of canine viruses and recommended for the cultivation of adenoviruses.

Primary cell line derived from naturally susceptible animals that are more susceptible to the infection is commonly used for virus isolation. Therefore, primary trypsinized baby dog kidney cell culture was selected for the first passage. Also, this cell culture was of priority for the virus isolation from pathological materials owing to high embryonic cell potential for growth.

MATERIALS AND METHODS

Biological samples (nasal and oral swabs) were collected from dogs suspected to have adenovirus infection, that were brought to veterinary clinics and shelters in the Vladimir, Vologda and Nizhny Novgorod Oblasts in 2019–2022.

Diagnosis. "ADENOVIR" commercial test system Central Research Institute for Epidemiology of the Rospotrebnadzor, Russia) was used to confirm CAV-2 presence in samples and to carry out differential diagnosis using polymerase chain reaction (PCR).

During CAV-2 virus passaging Asan Easy Test CAV2 immunochromatographic test system (Asan Pharmaceutical Co., Ltd., Korea) was used for the virus antigen detection according to the manufacturer's instructions.

Cell cultures. The following cell cultures were selected for testing: continuous MDCK cell cultures (NBL-2 and NBL-9

lines) and Vero cell culture; primary trypsinized baby dog kidney, baby dog spleen, baby cat kidney and baby cat spleen cell cultures. The initial cell concentration in the cell suspension for MDCK NBL-2 and NBL-9 lines was 400 ths cells/cm^3, for Vero cell culture – 200–250 ths cells/cm^3, for primary trypsinized cell cultures – 300–400 ths cells/cm^3. Completely formed monolayer without cell degeneration signs was a criterion for cell culture selection for inoculation. These cultures were subjected to stationary cultivation at a temperature of (37.0 ± 0.5) °C.

Plastic cell culture flasks (T25) with surface area 25 cm^3 were used for passaging.

Nutrient media. The following nutrient media indicated in the cell culture data sheets were used: semi-synthetic nutrient medium supplemented by 5% bovine serum, antibiotics (streptomycin 100 μg/cm^3 and penicillin 100 U/cm^3) was used as growth medium for and serum-free semi-synthetic nutrient medium was used as maintenance medium for MDCK cell culture; synthetic nutrient medium supplemented by glutamine (0.584 g/L), 10% bovine serum, antibiotics (streptomycin 100 μg/cm^3 and penicillin 100 U/cm^3) was used as growth medium for and serum-free synthetic medium supplemented by antibiotics was used as maintenance medium for continuous Vero cell culture and primary trypsinized cell cultures.

Preparation of the material for inoculation into cell culture. The virus-containing suspension was filtered using a Millipore membrane filter (MCE) 20 microns (Merck Millipore, USA), centrifuged at 3,000 rpm for 15 minutes. Then, the supernatant was collected and antibiotics (streptomycin 100 μg/cm^3 and penicillin 100 U/cm^3) were added to the supernatant and the supplemented supernatant was left at a temperature of 2–8 °C for 1 hour and then used for inoculation in cell cultures.

The virus isolation was carried out in primary trypsinized baby dog kidney cell culture.

Inoculation of cell cultures. Three T25 culture flasks were used for each passage of the canine adenovirus serotype 2. Before virus inoculation onto the cell monolayer, the growth nutrient medium was decanted, the monolayer was washed three times with Hanks solution and then the virus-containing suspension was inoculated; the multiplicity of infection was 0.01 $TCID_{50}$/cell. Cell culture flasks were placed in a thermostat at temperature of (37.0 ± 0.5) °C for 1 hour. Maintenance nutrient medium was added after 1 hour, cell culture flasks were placed in a thermostat at a temperature of (37.0 ± 0.5) °C and examined under an inverted microscope every 12 hours for characteristic morphological changes in cells: flasks showed more than 80% monolayer destruction were frozen at a temperature of minus (45 ± 5) °C until the next passage.

The following factors was also examined for their effect on the virus infectivity titre:

– cultivation time;
– cultivation temperature;
– age of cell culture monolayer;
– multiplicity of infection;
– preliminary holding time.

The virus was titrated according to the common procedure [23, 24] in triplicate for each sample. For this purpose, Costar® (Corning, USA) 96-well flat-bottomed microplates were used. After adding the reaction components, the plates were cultivated at a temperature of (37.0 ± 0.5) °C and 5% CO_2 concentration for 5 days (120 hours) and examined daily under an inverted microscope. The virus titre was assessed based on the number of wells with cytopathic effect. The titre was calculated using the Kärber method and expressed in lg $TCID_{50}$/cm^3.

Analysis of the results. The obtained examination results were processed using Microsoft Office Excel programme.

RESULTS AND DISCUSSION

Pathological material selected for virus isolation was previously tested with PCR for CAV-2 viral genome detection; PCR-positive samples were used for further work. Primary trypsinized baby dog kidney cell culture was used for canine adenovirus serotype 2 isolation. Data on infectivity titres of the virus isolated at the first passage are presented in Table 1.

It was shown that all isolates found positive for CAV-2 genome by PCR, were infectious to different degree and were considered suitable for further work.

Five serial passages were carried out in primary trypsinized baby dog kidney cell culture to assess the virus reproducibility and stability. Test results are presented in Table 2.

Based on the test results, isolate No. 5 virus-containing material (later called "Yunity") was selected for further cultivation, since it remained stable for five passages, the characteristic virus cytopathic effect (CPD) was observed at each passage level; the virus infectivity titre was in the range of (3.33 ± 0.29) up to (4.33 ± 0.29) lg $TCID_{50}$/cm^3. Virus-containing materials of isolates No. 1, 2, 3, 4 were not used for further testing since their titres were below 3.0 lg $TCID_{50}$/cm^3. At the next stages, third passage virus-containing material of isolate No. 5 with maximum titre for this isolate was used for testing the virus for its cultural properties.

At the first passage, CAV-2 caused CPE manifested 24 hours after the virus-containing suspension inoculation in the cell culture; 80% monolayer destruction was observed after 72 hours. At the second passage and subsequent passages, morphological changes in cells were detected after 24–48 hours, 80% monolayer destruction was registered after 72–120 hours.

Five serial passages were performed to test various cell cultures for their susceptibility to CAV-2. In susceptible cell cultures, the virus-induced CPE manifestations were similar and observed at the first passage. CAV-2 infectivity titre was determined by microtitration in MDCK cell culture. In case of cytopathic effect absence, the virus antigen in the culture fluid was detected using immunochromatographic test system. When the culture fluid

Table 1
CAV-2 infectivity in primary trypsinized baby dog kidney cell culture when the virus was isolated at the first passage

Isolate No.	Infectivity titre, lg $TCID_{50}$/cm^3
1	1.83 ± 0.14
2	1.67 ± 0.29
3	1.92 ± 0.38
4	2.42 ± 0.29
5	3.33 ± 0.29

Table 2
Serial CAV-2 cultivation in primary trypsinized baby dog kidney cell culture

Virus passage No.	Infectivity titre, lg $TCID_{50}/cm^3$				
	Isolate No. 1	Isolate No. 2	Isolate No. 3	Isolate No. 4	Isolate No. 5
1	1.83 ± 0.14	1.67 ± 0.29	1.92 ± 0.38	2.42 ± 0.29	3.33 ± 0.29
2	0.83 ± 0.58	1.67 ± 0.29	2.25 ± 0.25	2.50 ± 0.43	4.17 ± 0.14
3	–	1.17 ± 0.14	2.50 ± 0.25	2.17 ± 0.14	4.33 ± 0.29
4	–	–	2.58 ± 0.14	1.75 ± 0.25	4.08 ± 0.14
5	–	–	2.33 ± 0.29	–	3.92 ± 0.14

"–" – not tested.

Table 3
Susceptibility of different continuous and primary trypsinized cell cultures to CAV-2 Yunity isolate

Cell culture	Infectivity titre, lg $TCID_{50}/cm^3$				
	1st passage	2nd passage	3rd passage	4th passage	5th passage
Continuous cell cultures					
MDCK NBL-2	4.33 ± 0.29	4.00 ± 0.25	4.08 ± 0.14	4.17 ± 0.14	4.25 ± 0.25
MDCK NBL-9	4.00 ± 0.25	3.92 ± 0.38	3.83 ± 0.29	4.00 ± 0.00	3.83 ± 0.14
Vero	< 1.0*	< 1.0	–	–	–
Primary trypsinized cell cultures					
BDS	2.42 ± 0.14	2.17 ± 0.29	1.50 ± 0.25	1.17 ± 0.29	0.92 ± 0.14
BCK	< 1.0	< 1.0	–	–	–
BCS	< 1.0	< 1.0	–	–	–

* microtitration results; "–" – not tested; BDS– baby dog spleen; BCK – baby cat kidney; BCS – baby cat spleen.

was found negative during two passages, no further tests in the cell culture were performed. The virus infectivity titres in the selected cell cultures are given in Table 2 (for baby dog kidney cell culture) and Table 3 (for other cell cultures).

No CAV-2 accumulation was found in continuous Vero cell culture, primary trypsinized baby cat kidney and baby cat spleen cell cultures, no visible morphological changes were detected in these cells; no CAV-2 antigen was detected with immunochromatographic assay in culture fluid staring with the second passage. The virus titre was not determined during microtitration. The virus infectivity titre gradually decreased during the virus cultivation in the primary trypsinized baby dog spleen cell culture. Based on the obtained results the above cell cultures were considered non-suitable for the virus cultivation.

The virus infectivity in primary trypsinized baby dog kidney cell culture was (4.33 ± 0.29) lg $TCID_{50}/cm^3$ at the third passage; the virus titre in continuous MDCK cell culture was (4.25 ± 0.25) lg $TCID_{50}/cm^3$ at the fifth passage.

The following most susceptible cell cultures were identified based on the test results given in Tables 2 and 3: continuous MDCK cell cultures (NBL-2 line [22], the so-called parent line, and NBL-9 line), as well as the primary trypsinized baby dog kidney cell culture. However, use of a primary trypsinized culture for subsequent virus cultivation for specific vaccine and diagnosticum production is inexpedient since it is seasonal due to the use of tissue donors. Subcultivation of primary trypsinized cell cultures is impossible due to their low producibility.

Uneven cell distribution on the flask surface was noted during NBL-9 line MDCK monolayer formation that could potentially affect the virus infectivity accumulation and result in errors in CPE detection with microscopy (Fig. 1). The infectivity titre was slightly higher when the virus-containing material was cultivated in NBL-2 MDCK cell culture. Thus, continuous MDCK cells (NBL-2 line) was selected for further tests aimed at determination of optimal virus cultivation conditions (Fig. 2).

Intact MDCK cell culture (NBL-2 line) is shown in Figure 3. Figures 4 and 5 show the virus-induced CPE manifestations after 96 and 120 hours (magnification 200× and 400×): individual rounded refractive cells gradually detaching from the glass are clearly visible. At the beginning, cells showed focal morphological changes, then the monolayer destroyed and the cell detached from the culture flask surface. As the virus multiplied, the number of cells undergoing degeneration increased and voids

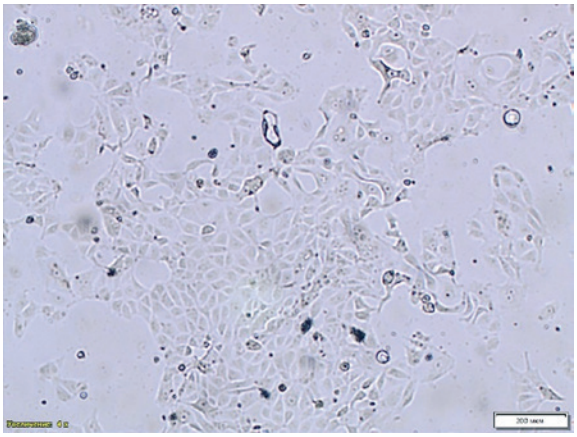

Fig. 1. Monolayer formation – age: 24 hours, MDCK cell culture NBL-9 line (200× magnification)

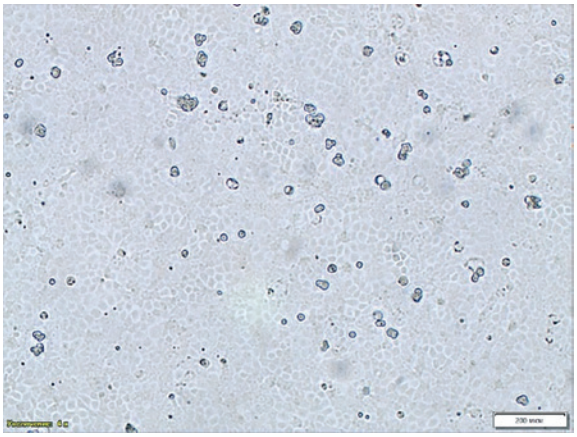

Fig. 2. Monolayer formation – age: 24 hours, MDCK cell culture NBL-2 line (200× magnification)

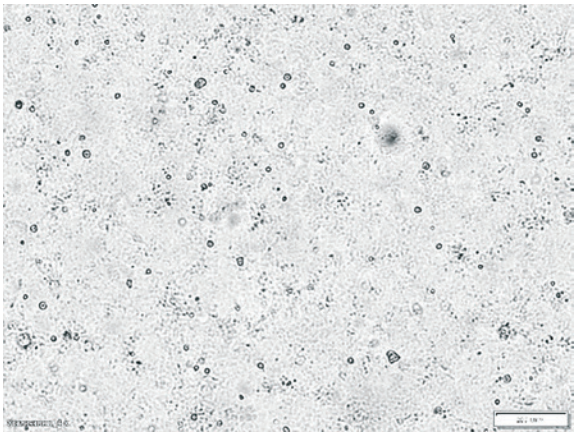

Fig. 3. Intact continuous MDCK cell culture NBL-2 line (200× magnification)

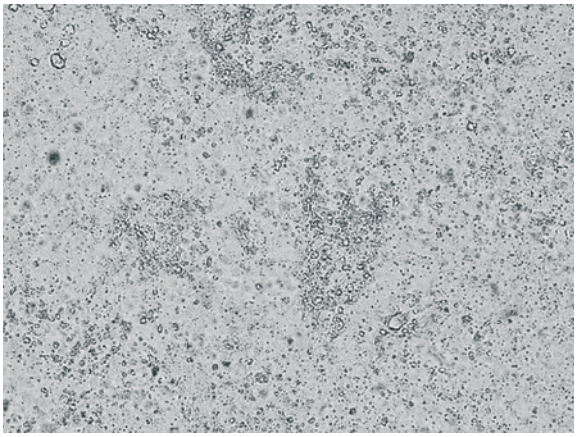

Fig. 4. CAV-2 CPE manifestation 96 hours after infection of continuous MDCK cell culture NBL-2 line (200× magnification)

formed in the monolayer. The affected cells concentrated along the edges of the remained monolayer areas, forming large conglomerates resembling grape bunches.

Cultivation time required for CAV-2 accumulation at maximum titres was to be determined for further testing. Test results are presented in Table 4.

It was found that the virus accumulated to maximum levels (4.08 ± 0.29 lg $TCID_{50}/cm^3$) on day 5 of cultivation.

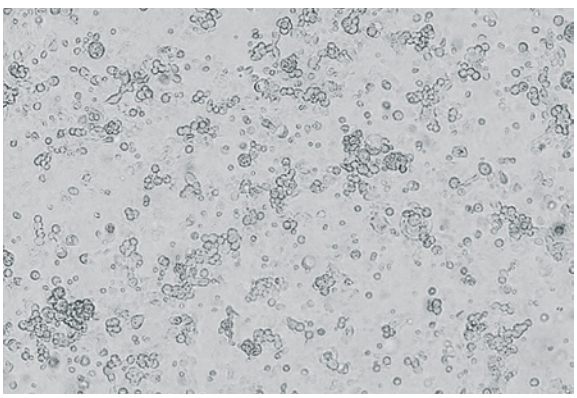

Fig. 5. CAV-2 CPE manifestation 120 hours after infection of continuous MDCK cell culture NBL-2 line (400× magnification)

The virus reproduction ranged from (1.25 ± 0.25) to (3.08 ± 0.29) lg $TCID_{50}/cm^3$ when the virus was cultivated for 48, 72 and 96 hours. The virus infectivity titre decreased to (3.42 ± 0.14) and (3.08 ± 0.14) lg $TCID_{50}/cm^3$ when the virus was cultivated for 144 and 168 hours, respectively. The decrease in the virus infectivity was accounted for slowdown in the metabolic processes in cells linked with the external environment during long-term cultivation.

Table 4
CAV-2 accumulation depending on the time of cultivation in continuous MDCK NBL-2 line cell culture

Cultivation time, hours	Infectivity titre, lg $TCID_{50}/cm^3$
48	1.25 ± 0.25
72	2.92 ± 0.38
96	3.08 ± 0.29
120	4.08 ± 0.29
144	3.42 ± 0.14
168	3.08 ± 0.14

Table 5
CAV-2 infectivity titre depending on temperature of cultivation in continuous MDCK NBL-2 line cell culture

Cultivation time	Infectivity titre, lg $TCID_{50}/cm^3$	
	(35.0 ± 0.5) °C	(37.0 ± 0.5) °C
1 day (24 hours)	0	0
2 days (48 hours)	0.92 ± 0.14	1.17 ± 0.14
3 days (72 hours)	2.67 ± 0.14	3.33 ± 0.14
4 days (96 hours)	2.75 ± 0.25	3.58 ± 0.14
5 days (120 hours)	2.83 ± 0.14	4.33 ± 0.29
6 days (144 hours)	2.83 ± 0.29	3.58 ± 0.29
7 days (168 hours)	1.75 ± 0.00	2.17 ± 0.14

Also, temperature (39.0 ± 0.5) °C was preliminary examined for its effect on MDCK cell culture viability. At this temperature, morphological changes were observed in the cell culture after 12 hours. After 24 hours, the monolayer detached from the surface. Therefore, this temperature was not used for the virus cultivation.

Results presented in Table 5, show that CAV-2 activity was lower and the maximum titre was (2.83 ± 0.29) lg $TCID_{50}/cm^3$ after 144 hours of cultivation at a temperature of (35.0 ± 0.5) °C. When the virus was cultivated at temperature (37.0 ± 0.5) °C in MDCK cell culture (NBL-2 line), the virus activity was maximum (4.33 ± 0.14 lg $TCID_{50}/cm^3$) after 120 hours of cultivation. Then, CAV-2 infectivity titre gradually decreased.

At the next stage, the optimal age of the cell monolayer for virus inoculation was determined. For this purpose, monolayers formed 24, 48, 72, 96 hours after the MDCK cells were placed into culture flask and the cell culture that was placed into culture flask immediately before inoculation of the virus-containing material were used. The results were recorded after 120 hours (5 days) of incubation in a thermostat at a temperature of (37.0 ± 0.5) °C.

Based on the obtained data (Table 6), the optimal time for the cell culture monolayer formation to be used for the virus inoculation was 48 hours, the virus titre was (4.08 ± 0.38) lg $TCID_{50}/cm^3$. When CAV-2 was inoculated into the cell suspension before monolayer formation, the virus titre was (2.42 ± 0.14) lg $TCID_{50}/cm^3$, the monolayer formed more slowly than in the intact cell culture, and the trend for formation of cell clusters was more evident. Cell metabolism and virus reproduction appeared to decrease during cultivation for 72 and 96 hours. The infectivity titre was (3.83 ± 0.14) and (3.08 ± 0.29) lg $TCID_{50}/cm^3$, respectively.

The following infection doses were used to test the effect of the multiplicity of infection on CAV-2 accumulation level in MDCK cell culture (NBL-2 line): 0.1; 0.01; 0.001; 0.0001 $TCID_{50}$/cell. Incubation was stopped when 80% of the cell monolayer area destroyed and the cells detached from the surface. The obtained results are shown in Table 7.

When the infection dose was 0.1 $TCID_{50}$/cell, CPE was observed as early as 48 hours after the virus suspension inoculation into the cell culture. However, CAV-2 titre was (3.08 ± 0.38) lg $TCID_{50}/cm^3$ and was lower than that

Table 6
Correlation of MDCK NBL-2 line cell culture monolayer age with CAV-2 infectivity titre

Monolayer formation time, hours	Infectivity titre, lg $TCID_{50}/cm^3$
0	2.42 ± 0.14
24	3.17 ± 0.14
48	4.08 ± 0.38
72	3.83 ± 0.14
96	3.08 ± 0.29

Table 7
CAV-2 infectivity titre depending on infectious dose in MDCK NBL-2 line cell culture

MOI, $TCID_{50}$/cell	Cultivation time, hours	Infectivity titre, lg $TCID_{50}/cm^3$
0.1	48	3.08 ± 0.38
0.01	72	4.33 ± 0.29
0.001	96	2.17 ± 0.14
0.0001	96	1.75 ± 0.25

MOI – multiplicity of infection.

one (4.33 ± 0.29 lg $TCID_{50}/cm^3$) when the infection dose of 0.01 $TCID_{50}$/cell was used that was accounted for the rapid monolayer destruction and, as a result, the lack of the possibility for the virus to accumulate to its maximum concentration. At a multiplicity of infection of 0.001 and 0.0001 $TCID_{50}$/cell, the virus infectivity titre was low and was (2.17 ± 0.14) and (1.75 ± 0.25) lg $TCID_{50}/cm^3$, respectively; CPE was observed in cell culture after 96 hours.

