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Ilya S. Tsyganov, Sergey V. Shcherbinin, Tatyana S. Galkina, Konstantin N. Gruzdev
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ABSTRACT

Introduction. Feline chlamydiosis is a disease caused by *Chlamydia felis*, it is characterized by conjunctivitis (unilateral or bilateral), lacrimation and lesions of the respiratory and reproductive tracts. This pathogen is a gram-negative bacterium with a strictly obligate intracellular parasitic nature. In the early 1940s, *Chlamydia* were considered an intermediate link between viruses and bacteria. Until the 1960s, *Chlamydia* were classified as viruses due to their small size and inability to grow on artificial nutrient media. Their unique two-phase developmental cycle allows the pathogen to persist in the body of an animal or human for a long time without clinical manifestations. Treatment of chlamydiosis must be systemic, addressing both etiological therapy (use of antibiotics) and symptomatic therapy. Immunity against chlamydiosis is weak, with cellular immunity being more important than humoral immunity. Currently, both attenuated and inactivated vaccines are available on the market, which can protect against the clinical manifestation of the disease but not against infection. Chlamydiosis is a globally widespread disease, with detection rates showing a consistent year-on-year increase.

Objective. To review and systematize current data on feline chlamydiosis caused by *Chlamydia felis*.

Results. The article reviews global prevalence data of feline chlamydiosis. It examines the biological properties of the infectious agent, the clinical signs of the disease, and the pathological findings. Data on immunity are presented, and disease control measures are discussed.

Conclusion. *Chlamydia felis* is a worldwide spread pathogen capable of infecting not only cats and other animals but also humans, which indicates its zoonotic potential. Such factors as complex life cycle, adeptness at host immune evasion and ability to establish persistent infections hinder its effective eradication. The required extended treatment regimens and propensity for chronic infections compromise companion animal welfare and pose a risk of transmission to humans. A more profound understanding of *Chlamydia felis* pathogenesis is essential for developing effective treatment and prevention strategies.

Keywords: review, feline chlamydiosis, *Chlamydia felis*, cats

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Хламидиоз кошек (обзор)

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РЕЗЮМЕ

Введение. Хламидиоз кошек – болезнь, вызываемая *Chlamydia felis*, характеризуется конъюнктивитом (одно- или двухсторонним), слезотечением, поражением респираторного и репродуктивного трактов. Данный возбудитель относится к грамотрицательным бактериям со строго облигатным внутриклеточным паразитизмом, однако в начале 40-х гг. XX века хламидии считали промежуточным звеном между вирусами и бактериями. До 1960-х гг. из-за малых размеров и неспособности развиваться на искусственных питательных средах хламидии классифицировали как вирусы. Необычный цикл развития, состоящий из двух фаз, позволяет возбудителю долгое время персистировать в организме животного или человека без клинических проявлений. Лечение хламидиоза должно быть системным и затрагивать как этиологическую терапию (применение антибиотиков), так и симптоматическую. Иммунитет против хламидиоза слабый, клеточный иммунитет имеет большее значение, чем гуморальный. В настоящее время на рынке представлены аттенуированные и инактивированные вакцины, позволяющие защитить от клинического проявления болезни, но не от заражения. Распространен хламидиоз практически повсеместно, при этом заметна тенденция к росту выявляемости из года в год.

Цель исследования. Актуализация и систематизация данных по хламидиозу кошек, вызываемому *Chlamydia felis*.

Результаты. В статье обозреваются данные о распространенности хламидиоза кошек в мире. Рассмотрены биологические свойства описываемого инфекционного агента, клинические признаки хламидиоза кошек и патолого-анатомическая картина, приведены данные об иммунитете, освещаются меры контроля болезни.