Duration of preliminary holding (adsorption) of the virus with the MDCK cell culture monolayer is also a factor of interest in view of its effect on the virus accumulation.

CAV-2 infectivity titre was found to be (2.16 ± 0.14) lg $TCID_{50}/cm^3$ when the cultivation was

carried out without adsorption. Preliminary holding of the virus with the monolayer enhanced CAV-2 reproduction manifested by an increase in its infectivity titre. Preliminary holding for 60 minutes was the optimal time for maximum virus accumulation: the infectivity titre was (4.33 ± 0.29) lg $TCID_{50}/cm^3$. When preliminary adsorption time was 30 and 90 minutes, the virus infectivity titre was (4.08 ± 0.38) and (4.08 ± 0.14) lg $TCID_{50}/cm^3$, respectively.

CONCLUSION

Canine adenovirus serotype 2 was isolated as a result of the study, the isolated virus remained stable for five passages and had high infectivity titre.

CAV-2 isolate was tested for its cultivation parameters in continuous and primary trypsinized cell cultures. MDCK cell culture (NBL-2 line) was selected as the most susceptible culture enabling yielding of the virus-containing material at high titre (4.33 ± 0.29 lg $TCID_{50}/cm^3$). The following conditions facilitating CAV-2 accumulation to maximum titres were found optimal: use of the cell culture monolayer formed within 48 hours for the virus inoculation; a multiplicity of infection of 0.01 $TCID_{50}$/cell; preliminary holding time – 60 minutes; cultivation at a temperature of (37.0 ± 0.5) °C for 120 hours.

The obtained results can be used for development of diagnostic test systems and vaccines for prevention of CAV-2 infection in dogs.

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Study of the vaccination effects against *Staphylococcus aureus*, causing mastitis and endometritis in cows

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ABSTRACT

The high contagiousness of staphylococcal infections and emergence of antimicrobial resistant strains call for search and development of new highly effective drugs and vaccines against infectious animal diseases. Twenty adult pregnant black pied cows were used to form a test and a control groups (10 animals per group). The vaccine was administered twice subcutaneously in the middle third of the neck of the test animals: the first dose in a volume of 3 mL 55–70 days before calving, the second dose in the same volume 25–30 days before the expected calving. Control animals were injected subcutaneously with the same volume of sterile saline at the same dates. To evaluate the antigenicity of the vaccine against *Staphylococcus aureus*, blood was collected from animals of both groups: in the test group 14–16 days after booster vaccination, in the control group 14–16 days after second injection of the sterile saline. For bacteriological testing, milk samples from both groups were collected during the first month of lactation after calving. According to the results of serological testing, the antibody titer against *Staphylococcus aureus* in the test group ranged from 4.01 to 4.61 lg, its mean value was (4.34 ± 0.06) lg. In the test group, the mean antibody titers against *Staphylococcus aureus* were 5.8 times lower and were equal to (0.75 ± 0.09) lg with fluctuations from 0.3 to 1.2 lg. The bacteriological tests of milk in the control group revealed *Staphylococcus aureus* in 5 out of 10 samples, which is 50%. In the test group, the pathogen was detected in 20% of cases, which is 2.5 times lower than in the control group.

Keywords: mastitis, endometritis, cows, vaccination, *Staphylococcus aureus*, antibodies, potency, milk

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Изучение воздействия вакцинации в отношении *Staphylococcus aureus*, вызывающего маститы и эндометриты у коров

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РЕЗЮМЕ

Высокая контагиозность стафилококковых инфекций и появление антибиотикоустойчивых штаммов микроорганизмов диктует необходимость поиска и разработки новых высокоэффективных средств лечения и профилактики инфекционных болезней животных. Для опытной и контрольной групп отобраны взрослые стельные коровы черно-пестрой породы по 10 гол. в каждой. Животным опытной группы вакцину вводили подкожно в область средней трети шеи двукратно: первую дозу в объеме 3 см^3 – 55–70 дней до отела, вторую – за 25–30 дней до предполагаемого отела в том же объеме. Животным контрольной группы подкожно вводили стерильный физиологический раствор в сопоставимом объеме и с тем же интервалом. Для изучения антигенной активности вакцины в отношении *Staphylococcus aureus* у животных обеих групп отбирали кровь: в опытной группе – через 14–16 сут после двукратной иммунизации, в контрольной группе – через 14–16 сут после двукратного введения животным стерильного физиологического раствора. Для проведения бактериологического исследования пробы молока в обеих группах отбирали в первый месяц лактации коров после отела. Как показали результаты серологических исследований, в опытной группе коров титр антител к *Staphylococcus aureus* колебался от 4,01 до 4,61 lg, его среднее значение составило

(4,34 ± 0,06) lg. В контрольной группе животных средние значения титра антител к *Staphylococcus aureus* были в 5,8 раза ниже и составили (0,75 ± 0,09) lg с колебаниями от 0,3 до 1,2 lg. В результате бактериологических исследований молока в контрольной группе *Staphylococcus aureus* выделили в 5 из 10 образцов, что составило 50%. В опытной группе возбудитель был обнаружен в 20% случаев, что в 2,5 раза ниже по сравнению с контрольной группой.

Ключевые слова: мастит, эндометрит, коровы, вакцинация, *Staphylococcus aureus*, антитела, иммуногенность, молоко

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INTRODUCTION

Staphylococcal diseases are the most frequent pathologies of animals and require highly qualified, long-term and expensive therapy [1, 2]. Staphylococci can affect any tissue or organ and cause more than 100 different diseases: mastitis, endometritis, dermatitis, pneumonia, arthritis, purulent and wound infections, food poisoning, sepsis, etc. Enterotoxins, secreted by staphylococci in large quantities, have a complex effect on the animal immune system, resulting in its limited resistance. All staphylococcal enterotoxins are proteins with a relatively small molecular weight: from 26,900 to 29,600 Da. The classic staphylococcal enterotoxins comprise five main types: A, B, C, D and E (SEA-SEE), which are believed to be responsible for 95% of all staphylococcal poisoning cases. The prevention of food-borne diseases relies on the effectiveness of early diagnosis, that is, on the detection of enterotoxigenic staphylococci in milk, dairy and other food products [3, 4].

The main causative agent of staphylococcosis in cattle is *Staphylococcus aureus*, which is isolated in 69.5% of staphylococcosis cases [5]. In biological samples collected from cows demonstrating signs of mastitis and endometritis, *S. aureus* bacteria are most frequent [6, 7, 8]. According to researchers, *S. aureus* was isolated from 8.8% of milk samples from healthy cows; in 59.3 to 62.8% of cases *S. aureus* was detected in milk samples from cows with subclinical mastitis; it was also found in udder secretions of mastitic cows and pooled milk in 28.8 and 18% of samples, respectively [6, 9]. The bacteriological testing of vaginal swabs showed that one of the most frequent species responsible for postpartum endometritis was *S. aureus* (15.3% of the total tested cultures) [10].

The recovery rate from diseases caused by *S. aureus* is lower compared to other bacteria, which is explained by antimicrobial resistance and biofilm formation ability [11, 12].

In recent years, *S. aureus* isolated from animals have become increasingly resistant to antimicrobials, including through the production of the enzyme *β*-lactamase, capable of cleaving penicillins and cephalosporins [13, 14, 15, 16, 17]. Staphylococcal mastitis caused by resistant strains of *S. aureus* is reported in almost 90% of large farms and commercial holdings where antibiotics are used [3, 18, 19, 20]. Penicillin-resistant *S. aureus* are most common strains, which are identified as the first wave of resistance, and the second wave of resistance are methicillin-resistant *S. aureus*. Artemyeva O. A. et al. found that the highest resistance of *S. aureus* isolates *in vitro* was observed to erythromycin (82.5%) and fusidin (75.7%). Only seven isolated strains (6.8%) showed susceptibility to all tested antimicrobials, whereas 96 isolates were resistant to at least one of them [21]. According to I. S. Abdina et al., *S. aureus* antimicrobial resistance varied significantly, with the highest resistance reported to ampicillin (up to 57%), benzylpenicillin (up to 45%), doxycycline (up to 38%), oxacillin (up to 48%), streptomycin (up to 55%) and tetracycline (up to 45%) [22]. Other researchers established that of the 64 isolated *S. aureus* strains, 60 (93.7%) showed resistance to one or more antimicrobials. Multidrug resistance was observed in the tested *S. aureus* strains [11].

Despite the large increase in the incidence of staphylococcal infections in animals, effective drugs against them have not yet been developed. The spread of multidrug resistant *S. aureus* strains only complicates the disease control [23]. The successful treatment of animals with antibiotics brings risks of their consumption by humans. The high contagiousness of staphylococcal infections and the emergence of antimicrobial-resistant strains are the most serious problems and emerging threats for cattle industry, leading to decreased milk yields, impaired hygiene and quality of milk, increased costs for veterinary medicines and services, early culling of cows and their restricted performance. In this regard, it is necessary to search for alternative options based on staphylococci suppression, limiting the use of antimicrobials and minimizing their negative effects on the organism. One of such options is specific prevention, i.e. vaccines that provide reliable protection of animals from infectious diseases, contribute to the reduction in the use of antibiotics and prevent antimicrobial resistance of microorganisms [24, 25]. Scientists have proven the effectiveness of the vaccines containing *S. aureus* antigen against mastitis and endometritis of cows. The studies revealed that immunization of animals against mastitis has a positive effect. After 6 months after first vaccination in the breeding facility and farm, the number of mastitis cases decreased by 16.6 and 7.3%, respectively, and somatic cell counts in the milk of high yielding cows decreased by 26.5 and 10.7%. The immunization remained effective even after 12 months post vaccination [26]. “Combovac-Endomast” vaccine (Vetbiochim, Russia) decreased the number of clinical mastitis cases by 7.8 times, of subclinical mastitis by 5.4 times, and

endometritis by 3.7 times [27]. The experiments with the "Mastivac" vaccine in Laboratorios Ovejero S. A. Spain established that clinical mastitis cases decreased by 32% in the experimental group compared to the control one [9].

The aim was to study the effect of immunization against *S. aureus*, which causes mastitis and endometritis in cows.

MATERIALS AND METHODS

The study has been performed starting from 2021 in a family-operated farm in the Republic of Mordovia. Twenty adult pregnant black pied cows were used to form a test and control groups (10 animals per group). The animals were selected based on conformity principle, that is, all animals in both groups had standard parameters of the body weight, age, health status and management conditions. The vaccine was administered twice subcutaneously to the middle third of the neck of test animals. The first dose in a volume of 3 mL was administered 55–70 days before calving, the second dose of the same volume 25–30 days before the expected calving. In one immunizing dose the vaccine contains the following strains: *Escherichia coli* UR-10, *Streptococcus agalactiae* UR-7, *Streptococcus dysgalactiae* UR-16, *Streptococcus uberis* OB-5, *Streptococcus pyogenes* OB-4, *Staphylococcus aureus* OB-I4, *Klebsiella pneumoniae* K-2 (at least 3.5×10^9 CFU of each), inactivated with formalin (0.3% solution) and adsorbed on carbomer gel (10% by volume). The vaccine is intended to prevent mastitis and endometritis in cows. Control animals were injected subcutaneously with the same volume of sterile saline at the same dates.

Before the start of the experiment, milk and blood samples were collected from 20 cows with clinical mastitis, not included in any of the groups, for microbiological testing for *S. aureus* and serological testing for antibodies against *S. aureus*.

Blood was collected from animals of both groups to evaluate the antigenicity of the vaccine against *S. aureus* and milk samples for bacteriological testing. Blood sampling in the test group was performed 14–16 days after booster immunization, in the control group 14–16 days after second administration of sterile saline (placebo).

Animals were handled in compliance with the ethical standards adopted by the European Convention ETS No. 123.

The vaccine antigenicity was evaluated by the increase in the antibody titer against *S. aureus* measured by agglutination test. For the purposes of testing serum was diluted with sterile saline from 1:2 to 1:4096 and 0.5 mL of *S. aureus* OB-I4 antigen with concentration of ~5×10^8 CFU/mL was added to 0.5 mL of each serum dilution. The serum-antigen mixture was thoroughly mixed, placed in a thermostat and kept for 16–18 hours at (37 ± 1) °C, and then for another 3–4 hours at room temperature. After that, they were examined for agglutination occurrence. The results were interpreted with the agglutination viewer and a four-point visual scale was used: ++++ is 100% of cells agglutinated, complete liquid clearing; +++ means 75% of cells agglutinated, slight turbidity of the liquid; ++ means 50% of cells agglutinated, medium turbidity of the liquid; + is 25% of cells agglutinated, heavy turbidity of the liquid; – means no agglutination, homogeneous bacterial suspension.

Milk was pooled from all four udder lobes, but milk samples from different animals were tested individually, that is, they were not pooled with each other. The bacteriological testing of milk was performed during the first month of lactation after calving in accordance with the "Guidelines for the bacteriological testing of milk and udder secretion of cows"[1]. *S. aureus* was identified in accordance with GOST 30347-2016 "Milk and milk products. Methods for determination of *Staphylococcus aureus*"[2], and by mass spectrometry (MALDI-ToF)[3].

The results were statistically processed using generally accepted methods with Microsoft Office Excel 2010, Stat Plus 2009 software.

Table
***S. aureus* antibody titer in blood of cows from test and control groups**

Microorganism	Antibody titer	
	test group (10 animals), lg	control group (10 animals), lg
S. aureus	4.31	0.6
	4.01	0.3
	4.61	1.2
	4.01	0.3
	4.61	0.9
	4.01	0.6
	4.31	1.2
	4.31	0.3
	4.61	0.9
	4.61	1.2
$M \pm m$	4.34 ± 0.06	0.75 ± 0.09

RESULTS AND DISCUSSION

According to the results of serological testing, the antibody titer against *S. aureus* in the test group ranged from 4.01 to 4.61 lg, its mean value was (4.34 ± 0.06) lg. In the test group, the mean antibody titers against *S. aureus* were 5.8 times lower and were equal to (0.75 ± 0.09) lg with fluctuations from 0.3 to 1.2 lg (Table).

As the data obtained show, the immunization facilitated the increase in antibodies against *S. aureus* in cows of the test group, which confirms the high potency of the vaccine. In addition, the titer of antibodies to *S. aureus* in the blood of animals during preliminary serological testing was almost identical to the titer in the control group and was equal to (0.70 ± 0.05) lg.

Loskutova I. V. et al. also found that clinically healthy cows immunized with the Mastivac vaccine (Laboratorios Ovejero S. A., Spain) containing *S. aureus* induced

[1] Guidelines for the bacteriological testing of milk and udder secretion of cows: approved by Chief Veterinary Department under the USSR Ministry of Agriculture No. 115-69 on 30.12.1983. https://base.garant.ru/72125912

[2] GOST 30347-2016 Milk and milk products. Methods for determination of *Staphylococcus aureus*. https://docs.cntd.ru/document/1200142424

[3] Guidelines for microorganism identification using MALDI Biotyper mass spectrometer when testing food raw materials and food products (approved by the Rosselkhoznadzor Scientific Technical Commitees on 03.04.2014).

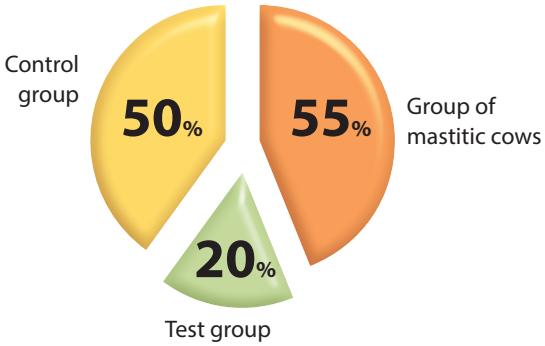

Fig. S. aureus isolation rate in the tested milk samples

antibodies to *S. aureus* enterotoxins which is evident of the vaccine ability to induce an immune response in animals against the bacterium [12]. Hadimli H. H. et al. evaluated the effectiveness of the staphylococcal vaccine on humoral immunity against *S. aureus* in vaccinated animals [28].

At the next stage, a bacteriological testing of milk for *S. aureus* was performed.

During a preliminary testing of milk from 20 cows with clinical mastitis, *S. aureus* was found in 11 samples, which amounted to 55% of the total number of samples tested. In the control group, *S. aureus* was isolated in half of the milk samples tested (the bacterium was detected in 5 out of 10 samples (50%). In the milk of cows from the test group, *S. aureus* was found in 20% of samples (2 samples), which is 2.7 and 2.5 times lower compared to the group of cows before the experiment and the control group, respectively.

The figure shows that *S. aureus* isolation rate from non-vaccinated animals (the control group and the group of cows with clinical mastitis) is almost the same; in immunized cows, it was significantly lower.

The effectiveness of *S. aureus* vaccination against mastitis was also established by other researchers, who isolated the bacterium from 73.3% of milk samples before vaccination, and 6 months after the first immunization the isolation rate decreased to 26.6%, that is, by 2.7 times [29].

CONCLUSION

It was found that double vaccination facilitated the increase in antibodies levels against *S. aureus*; the mean titers in the test group were 5.8 times higher than in the control group. In milk samples collected from vaccinated animals, the *S. aureus* isolation rate decreased by 2.7 and 2.5 times compared with groups of non-immunized animals. The results obtained show that the vaccination induces immune response in animals against *S. aureus*.

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Use of a microbial supplement containing live bacteria *Bacillus subtilis* and their metabolites in dairy farming

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ABSTRACT

The purpose of the research was to study parameters of immune status and milk yields in those cows that received a bacterial supplement containing live bacteria *Bacillus subtilis* strains B-239906 and B-249909 and their metabolites during a transition period. Animals in the experimental groups (10 animals in each) received the microbial supplement according to the following schemes: 14 days before calving (group 1), 14 days after calving (group 2), 14 days before and 14 after calving (group 3). Group 4 ($n = 10$) was a control one. On day 14 and day 28 after calving, the relative level of T-lymphocytes in blood of control cows and experimental group 2 did not significantly change compared with the level observed on day 1 after calving. While in groups 1 and 3, this indicator increased by 1.2–1.6 times throughout the whole experiment. In all cow groups, B-lymphocyte dynamics during the observation period was similar, i.e. an increase by day 14 and a decrease by day 28. The phagocytic activity of neutrophils in animals of all groups changed slightly. At the same time, the phagocytic index increased by 2.5–3.2 times throughout the experiment, which indicated an increase in nonspecific resistance of the body. Peak milk yields were recorded in cows of all experimental groups on day 90 of lactation. The maximum level (32.17 ± 3.33 kg) was observed in group 3. Within 150 days of observations, the average daily milk yields in animals of the experimental groups were: 24.50 ± 4.15 kg in group 1; 25.07 ± 4.38 kg in group 2; 25.33 ± 2.52 kg in group 3 and 22.75 ± 8.82 kg in the control group. The mass fraction of milk fat in all groups had no statistically significant differences throughout the entire observation period.

Keywords: live bacteria *Bacillus subtilis* and their metabolites, cows, cellular immunity, humoral immunity, milk yield, milk fat

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Применение композиции, содержащей живые бактерии *Bacillus subtilis* и их метаболиты, в молочном животноводстве

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РЕЗЮМЕ

Целью исследований явилось изучение показателей иммунного статуса и продуктивности у коров при применении композиции, содержащей живые бактерии *Bacillus subtilis* штаммов В-239906 и В-249909 и их метаболиты, в транзитный период. Животным опытных групп (по 10 гол. в каждой) композицию применяли: в течение 14 дней до отела (1-я группа), 14 дней после отела (2-я группа), 14 дней до и 14 после отела (3-я группа). Четвертая группа ($n = 10$) была контрольной. У коров контрольной и 2-й опытной групп на 14-й и 28-й дни после отела относительное содержание Т-лимфоцитов в крови по сравнению с уровнем в первые сутки после отела достоверно не изменялось, в то время как у особей 1-й и 3-й групп данный показатель возрастал на протяжении всего опыта в 1,2–1,6 раза. Во всех группах коров динамика относительного числа В-лимфоцитов за период наблюдений была аналогичной: увеличение к 14-му дню и сокращение к 28-му дню. Фагоцитарная активность нейтрофилов у животных всех групп изменялась незначительно.

Фагоцитарный индекс при этом возрастал в 2,5–3,2 раза на протяжении всего эксперимента, что свидетельствовало о повышении неспецифической резистентности организма. Пик молочной продуктивности регистрировали у коров всех опытных групп на 90-й день лактации. Максимальные значения (32,17 ± 3,33 кг) отмечали в 3-й группе. За 150 дней наблюдений среднесуточные удои у животных опытных групп составили: 24,50 ± 4,15 кг в 1-й; 25,07 ± 4,38 кг в 2-й; 25,33 ± 2,52 кг в 3-й и 22,75 ± 8,82 кг в контрольной. Уровень массовой доли жира в молоке у коров всех групп не имел статистически значимых различий на протяжении всего периода наблюдений.

Ключевые слова: живые бактерии *Bacillus subtilis* и их метаболиты, коровы, клеточный иммунитет, гуморальный иммунитет, молочная продуктивность, молочный жир

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INTRODUCTION

In recent years, the milk yield in Russian livestock holdings has exceeded the level of 8,000 kg of milk per year [1].

These achievements undoubtedly result from good breeding practice, appropriate conditions for realizing genetic potential of highly productive animals and introduction of physiologically and economically justified feeding schemes and control of zoohygienic parameters in animal housing [2, 3]. The progress became possible because recent observations into digestion processes (at the molecular, cellular and body levels) and biological protein synthesis had been put into practice. In addition, great attention was paid to the principles of adequate nutrition, which take into account health status of cows and their milk yields [4, 5].

When milk yield increases, vital functions often weaken: the immunity decreases and productive lifespan is reduced to 2–3 lactations. The animals are most likely removed from the herd due to metabolic disorders resulting from unbalanced diet in the pre-calving and post-calving periods [6, 7].

Products of microbiological synthesis, their development and use, are considered as a solution to the existing problems in the livestock sector of the Russian Federation. They have proved to be effective in preventing diseases, reducing animal culling and improving digestibility of feed components. Their indirect effect is associated with an increase in milk yields, improved product quality, thus, enabling to provide people with safe food [8, 9, 10].

Live *Bacillus subtilis* bacteria and their metabolites that stimulate the growth of indigenous intestinal microbiota tend to be a promising component in new supplements. During their production, it is required to ensure proper conditions to maintain long-term stability during storage of the finished products [11, 12, 13, 14, 15, 16].

The objective of the research was to study the effect of a microbial supplement containing live bacteria *B. subtilis* and their metabolites during the transition period on immunity status and milk yields in cows.

MATERIALS AND METHODS

The experiment was carried out with the support from the Department of Ecology and Non-Contagious animal pathogens of the Ural Scientific Research Veterinary Institute, a structural subdivision of the Ural Branch of the Russian Academy of Sciences. The work was done within the framework of the Basic Science Programme of the State Academies of Sciences in the field 4.2.1.5 "Development of technologies for lifetime quality management of livestock raw materials to obtain high-quality and safe food products".

The study was conducted in Holstein cows (n = 40), at the age of 2–3 lactations, kept in one of the livestock holdings of the Sverdlovsk Oblast.

Four groups, each consisting of 10 animals, were selected for the experiment according to the principle of equivalents. When forming the groups, their physiological status, weight, age, nutrition level and milk yields from the previous lactation were taken into account.

The cows were kept in one typical cattle house, in a tie stall, and received a balanced feed. In addition to the basic diet, the experimental animals were administered 5 g of a new, domestically produced supplement, containing live bacteria *B. subtilis* strains B-239906 and B-249909 (at a concentration of 10^3 CFU/g of each species) and their metabolites. They received the supplement at different times: 14 days before calving (group 1), 14 days after calving (group 2), 14 days before and 14 days after calving (group 3); group 4 was a control one.

Clinical status and behavioural responses of the animals were daily examined. Starting from day 15 after calving, milk quality was assessed using CombiFoss FT+ (FOSS, Denmark) instrument. Milk yields were measured for 150 days.

Hematological tests were done three times: on day 1, 14 and 28 after calving with blood sampling taken from the tail vein.

Blood morphological composition was analyzed in Abacus Junior Vet analyser (Diatron, Austria) using

Table 1
Hematological parameters in cows

Parameters		Red blood cells, 10^{12}/L	Hemoglobin, g/L	Thrombocytes, 10^9/L	White blood cells, 10^9/L	Lymphocytes, 10^9/L
Standard*		5.0–10	90–120	100–800	4.5–12	2.5–7.5
Control	Day 1	6.41 ± 0.50	100.80 ± 4.44	259.60 ± 179.33	8.44 ± 1.66	4.89 ± 1.22
	Day 14	6.75 ± 0.69	107.33 ± 3.51	284.00 ± 67.95	7.24 ± 0.76	4.26 ± 1.41
	Day 28	6.30 ± 0.68	99.50 ± 7.05	265.60 ± 115.70	7.54 ± 2.03	3.94 ± 1.37
Experimental group 1	Day 1	7.05 ± 0.40	111.00 ± 6.16	327.20 ± 121.91	11.44 ± 3.14	6.13 ± 1.92
	Day 14	6.87 ± 0.56	106.50 ± 8.89	426.00 ± 199.26*	9.04 ± 3.10	5.17 ± 0.97
	Day 28	6.05 ± 0.76	90.50 ± 6.36*	348.30 ± 110.90	7.05 ± 2.06*	3.65 ± 0.99*
Experimental group 2	Day 1	7.49 ± 0.10	113.43 ± 7.28	207.71 ± 86.71	10.41 ± 2.44	5.52 ± 1.32
	Day 14	7.12 ± 0.91	106.75 ± 9.78	405.25 ± 140.29*	9.86 ± 5.06	4.84 ± 0.83
	Day 28	6.74 ± 0.86	98.33 ± 10.02	310.70 ± 102.50	8.68 ± 0.70	4.37 ± 0.53
Experimental group 3	Day 1	6.67 ± 0.52	100.40 ± 10.64	301.40 ± 81.12	9.99 ± 2.15	5.26 ± 1.36
	Day 14	6.94 ± 0.60	102.00 ± 5.79	349.60 ± 85.12	7.59 ± 1.23	4.71 ± 1.27
	Day 28	6.20 ± 0.50	97.50 ± 2.38	305.60 ± 80.12	8.32 ± 0.55	4.17 ± 0.41

* differences are statistically significant at $p < 0.05$.

standard reagents (Diatron, Austria). Leukocytic formula was calculated in blood smears stained by the Romanovsky – Giemsa method (300 cells per smear) using an Olympus BX 43 microscope (Olympus, Japan). Immunological blood tests included assessment of T- and B-lymphocytes levels, T/B index, phagocytic index, phagocytic activity of neutrophils and monocytes using the method P. N. Smirnov et al. (2007)[1]. The reactions were observed in an Olympus BX 43 binocular microscope (Olympus, Japan).

The animals were manipulated in compliance with the norms and ethical principles of the European Convention ETS No. 123.

Experimental data were processed in Excel (Microsoft, USA) and Statistica 10.0 (StatSoft Inc., USA) programmes, arithmetic means and standard deviations were determined. Reliability of differences was calculated using Student's t-test ($p \leq 0.05$).

Table 2
Functional activity of neutrophils in cows

Group of animals	Parameters	Time after calving		
		Day 1	Day 14	Day 28
Control	PhA, %	37.60 ± 8.41	39.20 ± 8.41	36.00 ± 4.55
	PhI, c. u.	1.84 ± 0.19	2.31 ± 0.19	5.85 ± 0.17**
Experimental group 1	PhA, %	48.00 ± 8.37	52.60 ± 8.41	32.50 ± 0.71*
	PhI, c. u.	1.96 ± 0.22	4.38 ± 0.19*	6.25 ± 0.07**
Experimental group 2	PhA, %	42.29 ± 6.55	47.00 ± 8.41	34.00 ± 1.00
	PhI, c. u.	2.07 ± 0.37	2.62 ± 0.19	5.83 ± 0.64**
Experimental group 3	PhA, %	37.80 ± 9.76	42.80 ± 8.41	33.25 ± 7.45
	PhI, c. u.	2.36 ± 0.85	3.29 ± 0.19	5.92 ± 0.05**

PhA – phagocytic activity;
PhI – phagocytic index;
* differences are statistically significant at $p \leq 0.05$;
** differences are statistically significant at $p \leq 0.01$.

RESULTS AND DISCUSSION

The analyzed blood parameters in experimental and control groups were within the reference range (Table 1). Hemoglobin, red blood cell volume, leukocyte, lymphocyte and platelet counts on days 14 and 28 post calving insignificantly varied and did not exceed the normal range. Changes in hematological parameters suggested normalization of haemopoiesis and restoration of immunobiological reactivity during the experiment, which did not contradict the works of other researchers [17].

Dynamics of neutrophils functional activity in cows during the experiment are given in Table 2.

It was found that on day 14 after calving, phagocytic activity and phagocytic index in all groups increased slightly, if compared to day 1, thus suggesting an increase in the absorption capacity of neutrophils. By day 28, phagocytic activity returned to the level reported during the first test, while the phagocytic index continued to increase.

[1] Panel of the most informative tests for assessment of animal resistance: methodological recommendations. Compiled by. P. N. Smirnov et al. Novosibirsk; 2007. 37 p. https://elibrary.ru/qkpwdx (in Russ.)

Table 3
Indicators of cellular and humoral immunity in cows

Group of animals	Time after calving	Absolute number of lymphocytes, 10^9/L	T-lymphocytes		B-lymphocytes	
			10^9/L	%	10^9/L	%
Control	Day 1	4.89 ± 1.22	1.42 ± 0.41	36.20 ± 8.17	0.96 ± 0.42	24.80 ± 8.67
	Day 14	4.26 ± 1.41	0.65 ± 0.08*	29.20 ± 3.32	0.68 ± 0.11	30.40 ± 5.48
	Day 28	3.94 ± 1.37	1.60 ± 0.39	32.50 ± 6.61	1.21 ± 0.42	25.25 ± 10.21
Experimental group 1	Day 1	6.13 ± 1.92	1.62 ± 0.41	28.40 ± 8.02	1.08 ± 0.27	18.80 ± 2.68
	Day 14	5.17 ± 0.97	1.60 ± 0.24	30.52 ± 2.62	1.36 ± 0.17	26.00 ± 7.01
	Day 28	3.65 ± 0.99**	1.56 ± 0.69	35.00 ± 2.83*	1.09 ± 0.18	20.34 ± 5.66
Experimental group 2	Day 1	5.52 ± 1.32	2.03 ± 0.73	33.43 ± 6.73	1.31 ± 0.34	24.57 ± 8.56
	Day 14	4.84 ± 0.83	1.56 ± 0.26	32.20 ± 2.82	1.27 ± 0.11	26.24 ± 6.31
	Day 28	4.37 ± 0.53	1.64 ± 1.11	32.00 ± 18.52	0.79 ± 0.31	20.21 ± 4.51
Experimental group 3	Day 1	5.26 ± 1.36	1.26 ± 0.17	28.00 ± 4.64	0.88 ± 0.29	19.00 ± 5.92
	Day 14	4.71 ± 1.27	1.78 ± 0.42	37.82 ± 2.44*	1.20 ± 0.19	25.50 ± 5.23
	Day 28	4.17 ± 0.41	2.11 ± 1.10*	44.50 ± 15.86**	0.88 ± 0.27	18.75 ± 2.50

* differences are statistically significant at $p \leq 0.05$;
** differences are statistically significant at $p \leq 0.01$.

Thus, the parameter in cows increased by 2.5–3.2 times ($p \leq 0.01$), if compared to day 1. The obtained data can be regarded as positive and suggesting an increased body resistance to negative factors and a reduced risk of inflammatory processes [10].

The absolute number of lymphocytes in all groups decreased in different ways during the post-calving period (Table 3). The most significant decrease was observed on day 28, i. e. by 40.5% ($p \leq 0.01$) in group 1, if compared to day 1 after calving. In other groups, this parameter decreased by 19.4–20.8%, but did not exceed the reference range. Probably, these changes are associated with metabolic disorders and lack of energy after calving [4].

The relative content of T-lymphocytes in all groups on day 1 after calving ranged between 28.00 ± 4.64 and 36.20 ± 8.17%. On day 14 of observation, a 1.2-fold decrease was registered in the control group, and by day 28 it returned to the level of day 1. In animals of experimental groups 1 and 3, dynamical changes in the relative content of T-lymphocytes was opposite. Thus, throughout the whole observation period, this parameter increased by 1.2 ($p \leq 0.05$) and 1.6 ($p \leq 0.01$) times, respectively, which suggested the stimulation of cellular immunity.

The number of B-lymphocytes in experimental and control groups at the initial stage of the experiment (on day 1 after calving) ranged between 18.8 to 24.8%. During this period, a repeated pattern was revealed in all groups: an increase in B-lymphocyte synthesis by day 14 and a decrease by day 28.

Based on the above, we can assume that the dynamical changes in T- and B-lymphocytes levels in the post-calving period resulted from the indirect effect of the tested supplement and had a compensatory and restorative mechanism based on the regulation of intensity of biosynthetic processes, as was confirmed by a number of other researchers [4, 18].

Positive effect of the tested supplement was reported when assessing the milk yield. Data on average milk yield per month and milk fat mass fraction are given in Figures 1 and 2.

Analyzing the milk yield, we found a positive variation in the average daily milk yields in animals that were given the supplement containing live *B. subtilis* bacteria and their metabolites as compared to cows from the control group. The results obtained suggested the animals were able to recover better after calving and were able to withstand long physical stress due to continuous milk secretion. Thus, by the end of the 3rd month of lactation, peak milk yields were registered in cows of all experimental groups compared to the first month: month 1 – 28.70 ± 5.92 kg, month 2 – 28.94 ± 6.84 kg, month 3 – 32.17 ± 3.33 kg. The opposite situation was observed in control animals – a decrease to the level of 27.90 ± 7.25 kg.

In the following months, a regular decrease in average daily milk yields was observed in cows of all groups, but within different ranges. For five months of observations, we obtained the following average daily milk yields: Experimental group 1 – 24.50 ± 4.15 kg, Experimental group 2 – 25.07 ± 4.38 kg, Experimental group 3 – 25.33 ± 2.52 kg, control group – 22.75 ± 8.82 kg.

An important criterion for assessing milk quality is the mass fraction of milk fat (Fig. 2).

The mass fraction of milk fat in all experimental groups increased by the end of the 2nd month of observation and was: month 1 – 3.63 ± 0.28 g/100 g, month 2 – 3.62 ± 0.31 g/100 g, month 3 – 3.77 ± 0.35 g/100 g (more by 7.1; 4.9 and 4.4%, respectively). These parameters in the control group had no significant differences.

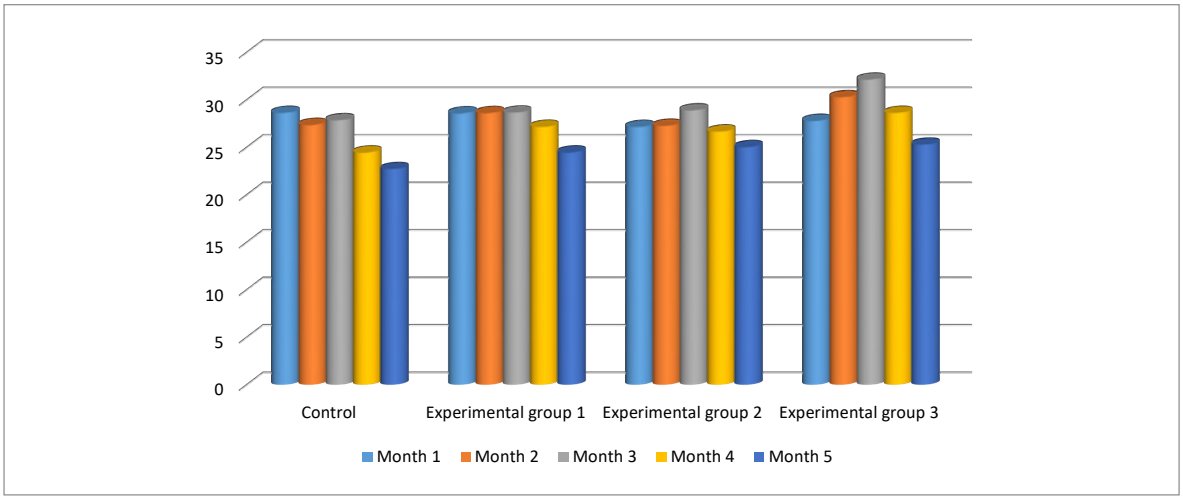

Fig. 1. Monthly dynamics of milk yields (kg)

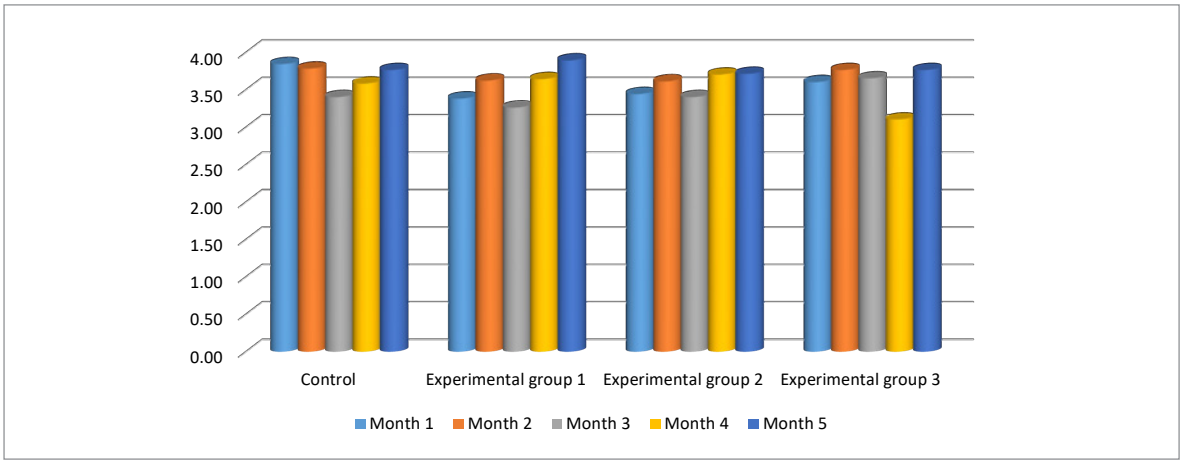

Fig. 2. Changes in the mass fraction of milk fat (g/100 g)

In experimental animals, such changes were an indirect sign of body fat mobilization into milk. A significant increase in fat level during the 2nd month of lactation and its pronounced decrease during the 3rd month may be suggestive of a lactational exhaustion in cows of group 1. In the following months, insignificant variations of the parameters were observed with a return to average values.

Thus, the most pronounced effect was registered in group 3, where the diet was supplemented in the pre-calving and post-calving periods with the tested supplement containing live bacteria *B. subtilis* and their metabolites. The use of the supplement resulted in an increase in milk quantity and quality and the observed parameters complied with the results obtained by other authors [2, 3, 19, 20].

CONCLUSION

The use of the supplement containing live bacteria *B. subtilis* strains B-239906 and B-249909 and their metabolites had a positive effect on immunohematological parameters of cows' blood. The revealed changes in immunity indicators (absolute number of lymphocytes, T- and B-lymphocytes, phagocytic activity and phagocytic index) should be regarded as a compensatory-adaptation mechanism aimed at maintaining and normalizing metabolism.

Higher average daily milk yields were reported in cows of Experimental groups 1, 2 and 3, i. e. 24.50 ± 4.15; 25.07 ± 4.38 and 25.33 ± 2.52 kg of milk, respectively, compared to control group (22.75 ± 8.82 kg).

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Metagenomic analysis of gut microbiota diversity in poultry before and after antibiotic administration

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ABSTRACT

The diversity of gut microbiota is an important ecological resource that plays a key role in maintenance of the host homeostasis. It is extremely important to preserve the existing gut microbiota diversity, which ensures its resistance to the negative effects of abiotic factors, while the study of the antibiotic role in the disturbance of microbiota diversity is a fundamental basis used not only to identify aspects responsible for microbiota-associated poultry diseases, but also to learn techniques of microbiota management. This study gives a characteristic of poultry gut microbiota diversity before and after antibiotic administration based on 16S rRNA gene sequencing analysis. *Firmicutes* and *Bacteroidota* species were predominantly detected in the chick microbiota during antibiotic administration and after its withdrawal. A significant increase in *Patescibacteria* abundance was observed on day 11 post enrofloxacin cessation. *Actinobacteriota* started appearing on day 11 after antibiotic discontinuation. An increase in *Cyanobacteria* abundance was detected on day 4 after the drug withdrawal. Taxonomic shifts in the chick microbial community structure at the class level both during the antibiotic treatment and after its withdrawal were observed. The abundance of *Clostridia* and *Bacteroidia* classes tended to decrease, while *Bacilli* class increased in its abundance, especially on day 8 after the drug withdrawal. It was found that a ten-day course of enrofloxacin treatment at the recommended doses leads to an increase in the abundance of *Bacillaceae, Gastranaerophilales, Lactobacillaceae, Bacteroidaceae, Bifidobacteriaceae* families, while the abundance of *Rikenellaceae, Erysipelatoclostridiaceae, Clostridiaceae, Ruminococcaceae* decreased and did not affect the abundance of *Lachnospiraceae* family. The revealed increase in the proportion of *Lactobacillaceae* during antibiotic treatment suggests the ability of a healthy organism to restore the microbiota balance. The results of metagenomic data bioinformatics (without truncation) showed the presence of 158 microorganism species in the chick microbiota, 38% of which were classified as nonculturable.