Заключение. *Chlamydia felis* является распространенным патогеном, способным поражать не только кошек и других животных, но и человека, то есть имеющим зоонозный потенциал. Сложный цикл развития, наличие способности обходить иммунитет хозяина, продолжительная персистенция в организме усложняет его эрадикацию. Длительный курс лечения и переход в хроническую форму снижают качество жизни животных-компаньонов и создают угрозу передачи патогена человеку. Для разработки схем успешного лечения и профилактики хламидиоза требуется более детальное практическое изучение *Chlamydia felis*.

Ключевые слова: обзор, хламидиоз кошек, *Chlamydia felis,* кошки

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INTRODUCTION

Feline chlamydiosis is a contagious disease of felids, characterized by ocular and respiratory tract lesions and caused by bacteria belonging to the genus *Chlamydia*. While the disease can be caused by different *Chlamydia* species, such as *C. pneumoniae*, *C. psittaci*, *C. abortus*, the primary agent is *Chlamydia felis* (previously known as *Chlamydophila felis* and earlier classified as *Chlamydia psittaci* variant *felis*). Given the historical classification of *C. felis* and *C. psittaci* as the same species, it can be assumed that past cases in domestic cats attributed to *C. psittaci* were indistinguishable from those caused by *C. felis* due to the limitations of molecular diagnostics at the time. Although *C. psittaci* and *C. felis* exhibit different host specificities and cause distinct clinical syndromes, these pathogens are closely related [1, 2, 3, 4, 5]. The reference strain for *C. felis* is the FP Baker strain [6, 7, 8, 9, 10, 11]. This species is also a zoonotic pathogen capable of causing infection in humans [12, 13, 14]. Regarding host range, *C. felis* can asymptomatically infect dogs, with the manifestation of conjunctivitis being a rare event; however, seroconversion to this pathogen does occur [13]. Consequently, dogs may play a potential role in the zoonotic transmission of *Chlamydia*, making *C. felis* an important pathogen to diagnose in dogs as well. In humans, *C. felis* can cause symptoms of keratoconjunctivitis, follicular conjunctivitis, respiratory tract pathology, hepatosplenomegaly, glomerulonephritis and endocarditis [15, 16, 17, 18, 19, 20]. It has also been reported that *C. felis* was detected in conjunctival swabs from an adult Eurasian lynx with unilateral ocular lesions [21].

In the early 1940s, *Chlamydia* were considered an intermediate link between viruses and bacteria. Until the 1960s, they were classified as viruses due to their small size and inability to grow on artificial nutrient media. At the beginning of the 21st century, after genome sequencing, this pathogen was classified as a bacterium [22, 23]. The first reports of *Chlamydia* infection in cats with symptoms of ocular and respiratory system lesions were in 1971 [17].

The relevance of this work lies in the fact that the review presents the biology of the pathogen *C. felis* and describes the features of the disease caused by this pathogen, which is widespread almost everywhere among stray and domestic cats (*Felis catus*). This is facilitated by inadequate pet care standards, uncontrolled breeding, and expanded stray population. The topic is critically important for developing strategies to enhance the health of companion and breeding animals – a significant concern given the high costs of veterinary care – and for mitigating the zoonotic risk to humans. As chlamydiosis is found on all continents, improving diagnostic and preventive measures is essential.

The novelty of this article lies in its analysis of current data on the epizootiology, clinical presentation, pathology, diagnosis, and vaccine prevention of feline chlamydiosis.

The objective of this review is to update and systematize data on feline chlamydiosis caused by *C. felis*.