Keywords: microbiota, metagenome, targeted sequencing, antibiotics

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Метагеномный анализ биоразнообразия микробиома кишечника птицы до и после медикаментозной нагрузки антибиотиком

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РЕЗЮМЕ

Биологическое разнообразие кишечной микробиоты представляет собой важный экологический ресурс, который играет ключевую роль в поддержании гомеостаза организма хозяина. Исключительно важное значение имеет сохранение существующего биоразнообразия кишечной микробиоты, которое обеспечивает ее устойчивость к негативному действию абиотических факторов, а исследование роли антибиотиков в нарушении биоразнообразия микробиомов является фундаментальной основой не только для выявления аспектов возникновения микробиом-ассоциированных болезней птицы,

но и освоения методов управления микробиомами. В данном исследовании представлена характеристика биоразнообразия микробиома кишечника птицы до и после медикаментозной нагрузки антибиотиком на основе биоинформатического анализа секвенирования гена 16S рРНК. Наибольшее количество прочтений в микробиоме цыплят в период выпаивания антибиотика и после его отмены составляли типы *Firmicutes* и *Bacteroidota*. Значительное увеличение *Patescibacteria* было отмечено на 11-й день отмены энрофлоксацина. Появление *Actinobacteriota* наблюдали на 11-й день после отмены выпаивания антибиотика. Увеличение *Cyanobacteria* выявлено на 4-й день после отмены препарата. Таксономические сдвиги в микробиоме цыплят на уровне классов как в период выпаивания антибиотика, так и после его отмены проявились тенденцией к снижению относительной доли представителей классов *Clostridia* и *Bacteroidia*, а также тенденцией к увеличению доли класса *Bacilli*, особенно на 8-й день после отмены препарата. Установлено, что десятидневный курс выпаивания энрофлоксацина в рекомендуемой дозе приводит к увеличению в микробиоме доли семейств *Bacillaceae*, *Gastranaerophilales*, *Lactobacillaceae*, *Bacteroidaceae*, *Bifidobacteriaceae*, снижению относительной численности семейств *Rikenellaceae*, *Erysipelatoclostridiaceae*, *Clostridiaceae*, *Ruminococcaceae* и не влияет на колебания относительной численности семейства *Lachnospiraceae*. Выявленное увеличение доли *Lactobacillaceae* при использовании антибиотика может говорить о возможностях здорового организма восстанавливать микробиоту самостоятельно. Результаты биоинформатического анализа метагеномных данных (без отсечения) показали присутствие в микробиоме цыплят 158 видов микроорганизмов, 38% из которых были отнесены к некультивируемым.

Ключевые слова: микробиом, метагеном, таргетное секвенирование, антибиотики

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INTRODUCTION

Normal intestinal flora is a quantitative and qualitative relationship between diverse microbial populations within individual organs and systems, maintaining the biochemical, metabolic and immunological balance needed to preserve the animal health [1]. In recent years, the role of the intestinal microbiota in the disease development has been established in various animal species, including humans, dogs, pigs, cattle, poultry and fur animals [2, 3, 4, 5, 6, 7, 8]. The gastrointestinal tract of chickens is densely populated with complex microbial communities (bacteria, fungi, archaea, protozoa and viruses), dominated by bacteria [9, 10]. Historically, selective culture techniques have been used to identify and characterize the microbial diversity of avian intestines. The novelty of this work is the use of bacterial 16S ribosomal RNA (rRNA) gene sequencing to study the poultry gut microbiota diversity before and after antibiotic treatment. Modern high-performance sequencing produce rapid data about microbial communities and is a powerful tool that brought an important novel understanding of the biological and ecological role of the intestinal microbiota [11, 12, 13, 14].

The use of antibiotics in veterinary medicine can contribute to the development of antimicrobial resistance [15]. According to the criteria proposed by the World Organization for Animal Health (WOAH), antimicrobials are classified into three categories: Veterinary Critically Important Antimicrobial Agents (VCIA), Veterinary Highly Important Antimicrobial Agents (VHIA) and Veterinary Important Antimicrobial Agents (VIA). However, a specific antimicrobial drug/class can be considered critically important for the treatment of a specific disease. For a number of antimicrobials, there are no or few alternatives to treat a particular disease. Fluoroquinolones are often used in veterinary practice to treat infectious animal diseases. They are also included in the list of the WOAH critically important antimicrobials for human and animal health [16]. The study of the effect of fluoroquinolones on a healthy organism, that is, a normal microbiota, is relevant.

The aim of the work was to study the diversity of the poultry intestinal microbiota before and after antibiotic treatment using 16S rRNA gene sequencing.

MATERIALS AND METHODS

The object of the study was the chick cecal microbiota.

The studies were conducted in 90 fifteen day-old Russian white layers from one batch, hatched from SPF eggs in the Manikhino hatchery with approximately the same live weight. The chicks were randomly divided into two groups (test and control), each was placed in a separate cage (5 chicks per cage). The chickens were kept in standard laboratory animal facility conditions, they were fed a standard diet and given water *ad libitum*.

The samples of the cecal contents from the test and control chicks were tested. The samples were collected immediately after killing by cervical dislocation on days 4, 8, 11 of antibiotic treatment and on days 4, 8, 11 after the drug cessation. The test chicks were individually given 1.0 mL of an antibiotic solution at a dose of 10.0 mg/kg of live weight using a probe. The drug was administered in the morning

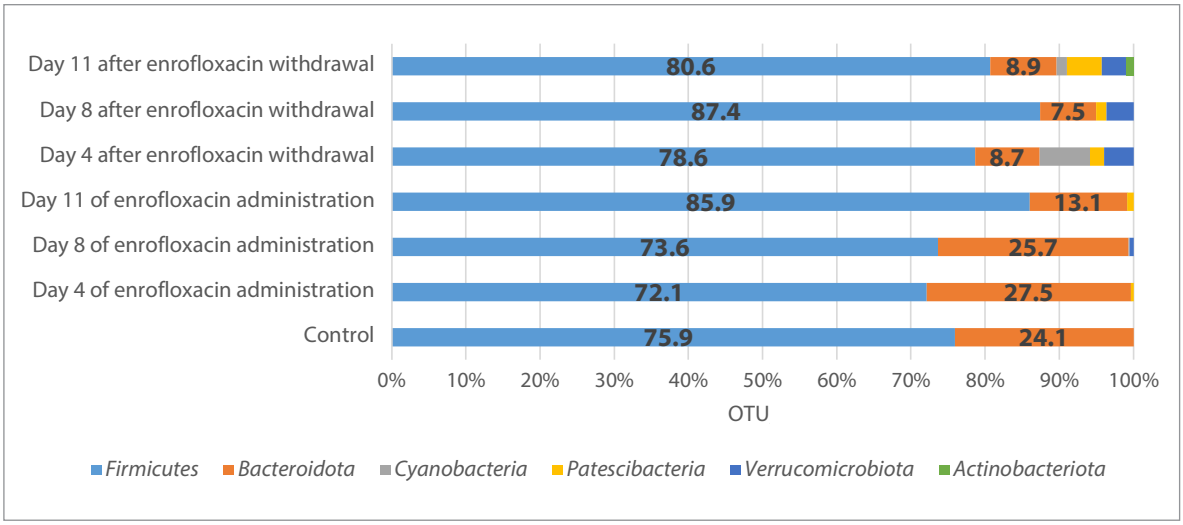

Fig. 1. Phylogenetic profile of the chick microbiome at the species level

Table 1
The cecal microflora composition at the bacterial species level demonstrated by 16S rRNA gene amplicon NGS-sequencing

Species	Control, %	Day 4 of enrofloxacin administration, %	Day 8 of enrofloxacin administration, %	Day 11 of enrofloxacin administration, %	Day 4 after enrofloxacin withdrawal, %	Day 8 after enrofloxacin withdrawal, %	Day 11 after enrofloxacin withdrawal, %
Firmicutes	75.9 ± 4.6	72.1 ± 10.6	73.6 ± 9.9	85.9 ± 2.5	78.6 ± 7.4	87.4 ± 7.2	80.6 ± 4.4
Bacteroidota	24.1 ± 4.6	27.5 ± 10.7	25.7 ± 9.5	13.1 ± 2.1	8.7 ± 1.8	7.5 ± 4.4	8.9 ± 2.2
Cyanobacteria	–	0.01 ± 0.01	0.08 ± 0.05	0.01 ± 0.01	6.8 ± 6.2	–	1.4 ± 0.7
Patescibacteria	–	0.4 ± 0.4	–	0.9 ± 0.6	1.9 ± 1.8	1.4 ± 0.7	4.7 ± 2.6
Verrucomicrobiota	–	–	0.6 ± 0.6	–	4.0 ± 2.2	3.7 ± 2.9	3.3 ± 2.8
Actinobacteriota	–	–	–	–	–	–	1.0 ± 0.5

before feeding. Control chicks in parallel to test chicks were given 1.0 mL of water for injection. The antibiotic used was enrofloxacin (Enrovec 10% for injection, Vector, Russia, batch OE011021, 1.0 mL contains 100 mg of enrofloxacin) at the rate of 10.0 mg/kg of weight.

All procedures involving animals complied with the ethical standards adopted by the European Convention ETS No. 123.

DNA was isolated from the samples using QIAamp DNA Microbiome Kit (QIAGEN, Germany) according to the manufacturer's recommendations. The quality of the extracted DNA was checked by electrophoresis in 0.8% agarose gel, as well as using the TapeStation 4200 system (Agilent Technologies, USA). The DNA concentration was measured using the Quantus fluorometer (Promega, USA). The DNA library was prepared according to the 16S Metagenomic Sequencing Library Preparation protocol using Nextera XT DNA Library Preparation Kit (Illumina, USA). MiSeq Reagent Kit v3 (Illumina, USA) was used for sequencing, which enables long read lengths (300 nucleotides).

QIIME2 software package was used to analyze the 16S rRNA sequencing data. For the initial processing of raw sequences, DADA2 package was used, which gives more reproducible and accurate results due to denoising algorithms, rather than clustering of phylotypes, in contrast to more classical approaches [17, 18]. The taxonomic affiliation of phylotypes was determined using the RDP classifier based on SILVA [19]. The data were normalised using the rarefaction algorithm in the QIIME2 software while analyzing alpha diversity according to major recommendations of the developers, and were stabilised by variation through the Deseq2 package [20] to compare the relative abundances of phylotypes in the samples. For the analysis of beta diversity, communities were compared using the construction of their dissimilarity matrix using the weighted UniFrac, unweighted UniFrac and Bray-Curtis algorithms.

Statistical processing of the results was performed by analysis of variance using Microsoft Excel 2010 software. The results are presented as the arithmetic mean (M) and the standard error of the mean (± SEM). The reliability of the differences was determined by the Student's *t*-test, the differences were considered statistically significant at $p \geq 0.95$.

RESULTS AND DISCUSSION

The bioinformatic analysis showed that the highest number of detected reads in the chick microbiome during the drug administration and after its withdrawal was produced by *Firmicutes* species. The second maximum

observed number of OTUs (operational taxonomic unit) was assigned to *Bacteroidota* species. A significant increase in *Patescibacteria* number was observed on day 11 post enrofloxacin cessation. *Actinobacteriota* started appearing on day 11 after the antibiotic discontinuation. An increase in *Cyanobacteria* abundance was detected on day 4 after the drug withdrawal (Fig. 1, Table 1).

Taxonomic shifts in the chick microbial community structure at the species level both during the antibiotic treatment and after its withdrawal were observed. The abundance of *Clostridia* and *Bacteroidia* representatives tended to decrease, while *Bacilli* species increased in its abundance, especially on day 8 after the drug withdrawal (Fig. 2, Table 2).

The change in the number of reads at the level of orders is consistent with the changes at the family level (Fig. 3, Table 3).

A ten-day course of enrofloxacin treatment at the recommended doses leads to an increase in the abundance of *Bacillaceae*, *Gastranaerophilales*, *Lactobacillaceae*, *Bacteroidaceae*, *Bifidobacteriaceae* families, while the abundance of *Rikenellaceae*, *Erysipelatoclostridiaceae*, *Clostridiaceae*, *Ruminococcaceae* decreased and did not affect the abundance of *Lachnospiraceae* family.

Of the 28 genera detected in the microbiome of test and control chicks, two genera belonging to families *Oscillospiraceae* and *Ruminococcaceae* could not be classified. The abundance of *Lactobacillus*, *Akkermansia*, *Blautia*, *Candidatus Saccharimonas* genera was significantly greater during the period of enrofloxacin administration and after its withdrawal, while *Faecalibacterium*, *Lachnoclostridium*, *Ruminococcus* abundance was reduced (Table 4, Fig. 4).

The results of metagenomic data bioinformatic analysis (without truncation) showed the presence of 158 microorganism species in the chick microbiota, 38% of which were classified as nonculturable.

The analysis of the beta diversity of the metagenomic community under study showed changes in the taxonomic diversity of microbiomes in the test and control chicks (Fig. 5A). The changes were expressed as an expansion in the taxonomic community composition within the groups (Fig. 5B).

Significant differences characterizing alpha diversity were found over time only within the groups, but not relative to each other at a specific time point. The most significant difference in alpha diversity was demonstrated for samples at point 19, corresponding to day 8 after the antibiotic cessation (Fig. 6).

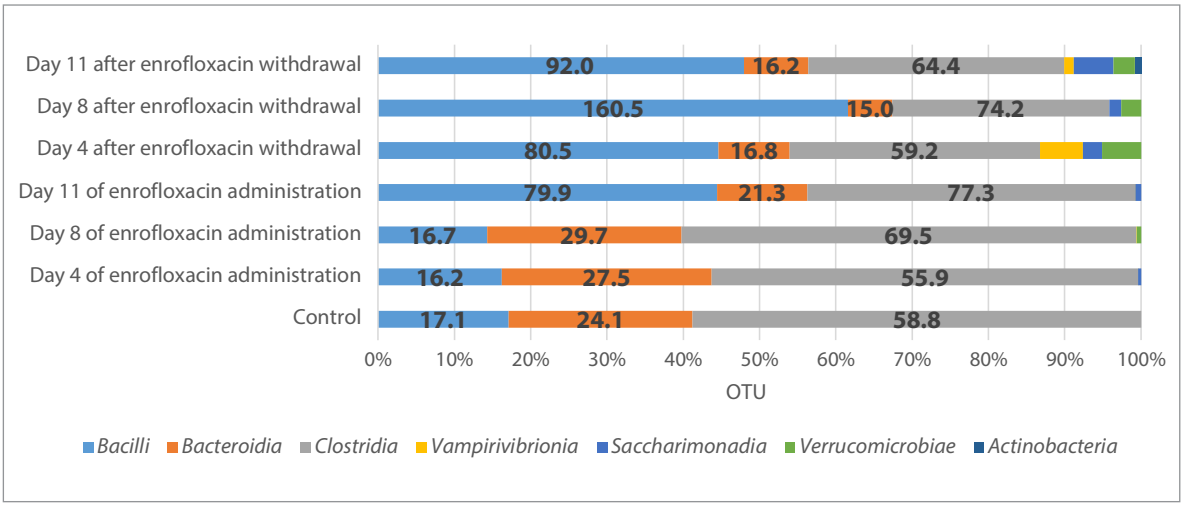

Fig. 2. Phylogenetic profile of the chick microbiome at the class level

Table 2
The cecal microflora composition at the bacterial class level demonstrated by 16S rRNA gene amplicon NGS-sequencing

Class	Control, %	Day 4 of enrofloxacin administration, %	Day 8 of enrofloxacin administration, %	Day 11 of enrofloxacin administration, %	Day 4 after enrofloxacin withdrawal, %	Day 8 after enrofloxacin withdrawal, %	Day 11 after enrofloxacin withdrawal, %
Bacilli	17.1 ± 6.3	16.2 ± 6.3	16.7 ± 5.5	79.9 ± 35.9	80.5 ± 23.6	160.5 ± 32.9	92.0 ± 17.2
Bacteroidia	24.1 ± 4.6	27.5 ± 10.7	29.7 ± 10.9	21.3 ± 2.0	16.8 ± 4.9	15.0 ± 6.6	16.2 ± 2.9
Clostridia	58.8 ± 5.2	55.9 ± 7.0	69.5 ± 11.3	77.3 ± 2.8	59.2 ± 14.1	74.2 ± 11.0	64.4 ± 7.3
Vampirivibrionia	–	0.01 ± 0.01	0.1 ± 0.06	0.02 ± 0.02	10.2 ± 8.7	–	2.4 ± 1.2
Saccharimonadia	–	0.4 ± 0.4	–	1.4 ± 1.0	4.5 ± 4.2	4.1 ± 2.0	10.0 ± 5.6
Verrucomicrobiae	–	–	0.7 ± 0.7	–	9.3 ± 5.3	6.8 ± 4.8	5.4 ± 4.4
Actinobacteria	–	–	–	–	–	–	1.6 ± 0.8

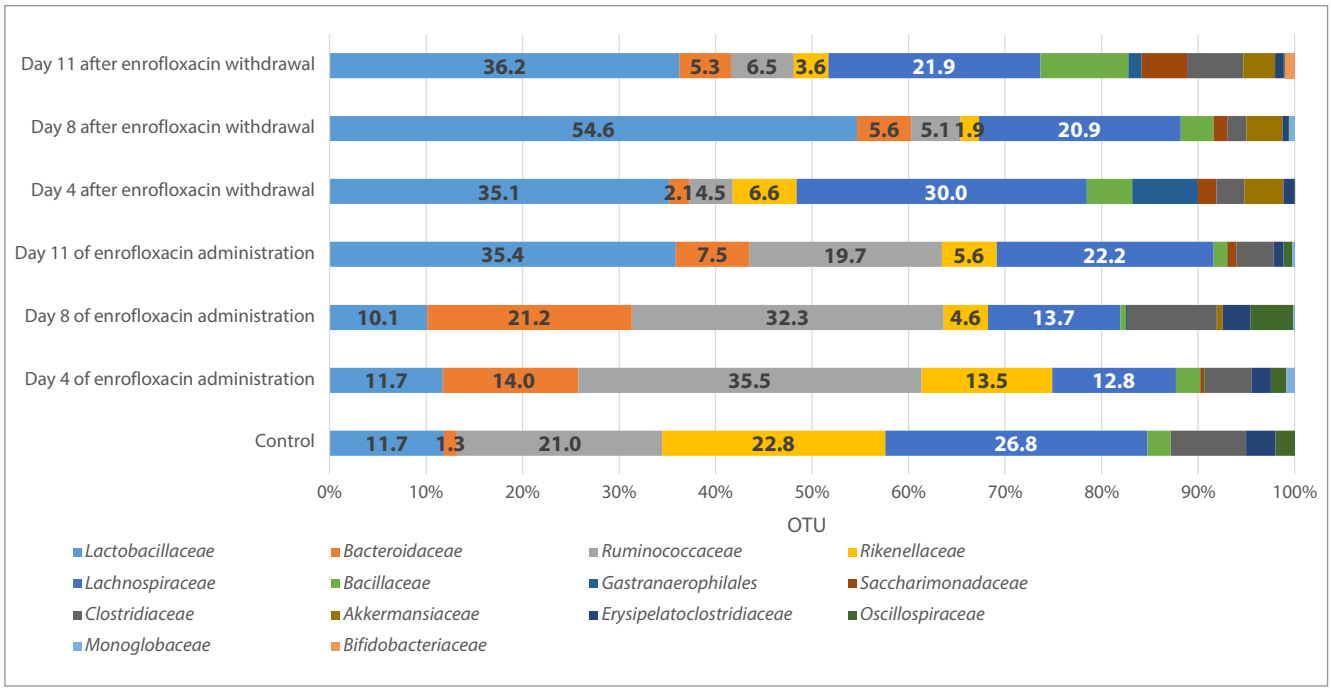

Fig. 3. Phylogenetic profile of the chick microbiome at the family level

Table 3
The cecal microflora composition at the bacterial family level demonstrated by 16S rRNA gene amplicon NGS-sequencing

Family	Control, % reed ± SEM	Day 4 of enrofloxacin administration, % reed ± SEM	Day 8 of enrofloxacin administration, % reed ± SEM	Day 11 of enrofloxacin administration, % reed ± SEM	Day 4 after enrofloxacin withdrawal, % reed ±SEM	Day 8 after enrofloxacin withdrawal, % reed ± SEM	Day 11 after enrofloxacin withdrawal, % reed ± SEM
Lactobacillaceae	11.7 ± 6.7	11.7 ± 6.3	10.1 ± 4.0	35.4 ± 8.2	35.1 ± 7.1	54.6 ± 5.4	36.2 ± 6.1
Bacteroidaceae	1.3 ± 1.1	14.0 ± 8.2	21.2 ± 8.0	7.5 ± 2.0	2.1 ± 0.6	5.6 ± 3.6	5.3 ± 0.6
Ruminococcaceae	21.0 ± 3.6	35.5 ± 7.1	32.3 ± 8.2	19.7 ± 5.2	4.5 ± 2.2	5.1 ± 1.7	6.5 ± 2.4
Rikenellaceae	22.8 ± 5.0	13.5 ± 3.1	4.6 ± 1.8	5.6 ± 1.7	6.6 ± 1.6	1.9 ± 0.9	3.6 ± 2.0
Lachnospiraceae	26.8 ± 2.7	12.8 ± 1.1	13.7 ± 3.5	22.2 ± 5.3	30.0 ± 8.8	20.9 ± 3.3	21.9 ± 3.4
Bacillaceae	2.4 ± 0.8	2.5 ± 0.8	0.6 ± 0.4	1.4 ± 0.8	4.7 ± 1.0	3.4 ± 0.6	9.1 ± 3.1
Gastranaerophilales	–	0.01 ± 0.01	0.1 ± 0.05	0.01 ± 0.01	6.8 ± 6.16	–	1.4 ± 0.7
Saccharimonadaceae	–	0.4 ± 0.4	–	0.9 ± 0.6	1.9 ± 1.8	1.4 ± 0.7	4.7 ± 2.6
Clostridiaceae	7.7 ± 2.0	4.9 ± 0.9	9.3 ± 4.9	3.8 ± 1.4	2.9 ± 2.3	2.0 ± 1.0	5.8 ± 1.9
Akkermansiaceae	–	–	0.6 ± 0.6	–	4.04 ± 2.2	3.7 ± 2.9	3.3 ± 2.8
Erysipelatoclostridiaceae	3.0 ± 0.8	2.0 ± 0.9	2.9 ± 0.9	1.0 ± 0.4	1.1 ± 0.5	0.6 ± 0.1	0.9 ± 0.4
Oscillospiraceae	2.0 ± 1.0	1.6 ± 0.5	4.4 ± 1.4	0.9 ± 0.4	0.1 ± 0.1	0.1 ± 0.07	0.1 ± 0.1
Monoglobaceae	–	0.9 ± 0.4	0.2 ± 0.1	0.3 ± 0.2	0.01 ± 0.01	0.62 ± 0.49	0.05 ± 0.05
Bifidobacteriaceae	–	–	–	–	–	–	1.0 ± 0.5

CONCLUSION

The change in the metagenomic taxonomy of the cecal microbiota of healthy chicks when using enrofloxacin antibiotic was analyzed. The most pronounced relative changes in the metagenomic taxonomy were recorded on day 8 after the start of the ten-day enrofloxacin administratopn course and on day 8 after its withdrawal.

The analysis of metagenome sequencing data revealed the presence of a significant number of nonculturable microorganisms that cannot be detected by microbiological techniques.

Table 4
The cecal microflora composition at the bacterial genus level demonstrated by 16S rRNA gene amplicon NGS-sequencing

Genus	Control, % reed ± SEM	Day 4 of enrofloxacin administration, % reed ± SEM	Day 8 of enrofloxacin administration, % reed ± SEM	Day 11 of enrofloxacin administration, % reed ± SEM	Day 4 after enrofloxacin withdrawal, % reed ± SEM	Day 8 after enrofloxacin withdrawal, % reed ± SEM	Day 11 after enrofloxacin withdrawal, % reed ± SEM
Lactobacillus	11.7 ± 6.7	11.7 ± 6.3	10.08 ± 3.97	35.39 ± 8.19	35.13 ± 7.07	54.59 ± 5.43	36.16 ± 6.08
Bacteroides	1.3 ± 1.1	14.0 ± 8.2	21.16 ± 8.0	7.54 ± 2.04	2.08 ± 0.64	5.58 ± 3.56	5.32 ± 0.61
Faecalibacterium	11.3 ± 2.8	25.4 ± 5.2	24.18 ± 7.75	9.48 ± 4.06	1.51 ± 0.44	1.02 ± 0.31	0.90 ± 0.46
Alistipes	22.8 ± 5.0	13.5 ± 3.1	4.56 ± 1.85	5.61 ± 1.7	6.59 ± 1.64	1.95 ± 0.87	3.63 ± 2.0
Subdoligranulum	1.0 ± 0.9	0.2 ± 0.1	1.75 ± 1.25	3.62 ± 2.46	2.26 ± 2.09	3.31 ± 1.85	5.37 ± 2.0
Ruminococcus	12.6 ± 1.7	3.6 ± 0.3	3.15 ± 0.56	5.29 ± 2.57	4.15 ± 2.16	2.30 ± 0.56	2.60 ± 0.64
Blautia	0.4 ± 0.2	0.3 ± 0.3	0.41 ± 0.25	1.12 ± 0.68	2.05 ± 0.68	3.51 ± 1.0	6.52 ± 1.14
Bacillus	2.4 ± 08	2.5 ± 0.8	0.60 ± 0.36	1.36 ± 0.80	4.73 ± 0.96	3.41 ± 0.65	9.09 ± 3.12
Lachnospira	9.7 ± 0.7	6.0 ± 0.8	6.50 ± 1.38	12.74 ± 3.76	15.59 ± 5.17	9.59 ± 1.5	6.25 ± 1.55
Ruminococcus	2.9 ± 0.8	2.7 ± 1.4	3.57 ± 1.28	2.66 ± 0.65	0.21 ± 0.08	0.06 ± 0.04	0.05 ± 0.05
Gastranaerophilales	0.0 ± 0.0	0.0 ± 0.0	0.08 ± 0.05	0.01 ± 0.01	6.82 ± 6.16	0.00 ± 0.0	1.43 ± 0.74
Candidatus saccharimonas	0.0 ± 0.0	0.4 ± 0.4	0.00 ± 0.0	0.90 ± 0.62	1.89 ± 1.77	1.41 ± 0.66	4.70 ± 2.56
Clostridium	7.7 ± 2.0	4.9 ± 0.9	9.29 ± 4.89	3.79 ± 1.38	2.94 ± 2.28	2.03 ± 0.95	5.83 ± 1.95
Akkermansia	0.0 ± 0.0	0.0 ± 0.0	0.62 ± 0.62	0.00 ± 0.0	4.04 ± 2.2	3.65 ± 2.93	3.30 ± 2.81
Erysipelatoclostridium	3.0 ± 0.8	2.0 ± 0.9	2.88 ± 0.94	0.96 ± 0.37	1.06 ± 0.46	0.59 ± 0.1	0.93 ± 0.37
Lachnoclostridium	2.1 ± 0.2	2.0 ± 0.3	2.29 ± 1.51	2.08 ± 0.71	1.22 ± 0.43	1.23 ± 0.34	0.81 ± 0.18
Sellimonas	1.8 ± 0.5	0.8 ± 0.3	1.21 ± 0.33	0.77 ± 0.29	3.32 ± 1.23	2.37 ± 1.18	1.75 ± 0.36
Ruminococcus	1.1 ± 0.5	1.0 ± 0.6	0.58 ± 0.24	2.45 ± 0.95	0.25 ± 0.13	0.00 ± 0.0	0.05 ± 0.05
Eubacterium hallii group	0.2 ± 0.1	0.0 ± 0.0	0.17 ± 0.07	0.19 ± 0.07	3.52 ± 1.48	1.45 ± 0.47	0.69 ± 0.27
CHKCI001	0.0 ± 0.0	0.0 ± 0.0	0.02 ± 0.01	0.02 ± 0.02	0.20 ± 0.1	0.48 ± 0.27	3.28 ± 1.89
Unclassified *Oscillospiraceae*	1.0 ± 0.5	0.7 ± 0.2	2.58 ± 0.99	0.92 ± 0.45	0.13 ± 0.06	0.08 ± 0.04	0.07 ± 0.05
Unclassified *Ruminococcaceae*	2.8 ± 0.8	3.8 ± 1.0	0.38 ± 0.24	0.00 ± 0.0	0.07 ± 0.07	0.36 ± 0.3	0.03 ± 0.03
Eubacterium coprostanoligenes group	1.0 ± 0.6	0.3 ± 0.3	0.09 ± 0.06	1.35 ± 0.99	0.00 ± 0.0	0.01 ± 0.01	0.05 ± 0.03
DTU089	0.8 ± 0.2	1.3 ± 0.2	0.39 ± 0.13	1.01 ± 0.34	0.24 ± 0.09	0.38 ± 0.17	0.15 ± 0.07
Unclassified *Ruminococcaceae*	1.3 ± 0.6	1.1 ± 0.5	1.49 ± 0.91	0.44 ± 0.32	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0
Monoglobus	0.3 ± 0.2	0.9 ± 0.4	0.17 ± 0.11	0.32 ± 0.25	0.01 ± 0.01	0.62 ± 0.49	0.05 ± 0.05
UCG-005	1.0 ± 0.5	1.0 ± 0.3	1.81 ± 1.32	0.00 ± 0.0	0.00 ± 0.0	0.03 ± 0.03	0.00 ± 0.0
Bifidobacterium	0.0 ± 0.0	0.0 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	1.00 ± 0.51

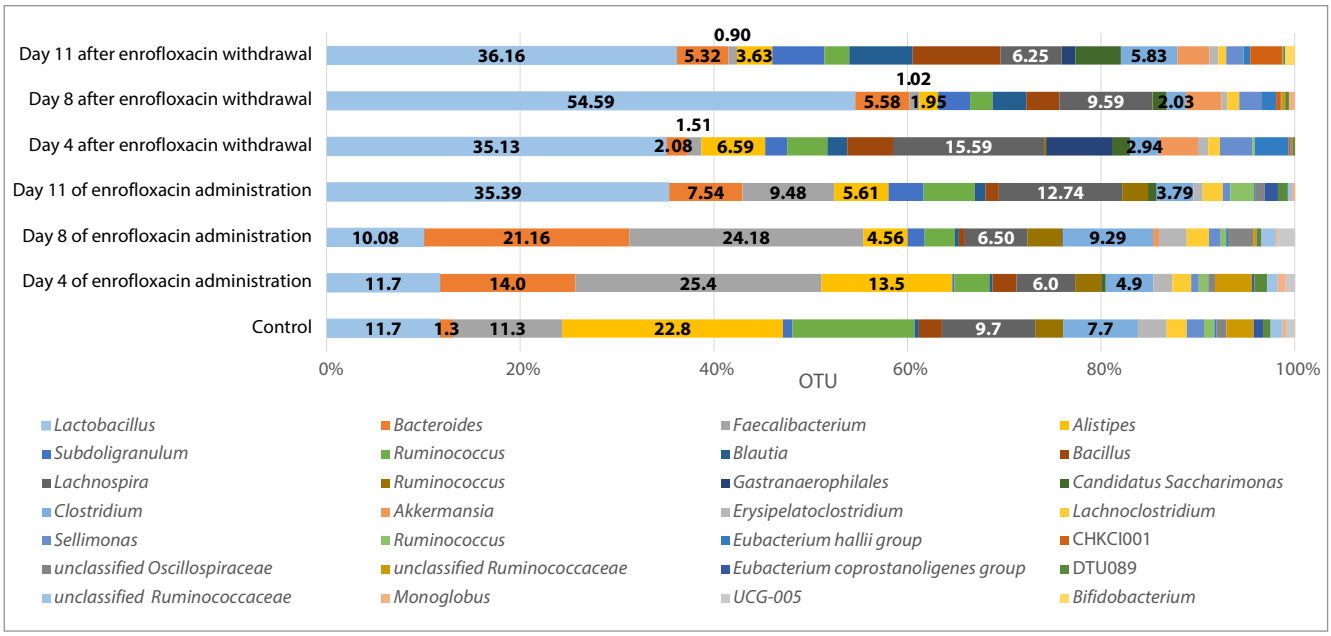

Fig. 4. Phylogenetic profile of the chick microbiome at the genus level

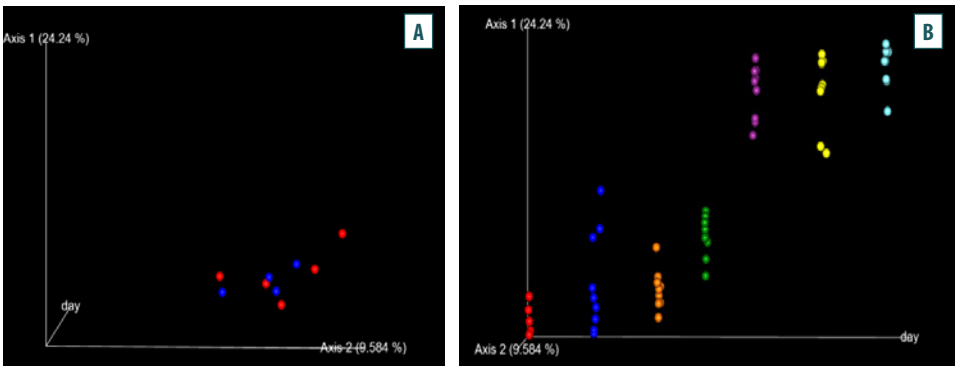

Fig. 5. A – changes in the beta diversity of the metagenomic community in the test (red) and control (blue) groups; B – changes in the beta diversity of the metagenomic community within the groups (red – control, blue – test, orange – day 4 of administration, green – day 8 of administration, purple – day 11 of administration)

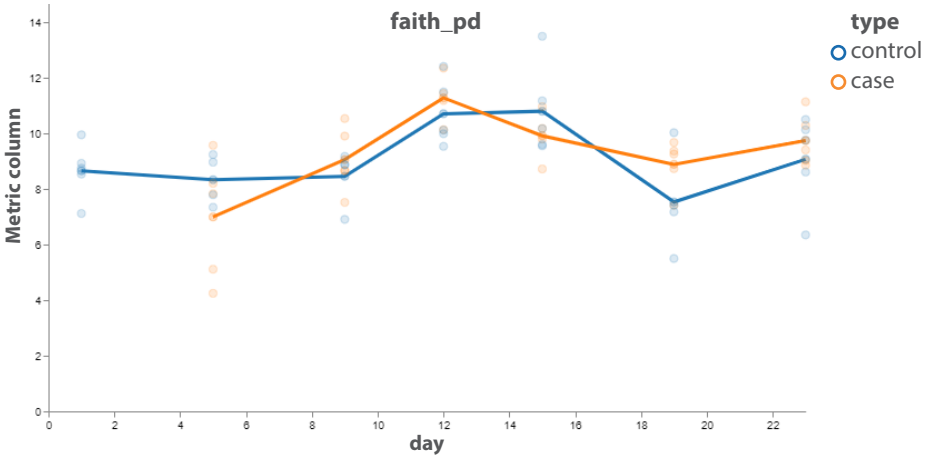

Fig. 6. Changes in the alpha diversity of the metagenomic community (blue – control, orange – test)

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Change in hepatocyte nuclear-cytoplasmic ratio at nontuberculosis mycobacteria infection against the background of immunomodulator action

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ABSTRACT

The study was targeted at the examination of the effect of the specific immunomodulator KIM-M2 on the morphostructure of liver cells of guinea pigs infected with nontuberculosis mycobacteria. The research was carried out in 15 guinea pigs selected by gender at the Diagnostic Research and Biotechnology Laboratory of the Department of Veterinary Medicine of the Omsk Agrarian Scientific Center. All animals were kept in a specialized animal keeping facilities according to standard housing and feeding regime. The experimental animals were subdivided into three groups of five animals in each: group 1 – challenge group (*Mycobacterium scrofulaceum*), group 2 – experimental group (*Mycobacterium scrofulaceum* and KIM-M2), group 3 – pure control group (saline solution). On day 30 after the start of the experiment, the animals were removed from the experiment, liver biopsy samples were collected and histologic specimens were prepared according to the classical method. During the experiment, it was found that KIM-M2 had a regenerative effect on the liver tissue of the guinea pigs infected with nontuberculosis mycobacteria, which was associated with 1.5-fold increase in the number of mononuclear hepatocytes, 3-fold increase in binuclear cells and 4.3-fold decrease in anucleate hepatocytes thus indicating the manifestation of compensatory reactions in the organ and increase in the depth of regenerative processes. As for animals in group 1; 1.8- and 1.3-fold increase in the area of the nucleus and cytoplasm as compared with the individuals in group 2, and 2.7- and 2-fold increase as compared with the animals in the control group, respectively, indicated the launch of the accumulation mechanisms of the potential reparative reserves and increase in their depth in the liver tissues.

Keywords: nontuberculosis mycobacteria, *Mycobacterium scrofulaceum*, guinea pig, liver, hepatocytes, immunomodulator

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Изменение ядерно-цитоплазматического соотношения гепатоцитов при заражении нетуберкулезными микобактериями на фоне действия иммуномодулятора

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РЕЗЮМЕ

Целью исследования явилось изучение влияния специфического иммуномодулятора КИМ-М2 на морфоструктуру клеток печени морских свинок, зараженных нетуберкулезными микобактериями. Работа проведена в лаборатории диагностических исследований и биотехнологии отдела ветеринарии ФГБНУ «Омский аграрный научный центр» на поголовье из 15 морских свинок, отобранных по гендерному признаку. Все животные находились в условиях специализированного вивария со стандартным режимом содержания и кормления. Опытных животных разделили на 3 группы по 5 гол. в каждой: 1-я – контроль заражения (*Mycobacterium scrofulaceum*), 2-я – экспериментальная (*Mycobacterium scrofulaceum* и КИМ-М2), 3-я – чистый контроль (физиологический раствор). На 30-е сут после начала эксперимента животных выводили из опыта, отбирали биоптаты печени и готовили гистологические препараты по классической методике. В ходе эксперимента установлено, что КИМ-М2 оказывает регенеративное действие на печеночную ткань зараженных нетуберкулезными микобактериями морских свинок, обусловленное увеличением в 1,5 раза количества одноядерных гепатоцитов, увеличением в 3 раза двухъядерных клеток и уменьшением в 4,3 раза безъядерных гепатоцитов, что указывает на проявление компенсаторных реакций в органе и увеличение глубины регенеративных процессов. У животных 1-й группы увеличение площади ядра и цитоплазмы в 1,8 и 1,3 раза в сравнении с особями 2-й группы

и увеличение соответственно в 2,7 и 2 раза по сравнению с животными из контрольной группы свидетельствует о запуске механизмов накопления потенциальных репаративных резервов и увеличении их глубины в тканях печени.

Ключевые слова: нетуберкулезные микобактерии, *Mycobacterium scrofulaceum*, морская свинка, печень, гепатоциты, иммуномодулятор

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INTRODUCTION

Currently, the role of non-tuberculosis mycobacteria has significantly increased globally. Mycobacterioses cause notable economic damage to animal production holdings, which is associated with a decreased performance, as well as with early culling of animals and their emergency slaughter [1, 2, 3]. High financial costs and lack of effective antiepidemic measures are a threat not only to the holding where mycobacteriosis has been detected, but also to the whole country [4, 5, 6, 7]. This issue has not been studied in depth enough and requires increased attention of the scientific and industrial organizations [8, 9, 10].

Scientists from Russia and many foreign countries have proved that nontuberculosis mycobacteria have not only sensitizing capacities, but they can also localize inducing specific changes in the animal body and cross-immune reactions to the PPD-tuberculin administration [11, 12, 13].

Toxins released by mycobacteria during their vital activity in the macroorganism affect the enzymatic activity of the liver, thus causing pathological changes in the liver tissue, which leads to a decrease in the level of the blood-bile barrier [14, 15, 16, 17].

One of the main functions of the liver is transformation of carbohydrates into glycogen, which is the most important energy resource of the body as a whole [18, 19]. Moreover, stellate reticulum endotheliocytes have a phagocytic effect that neutralizes the accumulation and transport of toxic substances in the animal's body [10, 20, 21].

According to the scientific data of many authors, one of the more effective methods of infectious pathogen control involves the use of immunoprophylaxis drugs [2, 22]. Currently, many modern immunomodulators do not demonstrate a sufficiently high ability to stimulate the effectiveness of the immune response to the infectious agents and their toxins in the organism.