EPIZOOTIC SITUATION

Information on feline chlamydiosis epizootic situation does not reflect the true prevalence of the disease. Data on the frequency of feline chlamydiosis cases caused by *C. felis* from various geographical regions are based on molecular test results. For instance, in the United States in 2014, the percentage of cats testing positive for *C. felis* was 24% [24]. In China, between November 2022 and October 2023, the proportion of PCR-positive samples from cats was 15.75% [25]. In European countries over the last 20 years, the leaders in *C. felis* detection rates have been the following countries: Slovakia (45.16%), Hungary (33.3%), Poland (25%), Italy (20%), Switzerland (16%) and Sweden (15.3%) [9, 13, 26, 27]. In Russia, from 2018 to September 2019, the proportion of positive results for *C. felis* was 11.2% [28]; specifically for Moscow in 2019,it was 7.2% [29]. According to some reports, bacteria of this species were detected in approximately 26.3% of stray cats in Japan [26]. These data, without considering sampling and diagnostic rates, demonstrate a high incidence of chlamydiosis in cats. Cases caused by *C. felis* are characterized as enzootic, and epizootiological studies conducted in various countries indicate the presence of this pathogen in 23% of cats suffering from conjunctivitis. Higher morbidity rates are observed in cats kept in groups, such as in animal shelters [2].

BIOLOGICAL PROPERTIES OF THE PATHOGEN

Chlamydia felis is a gram-negative, coccoid bacterium with strictly obligate intracellular parasitism. It belongs to the family *Chlamydiaceae*, genus *Chlamydia* (Fig.). Its size depends on the phase of the developmental

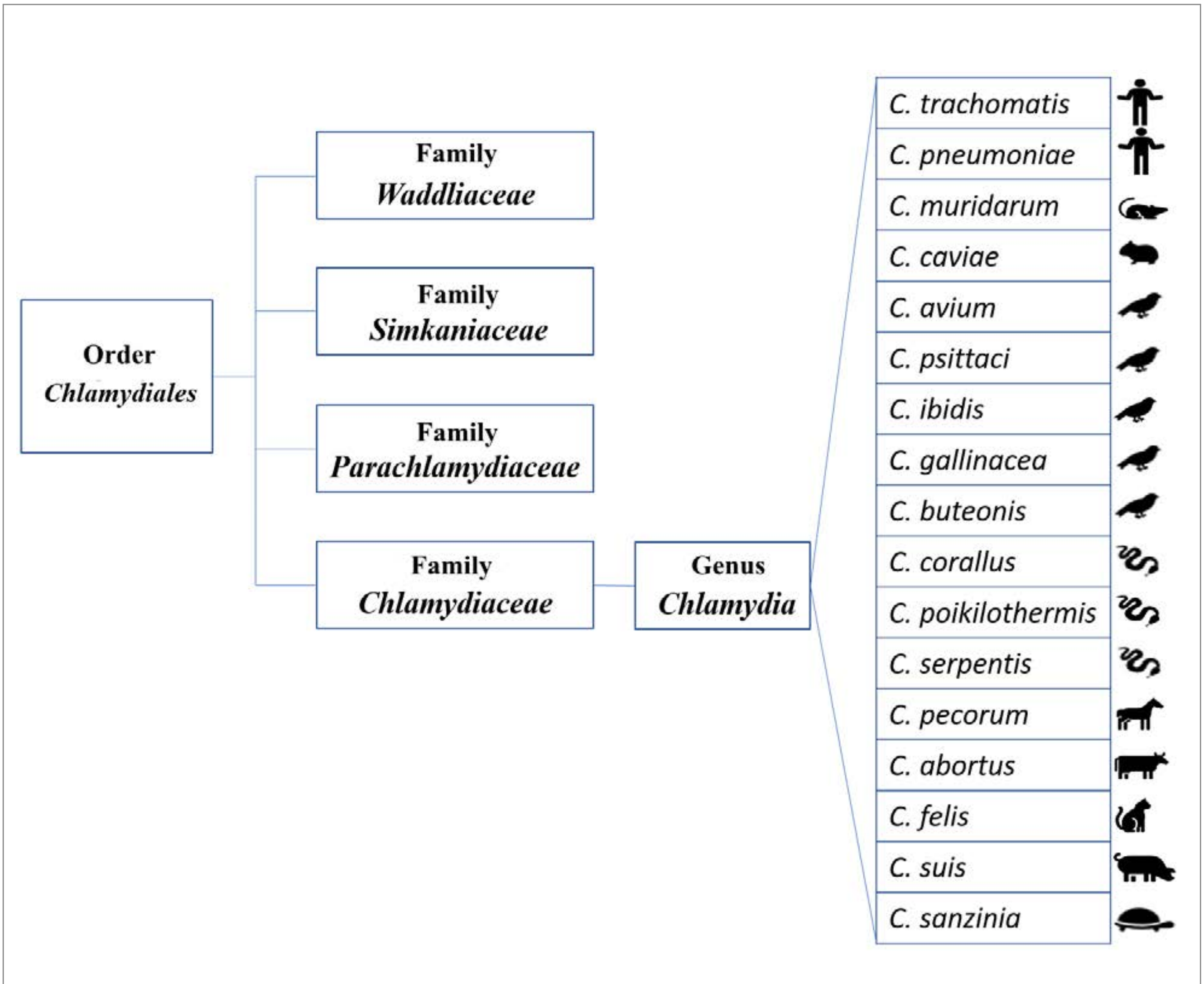

Fig. Phylogenetic classification of Chlamydia species according to 16S and 23S rRNA gene sequence analysis as of 2019 [32]. Icons indicate the primary host species for each strain, though all members of the Chlamydia genus are considered polyhostal cycle, which occurs in two stages: non-infectious reticulate bodies – RB (diameter from 0.5 to 1.6 μm) and infectious elementary bodies – EB (diameter from 0.2 to 0.6 μm) [7, 30].

Initially, all chlamydial isolates obtained from cats with upper respiratory symptoms since 1942 were classified as *C. psittaci*. Subsequent 16S rRNA sequence analysis, however, led to a reclassification of the family *Chlamydiaceae* into two genera: *Chlamydia* and *Chlamydophila*, with the feline agent being placed in the latter [8, 22, 30, 31]. This taxonomy was revised again in 2009 following whole-genome sequencing studies, which consolidated all pathogenic chlamydias into the single family *Chlamydiaceae* and a single genus, *Chlamydia*, within the expanded order *Chlamydiales* [11].

PATHOGEN RESISTANCE TO ENVIRONMENTAL FACTORS AND DISINFECTANTS

Chlamydias are inactivated by heat, with exposure to 55, 70, and 75 °C proving lethal after 45, 2, and 1 minute, respectively. While low temperatures have a preservative effect, the pathogen is susceptible to disinfectants (e.g., formaldehyde, chloramine, phenol) in standard concentrations that are effective against chlamydias [28, 32]. Low pH levels are detrimental, with an optimal pH range of 7.0–7.4. Chlamydias can persist in tap water at room temperature for 2–3 days and are sensitive to ultraviolet radiation [33].

MOLECULAR BIOLOGICAL CHARACTERISTICS OF THE PATHOGEN

The causative agent of chlamydiosis has a single circular chromosome with a genome size of just over 1.166 million base pairs; the guanine-cytosine content is 39.1–39.4%. It also contains a cryptic plasmid, *pCfe1*, approximately 7.6 thousand base pairs in size, with eight genes [31, 34].

The chlamydial genome is highly conserved and compact, a feature characteristic of *C. felis* as well [20, 35]. Due to their intracellular lifestyle, chlamydias have undergone genome reduction, which is an adaptation rather than degradation [22]. The chlamydial envelope consists of three layers: an inner bilipid membrane, an intermembrane structure (periplasmic space), and an outer lipopolysaccharide membrane containing inclusions of the major outer membrane protein (MOMP) and polymorphic outer membrane proteins (POMP – pmp1, pmp7, pmp13, and others), as well as cysteine-rich proteins. POMP includes the outer membrane proteins OMP1 and OMP2. These proteins are involved in the pathogen's adhesion to host epithelial cells [31, 34, 33, 36]. MOMP and OMP2 proteins contain genus-, species-, and type-specific epitopes, which can lead to cross-reactions. The MOMP protein (also known as OmpA – outer membrane protein A) is found in both EB and RB and functions as both an adhesin and a porin [31, 35, 37]. Outer membrane proteins are highly conserved among isolates [38].