Development of modern immunocorrecting agents and their use makes it possible to increase the body's resistance to the infectious pathogens. An increase in the immune response with the help of a specific immunomodulator enhances the regenerative properties of the cells, tissue and organ as a whole [23, 24, 25].

In view of the above material, the goal was set to study the effect of a specific immunomodulator on liver tissue under the laboratory infection with nontuberculosis mycobacteria.

MATERIALS AND METHODS

The research was carried out in a specialized animal keeping facility. Fifteen adult outbred guinea pigs of tortoiseshell color were used in the study. The animals demonstrated negative PPD test results.

A specific complex immunomodulator KIM-M2 was produced by cultivating BCG vaccine strain in a liquid synthetic Sauton medium, then the grown bacterial mass was destructed with an ultrasonic disperser UZDN-1 (Russia) and the resulting suspension was centrifuged at 15,000 rpm. The protein amount was determined in the collected supernatant after its incubation with formalin using bromophenol blue. Hereafter, the protein concentration was brought up to 1 mg/mL with saline solution. The resulting BCG antigen complex was conjugated with polyvinylpyrrolidone (PVP) and polyethylene glycol (PEG) [26].

The designed KIM-M2 series contained 320 mg of PVP and 80 mg of PEG polyions per 1 mg/mL of the protein. A 200 mL sample of the drug was prepared.

The animals were subdivided into three groups of five animals in each. The animals in group 1 (infection control) and group 2 (experimental) were inoculated with *Mycobacterium scrofulaceum* into the groin area at a dose of 0.001 mg/mL. In 2 weeks, the animals in the experimental group were subcutaneously injected with KIM-M2 immunomodulator into the inner thigh at a dose of 500 mg/mL of the protein. The animals in group 3 (pure control) were injected with a sterile 0.9% saline solution. The microorganisms of pathogenicity groups III–IV were handled in accordance with the sanitary rules and regulations (SanPiN 3.3686-21[1]). The specific immunomodulator is based on the antigenic complex of BCG vaccine strain. On day 30 after the start of the experiment, the animals were euthanized under ether anesthesia and bled. Pieces

[1] https://docs.cntd.ru/document/573660140?ysclid=lzck1dxyyc979388926 (in Russ.)

Table 1
Hepatocyte ratio in liver tissues of the experimental animals

Indicator	Group 1	Group 2	Group 3
Number of mononucleate hepatocytes	41.8%	63.5%	74.5%
Number of binucleate hepatocytes	8.3%	25.0%	18.0%
Number of anucleate hepatocytes	49.9%	11.5%	7.5%

of liver were extracted and fixed in 10% neutral formalin for further work. Further preparation was carried out at STP-120 automatic tissue processor (carousel type; Germany), paraffin blocks were embedded using EC 350 embedding center (Germany). Serial semi-thin sections (5–7 μm) were made using HM-340E microtome (Germany). Histological sections were stained with hematoxylin and eosin according to the generally accepted routine technique.

The work was carried out in compliance with the international principles set out in the Declaration of Helsinki for animals, Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes, as well as in accordance with the Guidelines for regulating the use of experimental animals.

Computer morphometry and digital images of micropreparations were performed in 10 fields using Axio Imager A1 (Zeiss, Germany) light microscope (ocular lens 10×, objective 40×), the area of hepatocytes and their nuclei was measured in micrometers (μm^2) using a software package and the AxioVision version 4.8 archiving system.

Statistical processing of the digital data was carried out using Microsoft Office 2010 and involved determination of the arithmetic averages (*M*) and calculation of the errors of arithmetic averages (*m*). The significance was determined by the Student's *t*-test and differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

The results of morphological examination demonstrated that in animals of group 1, the liver was visually enlarged and blood-filled, the edges did not converge along the incision, the organ was of loose consistency, dark brown color with large spilled gray lesions, the capsule was edematous and thickened. Beam structure was preserved. The hepatocytes were partially rounded. A large number of non-nuclear hepatocytes were observed. In 50% of the cases, nuclear karyopycnosis was reported, the cell wall was deformed, the cytoplasm was vacuolated and infiltrated into the intercellular space. Kupfer cells were not differentiated due to extensive overgrowth of loose connective tissue. The subendothelial space was expanded.

In KIM-M2 immunized animals in the experimental group, the liver was slightly enlarged, red in color, the capsule was within normal size, the liver lobules were clearly visible, the beam-radial structure was preserved. The centrilobular veins were varicosed. Small single focal hemorrhages were visible on the periphery of the hepatic lobes. Necrotic and dystrophic foci were not detected. There was a moderate connective tissue growth around the triads. The hepatocytes were identified, cell structure was determined, cytoplasm did not infiltrate into the intercellular space, karyopycnotic changes in the nucleus were not detected. Kupfer cells located in the periportal zone of the hepatic lobules were discernible in the sinusoids. No pathological lesions were detected in the intact animals.

The histological test results demonstrated that in the KIM-M2 immunized guinea pigs, the number of mononuclear hepatocytes was 63.5% of the total number of hepatocytes, which was 21.7% higher than in the infected control animals, and 11.0% less than in pure control animals. The proportion of binucleated hepatocytes from the total number of cells was 25.0%, which was 16.7% higher than in case of infection without the use of KIM-M2, and 7.0% higher than in the healthy animals. The number of anucleate hepatocytes was 11.5%, which was 38.4% less compared to infection controls and 4.0% higher compared to pure controls (Table 1).

Based on the data obtained, it can be seen that the specific immunomodulator of microbial origin KIM-M2 enhances the immune response owing to a significant increase in the number of binucleate hepatocytes during intensive mitotic division in animals of the experimental group. The number of mononucleate hepatocytes was lower in comparison with the pure controls, which can be explained by the abundant formation of binucleate hepatocytes. The number of anucleate hepatocytes increased insignificantly.

According to the morphometric study results, the average total area of mononucleate hepatocytes in animals in group 2 was established, which was 2,721.10 μm^2, which was 53.6% more than in the intact guinea pigs, and 22.8% less than in the infected animals in group 1. The average core area of mononucleated hepatocytes in animals inoculated with KIM-M2 was 289.83 μm^2, which was 47.6% more than in the intact guinea pigs and 45.0% less than in the animals in the infection control group. In animals in group 1, the average total area of mononucleate hepatocytes was 3,526.94 μm^2, the average core area of mononucleate hepatocytes was 526.76 μm^2. In intact animals, the average total area of mononucleate hepatocytes was 1,772.14 μm^2, the average core area of mononucleate hepatocytes was 196.35 μm^2 (Table 2).

Based on the results obtained, it can be concluded that the animals in group 2 accumulated potential restorative reserves and polyploidization reserves when exposed to the specific immunomodulator KIM-M2. In guinea pigs from the infection control group, a disturbed hepatocyte mitotic division was observed and the liver destruction process was reported, which slows down the recovery process.

According to the results of calculating the area of liver cells and their nuclei, the intracellular regeneration

Table 2
Morphometric characteristics of the hepatocytes

Groups	Hepatocyte cytoplasm area, μm^2 ($M \pm m$)	Area of hepatocyte nuclei, μm^2 ($M \pm m$)	Nuclear-cytoplasmic ratio
1	3,526.94 ± 243.200*	526.76 ± 28.147*	14.9
2	2,721.10 ± 44.757*	289.83 ± 15.474*	10.7
3	1,772.14 ± 45.124	196.35 ± 16.489	11.1

* differences are statistically significant as compared to group 3 (control), with $p \leq 0.01$

of the organ was determined by calculating the group nuclear-cytoplasmic ratio, which makes it possible to determine the level of metabolism and compensatory reactions in the body of laboratory animals.

CONCLUSION

Thus, in the animal experiment, it was established that a specific immunomodulator of microbial origin triggers and enhances the process of the liver cellular and intracellular regeneration, which stimulates the body to resist the mycobacterium toxins. In guinea pigs infected with nontuberculous mycobacteria, the destructive processes developed when KIM-M2 was not used, and the mechanism of liver tissue repair was underdeveloped.

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Serum biochemical profile in case of cardiorenal syndrome in cats with hypertrophic cardiomyopathy

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ABSTRACT

Veterinary specialists have lately observed comorbidity of heart and kidney diseases known as cardiorenal syndrome. Cardiorenal syndrome is typically defined as dysfunction of a primarily intact kidney under the influence of a complex of pathogenetic damaging factors that arise against the background of an underlying cardiac disease. The purpose of the work is to study the biochemical profile of blood serum in cats with cardiorenal syndrome arising against the background of hypertrophic cardiomyopathy. The studies were carried out on 24 physiologically healthy (control) cats, 24 diseased cats with uncomplicated forms of pathology (first group) and 25 cats with hypertrophic cardiomyopathy complicated by cardiorenal syndrome (second group). It was shown that cardiorenal syndrome may occur as a complication of hypertrophic cardiomyopathy in animals. Biochemical tests in animals with feline cardiorenal syndrome verified cytolysis of cardiomyocytes (increased serum activity of lactate dehydrogenase by 2.69 times, creatine phosphokinase by 2.02 times, increased serum concentration of cardiac troponin by 5.20 times as compared to healthy animals), azotemia (increased concentration in serum creatinine by 2.72 times, urea by 2.94 times, symmetric dimethylarginine by 2.60 times and cystatin C by 1.90 times as compared to healthy animals), enhanced ketogenesis, systemic inflammatory process (increased serum concentration of C-reactive protein by 1.55 times as compared to healthy animals), hypercholesterolemia, oxidative stress (decrease in serum activity of superoxide dismutase by 1.63 times, catalase by 4.67 times and glutathione peroxidase by 1.71 times, increase in the concentration of malondialdehyde by 1.79 times, ceruloplasmin by 2.50 times and diene conjugates by 1.85 times as compared to healthy animals), electrolyte imbalance in the form of hyperkalemia, hyponatremia, hyperphosphatemia and hypomagnesemia. Biochemical indicators such as serum concentrations of creatinine, troponin I, cystatin C, symmetric dimethylarginine and C-reactive protein can be considered reliable diagnostic markers for the presence of cardiorenal syndrome.

Keywords: cardiorenal syndrome, pathogenesis, biochemistry, pathochemistry, cats, hypertrophic cardiomyopathy

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Биохимическая картина сыворотки крови при кардиоренальном синдроме у больных гипертрофической кардиомиопатией кошек

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РЕЗЮМЕ

В последнее время ветеринарными специалистами отмечается коморбидное течение заболеваний сердца и почек – кардиоренальный синдром. Обычно кардиоренальный синдром определяется как дисфункция первично интактной почки под воздействием комплекса патогенетических повреждающих факторов, возникающих на фоне основного кардиозаболевания. Цель работы – изучить биохимический профиль сыворотки крови

у кошек при кардиоренальном синдроме, возникшем на фоне гипертрофической кардиомиопатии. Исследования проводили на 24 физиологически здоровых (контроль), 24 больных с неосложненными формами патологии (I группа) и 25 больных гипертрофической кардиомиопатией, осложненной кардиоренальным синдромом (II группа), кошках. Показано, что кардиоренальный синдром у животных может возникать как осложнение гипертрофической кардиомиопатии. Биохимическими исследованиями у больных кардиоренальным синдромом кошек верифицированы: цитолиз кардиомиоцитов (повышение сывороточной активности лактатдегидрогеназы в 2,69 раза, креатинфосфокиназы в 2,02 раза, увеличение сывороточной концентрации сердечного тропонина в 5,20 раза по сравнению со здоровыми животными), азотемия (повышение концентрации в сыворотке крови креатинина в 2,72 раза, мочевины в 2,94 раза, симметричного диметиларгинина в 2,60 раза и цистатина С в 1,90 раза по сравнению со здоровыми животными), усиленный кетогенез, системный воспалительный процесс (повышение сывороточной концентрации С-реактивного белка в 1,55 раза по сравнению со здоровыми животными), гиперхолестеринемия, оксидативный стресс (снижение сывороточной активности супероксиддисмутазы в 1,63 раза, каталазы в 4,67 раза и глутатионпероксидазы в 1,71 раза, повышение концентрации малонового диальдегида в 1,79 раза, церулоплазмина в 2,50 раза и диеновых конъюгатов в 1,85 раза по сравнению со здоровыми животными), электролитный дисбаланс в виде гиперкалиемии, гипонатриемии, гиперфосфатемии и гипомагниемии. Надежными диагностическими маркерами наличия кардиоренального синдрома можно считать такие биохимические показатели, как концентрация в сыворотке крови креатинина, тропонина I, цистатина С, симметричного диметиларгинина и С-реактивного белка.

Ключевые слова: кардиоренальный синдром, патогенез, биохимия, патохимия, кошки, гипертрофическая кардиомиопатия

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INTRODUCTION

Circulatory and excretory organs are morphologically and functionally closely related [1, 2, 3, 4, 5]. Combined heart and kidney dysfunction has often been noted by veterinary practitioners as a fundamentally new supranosological concept which is referred to as cardiorenal syndrome [6, 7, 8, 9]. It should be mentioned that the term "cardiorenal syndrome" is defined as a secondary kidney disorder occurring in the setting of the underlying heart failure [9]. Renocardial syndrome with cardiac dysfunction occurring against the background of the chronic kidney disease has also been described in the literature [6]. At the current stage of veterinary science development, many aspects of clinical manifestation, pathophysiology, methods of early diagnosis and highly effective treatment of cardiorenal syndrome in animals remain understudied. Generally, clinical symptoms appear in the late stages of pathology development [3]. Therefore, working out effective ways of predicting the cardiorenal syndrome development in pedigree animals is of particular relevance.

Hypertrophic cardiomyopathy (HCM) in domestic cats is frequently reported by veterinary practitioners. The risk of cardiorenal complications may increase in the setting of decompensated left ventricular heart failure in cats with HCM [10, 11, 12]. It should be stated that the basic pathobiochemical factors underlying the development and progression of cardiorenal syndrome in cats with HCM are not described in the scientific literature. Obviously, there is a high clinical demand for conducting large-scale studies on improvement of diagnostic and therapeutic methods, as well as giving a pathogenetic rationale for the use of nephroprotectors and cardioprotectors in cats with HCM.

The aim of the work was to study the serum biochemical profile in cats with cardiorenal syndrome occurring in the setting of HCM.

MATERIALS AND METHODS

The study was conducted in the Department of Veterinary Medicine of the Peoples' Friendship University of Russia named after Patrice Lumumba and on the basis of veterinary clinics in Moscow and the Moscow Oblast. Physiologically healthy (n = 24, control group) cats with HCM without cardiorenal complications (n = 24, group I), and animals with HCM complicated by cardiorenal syndrome (n = 25, group II) were used in the experiment.

The HCM diagnosis was verified comprehensively. Arterial tonometry was performed using petMAP graphic II device (Cardio Command, USA) according to the standard method [13]. Echocardiography and dopplerography were performed using Mindray DP-60 equipment (China) [14]. Electrocardiographic tests were performed using MIDAS-EK1T-04 device (Russia) [15]. A PCE-90Vet haemanalyser (High Technology Inc., USA) [16] and a SpotChem EZ SP-4430 biochemical analyzer (ARKRAY Factory Inc., Japan) [17] were also used. The level of azotemia (serum creatinine ≥ 200 μmol/L) was considered a reliable criterion for the presence of cardiorenal syndrome.

Blood was collected from the forearm saphenous vein of cats on an empty stomach in the morning hours and placed into vacuum tubes containing blood coagulation activator. Sodium, potassium, ionised calcium, phosphorus, magnesium, glucose, urea, creatinine, C-reactive protein, total protein, albumin, as well as serum activity of aspartate transaminase, alanine transaminase, creatine

phosphokinase, lactate dehydrogenase in feline sera were determined according to generally accepted methods. The concentrations of ketone bodies in blood were estimated using a FreeStyle Optium Xceed ketometer (Abbott Diabetes Care Ltd, UK) [18]. The level of symmetric dimethylarginine and cystatin C in serum was tested with enzyme-linked immunosorbent assay. The functional condition of cardiomyocyte membranes was assessed by serum concentration of troponin I [11]. The intensity of lipid peroxidation processes and antioxidant system in blood serum of cats with cardiorenal syndrome was assessed using test kits of Randox Laboratories Ltd. (UK) according to the manufacturer's instructions on a spectrophotometer UN2CO-WFT2100 (China).

Mann – Whitney and Kruskal – Wallis methods were used to statistically analyze the obtained numerical data in STATISTICA 7.0 [10]. The median (Me) and interquartile range (IQ) values were determined.

RESULTS AND DISCUSSION

The Kruskal – Wallis test performed for cats with feline cardiorenal syndrome showed high values of the H criterion and a high level of statistical significance in terms of the following biochemical parameters typical of such a phenomenon as cytolysis: serum activity of asparagine and alanine aminotransferases, lactate dehydrogenase, creatine phosphokinase, troponin I concentration (Table 1).

Thus, there was a statistically significant increase in serum activity of alanine aminotransferase (1.17-fold; $p < 0.001$), asparagine aminotransferase (1.95-fold; $p < 0.001$), lactate dehydrogenase (1.51-fold; $p < 0.001$), creatine phosphokinase (1.61-fold; $p < 0.001$) and troponin I concentration (4.00-fold; $p < 0.001$) in cats with non-complicated HCM forms (group I) as compared with healthy cats. In the sera of cats with HCM complicated by cardiorenal syndrome (group II), as compared with healthy cats, there was a statistically significant increase in activity of alanine aminotransferase (1.23-fold; $p < 0.001$), asparagine aminotransferase (2.83-fold; $p < 0.001$), lactate dehydrogenase (2.69-fold; $p < 0.001$), creatine phosphokinase (2.02-fold; $p < 0.001$) and troponin I concentration (5.20-fold; $p < 0.001$). It should also be added that a statistically significant increase in serum activity of aspartate aminotransferase (1.45-fold; $p < 0.001$), lactate dehydrogenase (1.79-fold; $p < 0.001$), creatine phosphokinase (1.26-fold; $p < 0.01$) and serum concentrations of troponin I (1.30-fold; $p < 0.01$) was found in cats with HCM complicated by cardiorenal syndrome, as compared with diseased cats without such a complication.

Statistically significant changes in serum concentrations of urea, creatinine, symmetric dimethylarginine and cystatin C in cats during the development of cardiorenal syndrome were observed using Kruskal – Wallis test (Table 2).

It has been revealed that a statistically significant increase in serum concentrations of urea (1.63 times; $p < 0.001$), creatinine (1.27 times; $p < 0.001$), symmetric dimethylarginine (1.40 times; $p < 0.001$) was registered in animals with uncomplicated forms of HCM (group I) as compared with healthy ones. There was a statistically significant increase in serum concentrations of urea (2.94-fold; $p < 0.001$), creatinine (2.72-fold; $p < 0.001$), symmetric dimethylarginine (2.60-fold; $p < 0.001$) and cystatin C (1.90-fold; $p < 0.001$) in cats with HCM complicated by cardiorenal syndrome (group II), as compared with healthy animals. A statistically significant increase in serum concentrations of urea (1.80-fold; $p < 0.001$), creatinine (2.15-fold; $p < 0.001$), symmetric dimethylarginine (1.86-fold; $p < 0.001$) and cystatin C (1.81-fold; $p < 0.001$) was established in cats with HCM complicated by cardiorenal syndrome, as compared with the diseased cats without such a complication.

Table 1
Biochemical parameters of the syndrome of hepatocyte and cardiomyocyte cytolysis in cats with feline hypertrophic cardiomyopathy depending on cardiorenal complications

Parameter	Animal groups						Kruskal –Wallis criterion
	control ($n = 24$)		I ($n = 24$)		II ($n = 25$)		
	Me	IQ	Me	IQ	Me	IQ	
Creatine phosphokinase, U/L	207.00	183.00–227.00	333.50*	279.50–360.00	419.00* #	381.00–457.00	H = 49.40 $p < 0.001$
Aspartate aminotransferase, U/L	31.50	29.50–34.00	61.50*	52.00–70.50	89.00* ##	66.00–108.00	H = 41.90 $p < 0.001$
Alanine aminotransferase, U/L	53.50	51.50–55.50	62.50*	58.00–64.50	66.00*	59.00–73.00	H = 22.70 $p < 0.001$
Lactate dehydrogenase, U/L	132.50	105.00–158.00	200.00*	152.00–230.00	357.00* ##	299.00–402.00	H = 33.80 $p < 0.001$
Troponin I, ng/mL	0.10	0.08–0.11	0.40*	0.30–0.45	0.52* #	0.40–0.60	H = 41.60 $p < 0.001$

Me – median; IQ – interquartile range;
*($p < 0.001$) – reliability of the difference between the indicators of groups I, II and clinically healthy animals according to the Mann – Whitney criterion;
($p < 0.01$), ## ($p < 0.001$) reliability of the difference between the indicators of groups I and II animals according to the Mann – Whitney criterion.

Table 2
Biochemical parameters of renal excretory function in cats with cardiorenal syndrome

Parameter	Animal groups						Kruskal –Wallis criterion
	control ($n = 24$)		I ($n = 24$)		II ($n = 25$)		
	Me	IQ	Me	IQ	Me	IQ	
Urea, mmol/L	5.45	5.05–6.40	8.90*	6.75–9.75	16.00* #	14.80–17.10	H = 57.80 $p < 0.001$
Creatinine, μmol/L	106.50	93.00–136.50	135.00*	121.00–147.00	290.00* #	257.00–313.00	H = 50.70 $p < 0.001$
Symmetric dimethylarginine, μg/dL	10.00	8.00–11.00	14.00*	11.00–17.00	26.00* #	24.00–30.00	H = 54.60 $p < 0.001$
Cystatin C, mg/L	1.00	0.75–1.40	1.05	0.85–1.45	1.90* #	1.40–2.20	H = 24.50 $p < 0.001$

Me – median; IQ – interquartile range;
* ($p < 0.001$) – reliability of the difference between the indicators of groups I, II and clinically healthy animals animals according to the Mann – Whitney criterion;
# ($p < 0.001$) reliability of the difference between the indicators of groups I and II animals animals according to the Mann – Whitney criterion.

Table 3
Biochemical parameters characterizing protein, fat, carbohydrate metabolism in cats with cardiorenal syndrome

Parameter	Animal groups						Kruskal – Wallis criterion
	control ($n = 24$)		I ($n = 24$)		II ($n = 25$)		
	Me	IQ	Me	IQ	Me	IQ	
Glucose, mmol/L	4.85	4.50–5.60	5.50*	4.95–5.90	5.10	4.60–5.50	H = 3.60 $p < 0.50$
Ketone bodies, mmol/L	0.10	0.03–0.14	0.12	0.00–0.18	0.85** #	0.59–0.85	H = 36.60 $p < 0.001$
Cholesterol, mmol/L	3.35	2.80–4.45	3.45	3.00–4.00	5.60** #	5.20–6.50	H = 39.30 $p < 0.001$
Triglycerides, mmol/L	0.70	0.70–0.85	0.90	0.50–1.10	0.70	0.60–0.80	H = 2.30 $p < 0.50$
Total protein, g/L	66.00	61.00–72.00	64.00	61.00–68.00	62.00*	57.00–64.00	H = 7.90 $p < 0.05$
Albumin, g/L	33.00	29.50–36.50	31.50	30.50–36.00	30.00*	28.00–32.00	H = 9.60 $p < 0.01$

Me – median; IQ – interquartile range;
* ($p < 0.05$), ** ($p < 0.001$) – reliability of the difference between the indicators of groups I, II and clinically healthy animals according to the Mann – Whitney criterion;
# ($p < 0.001$) – reliability of the difference between the indicators of groups I and II animals according to the Mann – Whitney criterion.

Statistically significant changes in the concentrations of ketone bodies, cholesterol, total protein and albumin in the sera of cats with cardiorenal syndrome were detected using the Kruskal – Wallis test.

The data in Table 3 show that a statistically significant increase in serum glucose concentrations (1.13-fold; $p < 0.05$) was registered in cats with uncomplicated forms of HCM (group I) as compared with healthy cats. A statistically significant increase in the serum concentrations of ketone bodies (8.50-fold; $p < 0.001$), cholesterol (1.67-fold; $p < 0.001$), and a decrease in the concentrations of total protein (1.06-fold; $p < 0.05$) and albumin (1.10-fold; $p < 0.05$) were noted in the sera of cats with HCM complicated by cardiorenal syndrome (group II), as compared with healthy cats. A statistically significant increase in serum concentrations of ketone bodies (7.08-fold; $p < 0.001$), cholesterol (1.62-fold; $p < 0.001$) was found in cats with HCM complicated by cardiorenal syndrome, as compared with diseased cats without such a complication.

Statistically significant changes in pathobiochemical parameters indicating oxidative stress were observed in cats with cardiorenal syndrome, using the Kruskal – Wallis method.

The numerical data presented in Table 4 show that a statistically significant increase in serum concentrations of malondialdehyde (1.63-fold; $p < 0.001$), diene conjugates (1.49-fold; $p < 0.001$), and a decrease in activity of superoxide dismutase (1.53-fold; $p < 0.001$), catalase (2.33-fold; $p < 0.001$), glutathione peroxidase (1.23-fold; $p < 0.001$) were observed in animals with uncomplicated forms of HCM (group I), as compared with healthy cats. A statistically significant increase in the serum concentrations of malondialdehyde (1.79-fold; $p < 0.001$), ceruloplasmin (2.50-fold; $p < 0.001$), diene conjugates (1.85-fold; $p < 0.001$) and simultaneous decrease of activity of superoxide dismutase (1.63-fold; $p < 0.001$), catalase (4.67-fold; $p < 0.001$), glutathione peroxidase (1.71-fold; $p < 0.001$) were observed in sera of cats with HCM complicated with cardiorenal syndrome (group II), as compared with healthy animals. A statistically significant increase in the serum concentrations of malondialdehyde (1.10-fold; $p < 0.001$), ceruloplasmin (2.14-fold; $p < 0.001$), diene conjugates (1.25-fold; $p < 0.001$), a decrease in activity of catalase (2.00-fold; $p < 0.001$), glutathione peroxidase (1.35-fold; $p < 0.01$), an increase in activity of glutathione reductase (1.36-fold; $p < 0.01$) were established in cats with complicated cardiorenal syndrome, as compared with diseased cats without such a complication.

The Kruskal – Wallis test revealed statistically significant changes in biochemical parameters of electrolyte metabolism in cats with cardiorenal syndrome (Table 5).

Hyponatremia, hyperkalemia and hyperphosphatemia occurred in cats with uncomplicated forms of HCM as compared to healthy animals. A trend for development of hyponatremia, hyperkalemia, hyperphosphatemia and hypomagnesemia was observed in sera of cats with HCM complicated by cardiorenal syndrome, as compared to healthy ones. It was established that insignificant hypercalcaemia, as well as significant hyperphosphatemia and hypomagnesemia were found in cats with HCM complicated by cardiorenal syndrome, as compared with diseased cats without such a complication.

The test results showing changes in C-reactive protein concentration in cats during the development of cardiorenal syndrome are presented in the figure.

Reliable changes in C-reactive protein concentration in sera of cats in different experimental groups were established (H = 50.50; $p < 0.001$; Kruskal – Wallis test). A statistically significant increase in C-reactive protein concentration in sera (1.15-fold; $p < 0.001$) was registered in animals with uncomplicated forms of HCM as compared to healthy animals. A statistically significant increase in C-reactive protein serum concentration (1.55-fold; $p < 0.001$) was registered in sera of cats with HCM complicated by cardiorenal syndrome as compared with healthy ones. It was also revealed that there is a statistically significant increase in C-reactive protein serum concentration in cats with HCM complicated by cardiorenal syndrome, as compared with diseased cats without such a complication.

Our study showed that the serum aminotransferase and lactate dehydrogenase activity is increased in cats with HCM. A significant increase in serum troponin concentration in HCM animals is indicative of damage to

Table 4
Oxidative stress in cats with hepatorenal syndrome

Parameter	Animal groups						Kruskal – Wallis criterion
	control ($n = 24$)		I ($n = 24$)		II ($n = 25$)		
	Me	IQ	Me	IQ	Me	IQ	
Malondialdehyde, μmol/L	2.80	2.60–3.05	4.55*	4.05–4.95	5.00* ##	4.80–5.20	H = 51.80 $p < 0.001$
Ceruloplasmin, mmol/L	0.60	0.40-0.70	0.70	0.50–1.20	1.50* ##	1.30–1.90	H = 32.50 $p < 0.001$
Diene conjugates, U/mL	2.05	1.55–2.35	3.05*	2.70–3.55	3.80* ##	3.60–4.00	H = 47.40 $p < 0.001$
Superoxide dismutase, U/mL	50.50	40.50–65.00	33.00*	30.50–35.50	31.00*	23.00–35.00	H = 28.90 $p < 0.001$
Catalase, U/mL	1.40	1.25–1.50	0.60*	0.50–0.75	0.30* ##	0.20–0.40	H = 59.70 $p < 0.001$
Glutathione reductase, U/mL	1.35	1.10–1.75	1.10	0.80–1.45	1.50 #	1.20–1.60	H = 7.20 $p < 0.05$
Glutathione peroxidase, U/mL	2.90	2.60–3.60	2.30*	2.00–2.65	1.70* #	1.50–2.10	H = 38.30 $p < 0.001$

Me – median; IQ – interquartile range;
* ($p < 0.001$) – reliability of the difference between the indicators of groups I, II and clinically healthy animals according to the Mann – Whitney criterion;
# ($p < 0.01$), ## ($p < 0.001$) reliability of the difference between the indicators of groups I and II animals according to the Mann – Whitney criterion.

Table 5
Electrolyte metabolism in cats with cardiorenal syndrome

Indicator	Animal groups						Kruskal –Wallis criterion
	Control ($n = 24$)		I ($n = 24$)		II ($n = 25$)		
	Me	IQ	Me	IQ	Me	IQ	
Sodium, mmol/L	155.00	151.50–158.50	149.00**	146.00–152.50	148.00**	143.00–154.00	H = 12.40 $p < 0.01$
Potassium, mmol/L	3.60	3.35–3.75	4.05**	3.65–4.75	4.40***	3.80–5.50	H = 18.40 $p < 0.001$
Ionised calcium, mg/dL	9.52	8.95–9.40	9.20	8.75–9.80	10.30*** #	9.90–10.60	H = 25.40 $p < 0.001$
Inorganic phosphorus, mg/dL	4.50	3.70–4.80	5.70*	5.25–6.45	11.00*** #	8.10–13.80	H = 41.10 $p < 0.001$
Magnesium, mg/dL	2.10	1.80–2.30	2.20	1.85–2.40	1.50*** #	1.30–1.80	H = 20.50 $p < 0.001$

Me – median; IQ – interquartile range;
* ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$) – reliability of the difference between the indicators of groups I, II and clinically healthy animals according to the Mann – Whitney criterion;
# ($p < 0.001$) – reliability of the difference between the indicators of groups I and II animals according to the Mann – Whitney criterion.

the cardiomyocyte cell membranes. Similar changes were previously described in the literature [1, 10, 12].

Azotemia developed in cats with cardiorenal syndrome occurring in the setting of HCM. It is obvious that this pathological process initially develops as prerenal azotemia, against the background of chronic circulatory insufficiency and, as a consequence, poor renal perfusion. However, as the pathology progresses, there is likely to be additional damage and death of nephrons, constituting a renal component in the pathogenesis of azotemia. Renal excretory dysfunction aggravates neuroendocrine shifts in the organism of diseased cats, which is also manifested by electrolyte metabolism disturbance (hyperkalemia, hyponatremia, hypomagnesemia, hyperphosphatemia). Obviously, the pathophysiological mechanism for a marked increase in serum concentration

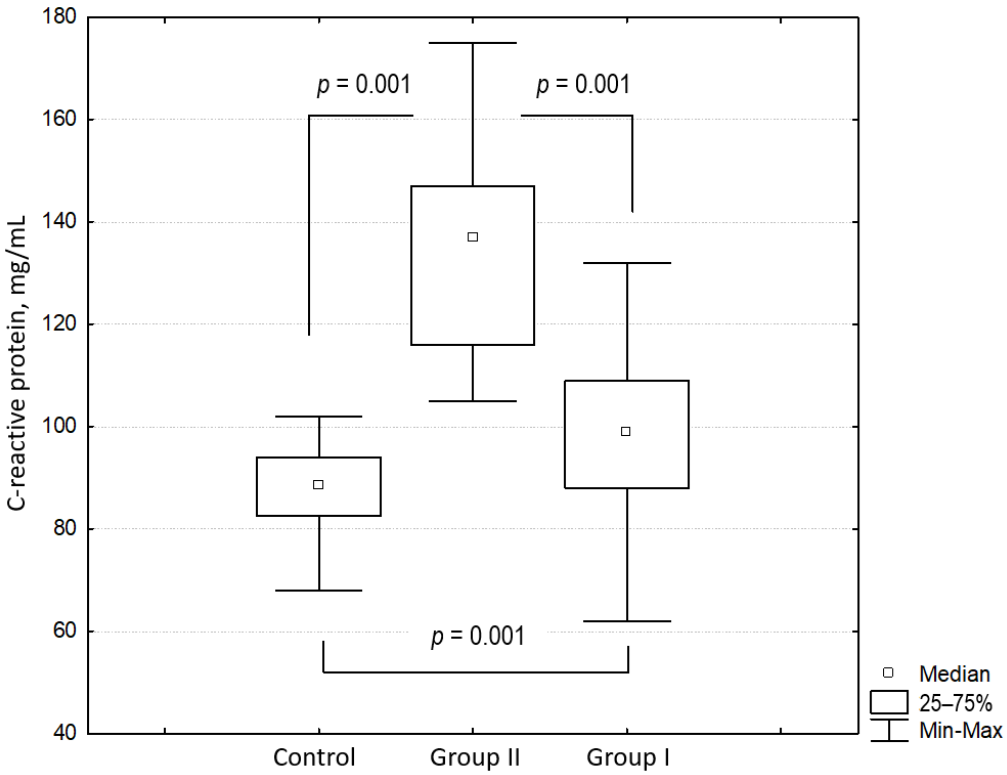

Fig. C-reactive protein concentration in cats with cardiorenal syndrome

of inorganic phosphorus in animals with cardiorenal syndrome is secondary hyperparathyroidism, which often complicates the course of renal failure [18]. Uraemic toxins accumulating in the body during cardiorenal syndrome have an additional damaging effect on both cardiomyocytes and nephrons, which leads to further progression of the pathological condition.

The detection of ketosis in cats with cardiorenal syndrome was unexpected. It is obvious that there are profound changes in metabolism of animals with cardiorenal syndrome, that are manifested in adipose tissue mobilisation, increased protein and lipid catabolism, energy deficit. In such cases ketogenic amino acids and triglycerides may be the source of synthesis of ketone bodies. This phenomenon requires further large-scale studies. In our case, hypercholesterolemia, moderate hypoproteinemia and hypoalbuminemia were detected in diseased cats.

Systemic inflammation is the key pathogenetic link in the formation of cardiorenal syndrome in cats with HCM. Proinflammatory cytokines, which are produced during the development of the inflammatory response, have an additional damaging effect on cardiomyocytes and nephrons. High C-reactive protein serum concentrations can be considered as a marker of inflammation in cats with cardiorenal syndrome.

It is obvious that activation of the neurohumoral system against the background of circulatory failure and increased myocardial tissue oxygen demand can initiate cardiac cell apoptosis and fibrosis in cardiorenal syndrome. In this direction, further studies are required for morphological verification of the above pathological processes. Metabolic disorder in cardiomyocytes causes the development of oxidative stress, which increases the alterative effect on the myocardium.

CONCLUSION

Hypertrophic cardiomyopathy results in chronic circulatory failure in cats and may be complicated by cardiorenal syndrome. The activity of aspartate aminotransferase, lactate dehydrogenase, creatine phosphokinase, glutathione reductase significantly increases, and the concentrations of cardiac troponin, urea, creatinine, symmetric dimethylarginine, cystatin C, ketone bodies, cholesterol, malondialdehyde, ceruloplasmin, diene conjugates, potassium, calcium, phosphorus, C-reactive protein increase, the activity of superoxide dismutase, catalase and glutathione peroxidase, as well as the concentrations of total protein and albumin, sodium and magnesium decrease in the sera of cats with cardiorenal syndrome. Cardiorenal complications in cats with feline hypertrophic cardiomyopathy are characterised by the following biochemical syndromes: azotemia, cardiomyocyte cytolysis, electrolyte imbalance, systemic inflammatory reaction, oxidative stress.

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Situational analysis on porcine diseases of priority to pig industry: methods for biosecurity improvement in the Russian Federation holdings

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ABSTRACT

The analysis of the situation on management of main epizootic threats significant for pig industry in the Russian Federation was carried out. The features and effectiveness of various biosecurity measures used in pig holdings and possible ways for their improvement were examined. Options for improvement of biosecurity measures in Russian pig holdings and development of the systems in the Russian Federation having favorable effect on the population protection were discussed focusing on four target aspects related to infection introduction and spread routes: isolation of the population from external sources of threats (elimination of probable environmental impact scenarios); isolation of the population from internal sources of threats (elimination of probable impact scenarios during production process); isolation of the pig population or pig farming system from the human factor impact (elimination of possible scenarios of human factor impact on the population); isolation of the population from the conditions under which the threat potential is manifested (changing of conditions). The analysis results were presented graphically in the form of a schematic diagram "Sources of threats to biosecurity systems and measures aimed at these sources for the protected population creation in pig industry". Based on the discussion results, automation and digitalization of all processes in pig industry, generation of genetically modified pigs not susceptible to the most significant pathogens such as African swine fever, classical swine fever, porcine reproductive and respiratory syndrome viruses and their use for production purposes, further research and implementation of integrated technological solutions for feed sanitation have been concluded to be the evolutionarily significant ways for effective pig farming intensification in the Russian Federation.

Keywords: porcine diseases, epizootic situation, pig industry, biosecurity, veterinary and sanitary measures

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Ситуационный анализ по приоритетным для промышленного свиноводства болезням свиней: пути улучшения биозащиты предприятий в Российской Федерации

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РЕЗЮМЕ

Проведен анализ ситуации по управлению основными значимыми для промышленного свиноводства Российской Федерации эпизоотическими угрозами. Рассмотрены особенности и эффективность различных мер, используемых в системах биозащиты свиноводческих предприятий, и возможные пути их улучшения. Обсуждение путей развития мер биозащиты свиноводческих предприятий и систем в Российской Федерации, способных повлиять на защищенность популяции, вели по четырем направлениям, связанным с путями заноса и распространения инфекций: изолированность популяции от внешних источников угроз (устранение вероятных сценариев воздействия из внешней среды); изолированность популяции от внутренних источников угроз (устранение вероятных сценариев воздействия в потоке производства); изолированность популяции или системы свиноводства от воздействия

человеческого фактора (устранение вероятных сценариев воздействия на популяцию человеческого фактора); изолированность популяции от условий, при которых проявлен потенциал угроз (изменение условий). Результаты анализа представили графически в виде принципиальной схемы «Источники угроз для систем биозащиты и меры, направленные на эти источники для формирования защищенной популяции в промышленном свиноводстве». По итогам обсуждения сделано заключение, что автоматизация и цифровизация всех процессов в свиноводстве, создание и внедрение в производство генетически модифицированных свиней, не восприимчивых к таким наиболее значимым возбудителям, как вирусы африканской чумы свиней, классической чумы свиней, репродуктивно-респираторного синдрома свиней, дальнейшие исследования и внедрение комплексных технологичных решений по санитарии кормов представляются на сегодня эволюционно значимыми путями, которые позволят эффективно интенсифицировать свиноводство в Российской Федерации.

Ключевые слова: болезни свиней, эпизоотическая ситуация, свиноводство, биозащита, ветеринарно-санитарные меры

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INTRODUCTION

Pork remains the main source of animal protein in the world, its output annually exceeds the production of meat of any other type. In 2019–2020, global animal protein production declined sharply due to large-scale outbreaks of African swine fever (ASF) in China and Southeast Asian countries. The Russian Federation has become self-sufficient in pork since 2020, and in 2022, commercial holdings produced 5,275.1 thousand tons of pork (slaughter weight), and proportion of large holdings was 76%. According to the predictions, domestic pork consumption will remain high and continue to grow up to 2025 in the Russian Federation [1, 2]. Pigs are susceptible to a wide range of diseases that affect their performance and pig producers' income. The spread of porcine diseases in the Russian Federation had a significant negative impact on pork production in the country during the last 10 years (since 2013), when, according to official data from the World Organization for Animal Health (WOAH), (214,985 ± 165,558) domestic pigs were annually destroyed due to ASF only with peak in 2020, when 615,239 pigs were destroyed. The ASF epizooty has been effectively contained for all these years owing to the coordinated joint activities of both State Veterinary Service with Regional Administrations and in-house veterinary staff with the holding managers as well as all pig holding sites. At the same time, any biosecurity system cannot be established based on template solutions only and is developed taking into account the holding characteristics, animal rearing practice, climatic conditions, animal disease situation in the region, sources of raw materials and feed. However, in any case, the biosecurity system consists of two components. External biosecurity is aimed at prevention of pathogen introduction into the herd, and internal biosecurity is aimed at prevention of the disease spread within the herd or production system. The study of the epizootic patterns of porcine diseases in pig industry and prioritization of porcine diseases allow for development of adequate biosecurity measures for the pig sector [3, 4].

In this context, description and assessment of options for the biosecurity system evolution in holdings remain an urgent task in the framework of discussion on organization of biosecurity measures and ways for their improvement for pig holdings and pig rearing systems in the Russian Federation.

MATERIALS AND METHODS

Official information on measures against porcine diseases taken in the country, scientific literature data, data from open official sources, special round tables and the mass media were used. The measures were assessed and discussed using elements of risk analysis and expert assessment with the consensus achievement [5]. The options for biosecurity measures improvement in pig holdings/pig rearing systems in the Russian Federation that could have impact on pig population protection were discussed taking into account four target areas related to the infection introduction and spread routes.

1. Isolation of the population from external sources of threats (elimination of probable environmental impact scenarios).

2. Isolation of the population from internal sources of threats (elimination of probable impact scenarios during production process).

3. Isolation of the pig population or pig farming system from the human factor impact (elimination of possible scenarios of human factor impact on the population).

4. Isolation of the population from the conditions under which the threat potential is manifested (changing of conditions).

The analysis results were presented in the form of a schematic diagram "Sources of threats to biosecurity systems and measures aimed at these sources for the protected population creation in pig industry" (Fig.).

RESULTS AND DISCUSSION

There are quite a few scenarios for known transmission routes for various porcine pathogens. Therefore, a set

of standard potentially effective measures aimed at epizootic chain breaking could be provided for the most diseases. But there is no common universal biosecurity system for holdings. An effective system is created with the participation of veterinarians and other specialists who have complete information about the holding, processes, employees and risk factors. The biosecurity plan is unique for each pig holding and includes prioritization and succession of measures based on their potential effectiveness and appropriateness for the production process [4, 6]. Quantitative data on each route or element impact and relative importance for the transmission scenario implementation are always required for development of point-based technological solutions. The actual effectiveness of applied biosecurity measures is influenced by economic, sociological and even psychological factors as well as their harmonization with the governmental official disease surveillance policy [7], that can be taken into account as negative factors or used for development of policies for biosecurity measures correction in holdings.

Biosecurity system strengthening in holdings is aimed at:

1. Isolation of the population from external sources of threats. Since domestication of pigs (about 10,000 years ago) [8] isolation of pig population from aggressive environmental factors has been historically the first effective measure used by humans in pig farming.

In an evolutionarily short period, the development of technical means and methods for pig population isolation from the external environment and the emergence of more and more modern technologies in pig farming industry (in genetics and artificial insemination; feed production; pharmacology and vaccinology) had enabled development of industrial pig production strategies and concentration of up to 84,000 sows producing over 2 million pigs per year in megaholdings by 2020 (in the People's Republic of China). Industrial pig production has provoked changes in susceptible population features (genetic homogeneity of the population, immune homogeneity); had an effect on the prevailing pathogen transmission mechanisms (increased number of direct and indirect contacts, emerged iatrogenic risks, risks of pathogen spreading through artificial insemination, feed-associated risks, etc.); enabled accelerated pathogen evolution during the production process and in large populations (reassortment, including reassortment with vaccine strains; associated infections; antibiotic resistance; changes in the epizootiology of many infectious and non-infectious diseases, etc.).

The evolution of the epizootiological triad components has made traditional approaches to population segregation ineffective due to globalization of pathogen spread risks in industrial pig production systems and necessitated development of new tools – biosecurity system and compartmentalization, effective against both new pathogens and pathogens that have changed their epizootological characteristics [9].

There are infections new to pig industry such as the widespread porcine reproductive and respiratory syndrome (PRRS), ASF caused by the genotype II virus, porcine epidemic diarrhea (PED) and swine influenza, as well as recurrent infections – classical swine fever (CSF) and foot-and-mouth disease (FMD). Isolation of the population from the external environment in pig farming systems is found insufficient for agents of the above-mentioned infections,

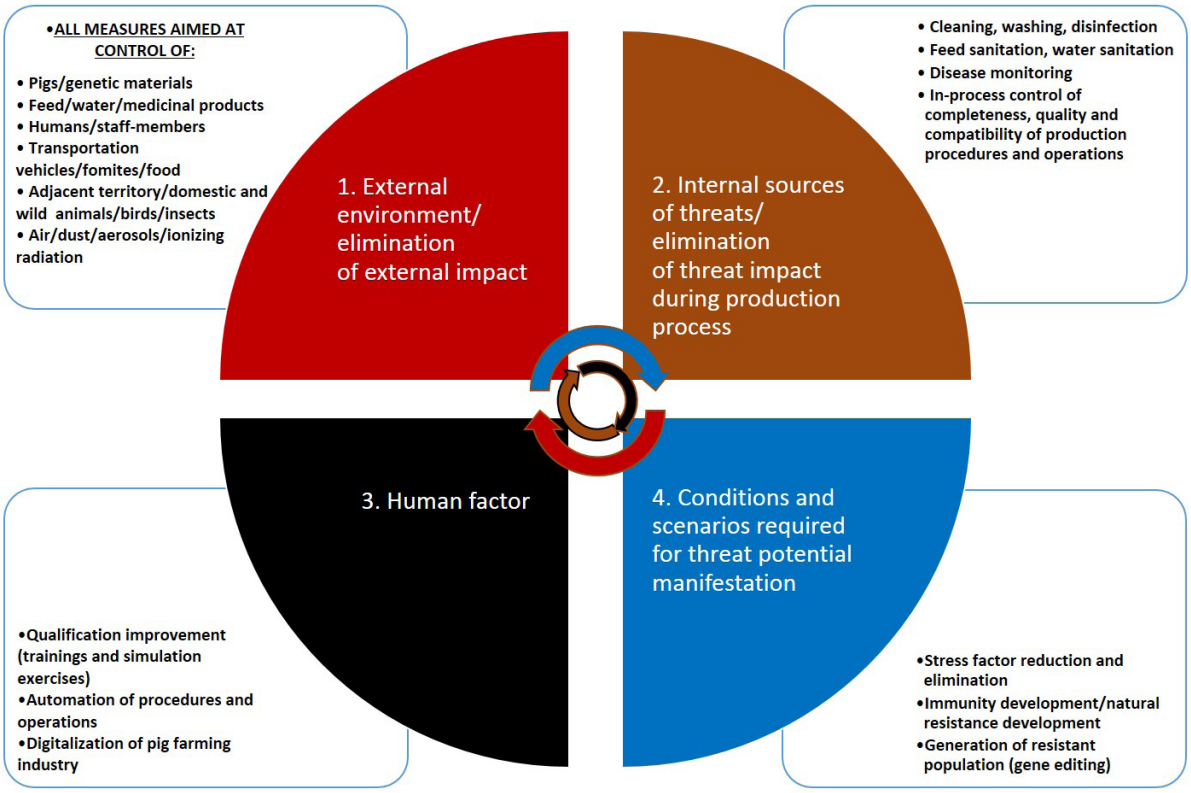

Fig. Schematic diagram "Sources of threats to biosecurity systems and measures against these sources for protected population creation in pig industry"

and, first of all, highly contagious pathogens (PRRS, FMD, swine influenza, CSF, ASF viruses, mycoplasmosis agent) that are transmitted by both direct and indirect routes [10, 11, 12, 13, 14, 15].

Considering this, population isolation methods take the new level. For example, taking into account possible airborne transmission of PRRS virus in indoor pig keeping systems much attention is paid to use of air filters and other air filtration technologies for the population protection [16]. Some studies have shown that air filtration system installation on pig farms contributed to an 80% reduction of the risk of PRRS virus introduction into production systems [17, 18].

It should be noted that Chapters 5.4, 5.5, 5.7 and 7.3 of the WOAH Terrestrial Animal Health Code [19] address to safe transportation of animals in isolated transportation vehicles preventing transboundary disease spread. It shows that likelihood of infection and pathogen spread during transportation shall be taken into account. Therefore, it is recommended to take into account meteorological risk factors associated with the presence of each of the pathogens in the air, as well as the ability of mechanical and antimicrobial filters to protect susceptible populations from RRRS virus and other pathogens for which aerosol spread has been proven (mycoplasma, swine influenza, CFS) in holdings and during transportation.

Compartmentalization (along with zoning) is also currently considered as a measure sufficient for creation of the population free from most transboundary diseases, including panzootic genotype II virus-caused ASF – a relatively new problem for global pig industry [9]. At the same time, fomites, transportation vehicles and animals (including rats, birds and insects) coming in contact with the pig population are considered to play important role in the "domestic cycle". Therefore, measures for exclusion of scenarios of potential exposure environmental through transportation vehicles, fomites, mechanical carriers, etc. should be periodically reviewed. The main focus is shifted to discussion the reliability of control of sanitary measures aimed at the population isolation from the external environment and potential for automation of biosecurity systems in holdings (for example, online monitoring of in-house transportation vehicle movements between compartments, the integrity of the holding perimeter, protection against mechanical vector penetration and activities; control of cleaning, washing and disinfection quality in technical facilities and transportation vehicles using automated devices for residual contamination detection, etc.). Automated control will allow for improvement of biosecurity system and isolation measures effectiveness.

Automation and digitalization of systems for effective air filtration, microclimate maintenance and for their control in facilities as well as when animals arrive to the holding or moved between compartment sites using public roads running through rural territories are also the most modern isolation measures contributing to elimination of gaps in management of airborne infection risk (primarily PRRS and mycoplasmosis).

The assessment of measures for segregation control in biosecurity systems and the experience accumulated by holdings in this field are of scientific interest for development of basic recommendations to put them into practice.

2. Isolation of the population from internal sources of threats. Likelihood of such threats affecting pigs during production process is eliminated by monitoring the completeness, quality and consistency of procedures and operations used within the production (technological) chain (in-process control). This also includes internal quarantine when moving adult animals between groups, and the "all in – all out" approach, sanitation and containment measures, etc. However, we will focus on three independent areas.

Cleaning, washing, disinfection. Management of risks of pathogen introduction and spread in herds through production chain is based on both general measures for holding biosecurity ensuring and targeted techniques to control the effectiveness of measures taken. Hygiene management is becoming increasingly important as an independent biosecurity tool. Infectious activity of most porcine viral and bacterial pathogens can be significantly reduced as early as at the cleaning stage, where up to 90% of microorganisms are destroyed that significantly increases the effectiveness of further disinfection [20] and facilitates elimination of scenarios of probable exposure during production process by "cutting off" probable indirect contacts. Therefore, the triad components: "cleaning – washing – disinfection" in processes where they are applied (treatment of transportation vehicles, footwear, equipment, facilities, etc.) should be as one measure (a three-stage process that includes sequential cleaning, washing and disinfection), and each operation should be subject to control.

In-process monitoring of pathogens. Porcine factor pathogens are characterized by circulation in herds with clinical disease cases manifesting only in isolated sex and age groups of animals (porcine parvovirus and rotavirus infections, actinobacillosis pleuropneumonia, streptococcosis, salmonellosis, pasteurellosis, escherichiosis, leptospirosis), therefore, control of measures against such infections can be focused on isolation of animal subpopulations during production process for a period of time sufficient for immunity (resistance) development [21, 22]. Consequently, the production processes for different groups of pigs should be monitored and such monitoring should be the part of a biosecurity plan. In addition, in-process monitoring based on serological tests with the mandatory use of polymerase chain reaction followed by sequencing of the detected isolates allowing for accurate determination of subpopulations status and potential scenarios for the pathogen spread in the system is to be carried out at all points critical for pathogen transmission, for example, for PRRS virus (replacement animals, genetic material, transfer of animals from one group to another, control of "all in – all out" system operation). This will ensure in-process isolation of the subpopulation.

Feed sanitation. Sanitation of feed for pigs is the third key point in biosecurity programs of establishments for reducing likelihood of threat scenarios during production process [23, 24, 25, 26]. Despite the fact that feeds can be produced by methods ensuring pathogen destruction, they are often cross-contaminated during storage, transportation or feeding. Some studies have shown long-term persistence of following infectious viruses in the feed during its transatlantic transportation: foot-and-mouth disease virus, ASF, swine vesicular disease virus, Aujezsky's disease virus, PRRS, and porcine vesicular exanthema virus,

whereas there is no evidence of such long-term survivability for CFS, Nipah, and swine influenza viruses [27].

The main measures for feed sanitation currently discussed worldwide are: 1) monitoring of feed directly supplied to pigs for pathogens; 2) holding of components of animal origin to decrease virus titre; 3) monitoring of feed and feed additive storage conditions; 4) application of additives (based on formaldehyde, fatty acids, essential oils and organic acids) for feed and water decontamination for bacteria and viruses removal (including ASF, PED virus), as well as application of mycotoxin adsorbents [23, 26].

Therefore, feed sanitation should include, among other things, supplier reliability control, raw material quality control and measures for mitigation of such risks as: a) presence of pathogens in source materials; b) the presence of infectious agents in ready-to-use feed and high-risk feed components (components of animal origin); c) recontamination of finished feed during its production, transportation and storage [24].

3. Isolation of the population from human factor effect. There are no commercial pig herds in the world that are 100% isolated from the human factor associated with production process maintenance, from engineering aspects up to control of technological processes, including veterinary and zootechnical aspects. It should be noted that the human factor is one of the most difficult to control, but at the same time one of the key factors for effective prevention of infection introduction and spread.

Automation and digitalization in pig farming sector. The strategy for reducing the human factor impact is the most knowledge-intensive and rapidly growing area of the developing biosecurity system management. With the advent of new information technologies and technical capabilities, this area has been further developed into the whole separate segment called "precision animal farming", that is widely discussed from the scientific and applied point of view in the pig breeders' community as a promising practice for future production [28, 29, 30, 31, 32, 33]. The priority task of the science today is to fill gaps in new technical solutions due to the complexity of validation of technologies for assessing affective states in pigs (both positive and negative) evaluated in process control systems using data obtained from recording sensors (strain gauges, thermal imagers, microphones, photometers, infrared, radio frequency sensors), as well as to reduce the cost of these technologies [34, 35].

Automation and exclusion of the human factor impact on such processes as animal movements, control of crossing dirty and clean zones, assessment of cleaning, washing and disinfection effectiveness, microclimate control, weighing of animals, feed distribution, monitoring of the clinical state of animals (body temperature, behavior, feed and water consumption) are undoubtedly of high importance for qualitative improvement of biosecurity systems in the holdings in future.

Technological innovations have the strongest positive impact on production. The territories of the Russian Federation with the highest technological innovation level hold leading positions as for index of agricultural production [36]. Ministry of Agriculture of the Russian Federation is going to introduce digital technologies into the national agricultural sector, develop a platform for domestic agricultural product promotion as well as launch modeling and predicting systems ("Digital Agriculture") by 2030. Obviously, advent of predicting technology allowing for creation of predictive models for animal diseases will enable a breakthrough in this area.

In other words, with data accumulation, it will become possible to create a basis for predictive models in existing pig farming systems allowing individual management decision-making modeling and predicting the response of all related estimated system indicators to these decisions, thereby reducing managerial errors at the stage of their development.

The expert community notes that the main consumers of new technologies in Russian pig farming industry are limited by top 50 pig producers. This is accounted for lack of funds and insufficient personnel qualifications and skills, the factors that impede large-scale automation and digitalization of pig farms. Small farms remain at the technological level of the last century. In Russian pig farms, veterinary manipulations account for large proportion of manual labor. Automation of animal censuring and remote diagnosis is in little demand, as it requires significant re-equipment of facilities and financial investments. Most of the technological processes in pig farming sector have been already mechanized, and currently they require automation. Full automation of production process on a smart farm is theoretically and practically possible, but it is still economically non-profitable [36, 37, 38].

The main challenge for domestic developers today, in our opinion, is the integration of systems of different producers put in place for various targets (microclimate control, feed control, control of hunting periods, livestock turnover, inventory, etc.) into common online management system, development and integration of automated systems for veterinary manipulations. Their creation and digitalization are carried out in consultation with experts and veterinary scientists, as this a priori will impact on the overall biosecurity level of holdings and will enable control of porcine infectious diseases in pig holdings [36, 37, 38].

Employee qualification improvement. Theoretically, the effectiveness of human factor elimination depends on the degree of the person involvement (awareness) in professional life and commitment to risk management practice in the field.

Continuous training and improvement of skills of the personnel involved in technological processes posing human factor – associated risks affecting committed implementation of biosecurity measures is an effective solution, as well as a measure complementing automation and digitalization of processes in pig farming industry. Trained personnel carrying out monitoring of each automated procedure, is the basis for the introduction of automation and informatization in pig farming sector.

Employee competence improvement throughout the whole production chain is the key aspect for elimination of barriers to digitalization, but also for reducing the impact of "advertising approach" to selection of measures and tools for disease prevention, when the ineffectiveness of medicines is masked by sales consultants with arguments about the imperfection of the applied preventive measures and tools and by requirements for the implementation of disease eradication programs that a priori are impossible at the level of a separate pig holding (refusal of vaccination, etc.).

4. Isolation of the population from the conditions under which the threat potential is manifested (changing conditions). The following is currently applied to exclude propagation and accumulation of pathogenic microorganisms: vaccination, acclimatization, the elimination of feed, heat, noise and behavioral stresses, breeding of animals resistant to pathogens and stress factors, changing of the design features/physicochemical properties of surfaces and materials coming in contact with pigs (floors, stalls, walls, bedding, drinking bowls, feeders, feed pipelines) [39].

Stress reduction/elimination. Type of feeding, type of microclimate in facilities where different age and sex groups are kept, and contamination of facilities are considered to be associated with the development of stress of various levels, morphological and biochemical changes in blood, changes in general animal resistance to stress and to pathogens, and animal performance, and the control of these parameters is akin to the control of factors contributing to unfavorable events [40, 41, 42, 43]. Interdisciplinary approach has enabled generation of new materials and their introduction into pig production industry. Monitoring and management of animal keeping conditions and general resistance allow for reducing stress and neutralizing pathogen exposure, however, they are high-tech, knowledge-intensive and, as a rule, interdisciplinary areas for commercial pig farming.

Immunity development. Registered and approved vaccine shall be selected for vaccination taking into account genetic characteristics of the pathogen circulating in the region. Information about PRRS virus strain genotypes and the infection spread in the herd, for example, will help to timely select the most appropriate disease control strategy in the particular Russian Federation subject or production system [44, 45]. Application of vaccines against CSF and Aujezsky's disease remains one of the most effective disease control measures and correlates with current biosecurity and biosafety measures used in pig farming industry in the Russian Federation. Improvement of animal health status involving the refusal of vaccination without the federal program and involvement of all regions and all business operators in the country appears to be ineffective based on the experience of other countries and in the context of current Russian Federation regionalization policy (the most effective quarantine tool in place in veterinary field in the Russian Federation).

Genetically modified pigs. Gene editing, which can be used for animal disease resistance inducing and maintaining is the most effective out of all measures that radically changes conditions in the pig farming system. In view of successful experiments in public health sector (HIV, sickle cell anemia and cancer of various types), gene editing technology is considered to have a great potential in animal disease treatment and prevention. This is, theoretically, generation of genetically modified pigs not susceptible to ASF or PRRS [46, 47]. In case of any successful genetic improvement, it will only take just a few time to reproduced genetically enough virus-resistant progenitor pig populations to meet global demand.

CONCLUSION

Biosecurity of pig production systems is currently paid much attention in science and practice. The adequacy of the assessment and monitoring of external threats is a priority task for ensuring effective pig farming system biosecurity. The surveillance system, as the basis of all animal disease control measures already taken and implemented, should provide the pig industry and biosecurity systems of holdings with, first of all, accurate, measurable and interpretable data on the population state and existing external threats.

An important gap in the most technological solutions is the dependence on foreign software programs for equipment despite the presence of Russian developers on the market.

Taking into account the lack of standard technologies for pig farming sector and attempts to integrate equipment and software from different countries into a single project, the elimination of the "advertising approach" (unjustified transfer of some digital technologies from cattle farming to pig farming sector) is also the task for domestic developers of equipment and software programs (systems).

Employee competence improvement throughout the whole production chain is the key aspect for elimination of barriers to digitalization as well as for reducing the impact of "advertising approach" to selection of measures and tools for disease prevention when the inefficiency of medicines is masked by sales consultants with arguments about the imperfection of the applied preventive measures and tools and by requirements for the implementation of disease eradication programs that is a priori impossible at the level of a separate pig holding (for example, refusal of vaccination, etc.).

It can be concluded that the automation and digitalization of all processes in pig industry, creation of genetically modified pigs not susceptible to the most significant pathogens such as African swine fever, classical swine fever, porcine reproductive and respiratory syndrome viruses and their use for production purposes, further research and implementation of integrated technological solutions for feed sanitation are the evolutionarily significant ways for effective pig farming intensification and that they will facilitate the development of production data monitoring and porcine disease control systems and reduce the impact of manual control errors.

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