The chlamydial envelope lacks peptidoglycan but contains a polypeptide that, together with covalently bound lipoproteins, lipopolysaccharides, and outer membrane proteins, provides mechanical strength to the envelope [26, 31, 39]. Experimental data show that polymorphic membrane proteins (pmp) possess multiple biological and immune functions, including evasion of the host immune response, induction of inflammatory and immune reactions, and tissue tropism. The pmp genes show a high degree of intra- and interspecies heterogeneity in their amino acid sequences and sizes, but they can be identified by the presence of conserved amino acid sequences GGA(I, L, V) and FxxN, repeated in the N-terminal region and the C-terminal translocation block / β-domain [40, 41, 42]. The number of pmp genes varies among *Chlamydia* species, ranging from 9 to 21; 12 have been identified in *C. felis*. The similarity between the nucleotide sequences of pmp is high, with non-synonymous single nucleotide polymorphisms (SNPs) limited to one site in each of the pmp1, pmp9, and pmp20 genes. It can be assumed that the pmp genes of *C. felis* are highly conserved among strains from different geographical regions [9, 37, 41, 42]. Pmp1 and pmp7 are likely immunodominant proteins [42].

Cryptic plasmid genes were found in cats with severe clinical symptoms of chlamydial infection. The cryptic plasmid is often associated with chlamydial virulence [18, 43]. The plasmids contain non-coding RNA and 8 open reading frames (ORF1–8), with the functions of 5 of them being sufficiently studied. Analysis of the nucleotide sequences of the open reading frames showed that the proteins encoded by ORF1 (pgp7) and ORF2 (pgp8) are homologs of integrase and recombinase, respectively, and are responsible for regulating plasmid replication, while ORF3 (pgp1) is an analog of DnaB helicase and is involved in unwinding double-stranded DNA during replication. ORF4 encodes the pgp2 protein, whose function has not yet been determined. One of the main frames is ORF5, encoding the pgp3 protein, which may be a marker of chlamydial infections. ORF6 encodes the pgp4 protein, containing 101–102 amino acids, and ORF7 and ORF8 encode the pgp5 and pgp6 proteins, respectively, which may be involved in plasmid replication. The plasmids encode various proteins involved in the replication of the plasmid itself, but it is not established whether they affect communication with the host cell; it has only been demonstrated that the pgp3 protein is secreted in the cytosol of *Chlamydia*-infected cells. The role of plasmids is not yet fully understood, although it has been shown that pgp1 (ORF3), pgp2 (ORF4), pgp6 (ORF8), and pgp8 (ORF2) are essential genes for plasmid maintenance, while pgp3 (ORF5), pgp5 (ORF7), and pgp7 (ORF1) are less essential [43].

Chlamydiae possess a specialized type III secretion system (T3SS), which allows these bacteria to inject virulence factors directly into the cytosol of the target cell of a susceptible organism, leading to disruption of adaptive immune response mechanisms (hindering the fusion of the phagosome with the host cell lysosome) [32, 33]. Type III secreted effectors include the translocated actin-recruiting phosphoprotein, necessary at the initial stage of infection, the family of inclusion membrane proteins (internal compartments) required for the development of this inclusion, and the effector chlamydial outer protein N (CopN), involved in the late disruption of type III secretion activity. Other key factors include proteins playing a central role in chlamydial development and gene regulation, such as histone-like proteins HctA and HctB; cysteine-rich proteins (OmpA and OmpB); proteins responsible for the permeability of the outer membrane to nutrients (OmpA and PorB), as well as the global gene regulatory factor Euo [11].

Comparison of the *C. felis* genome with other members of the genus *Chlamydia* revealed that 795 genes are common to them, while 47 genes are unique to *C. felis*. It was also found that orthologous genes show a similar divergent pattern, except for 14 genes that have accumulated the most mutations, suggesting that these *C. felis* genes may be involved in evolutionary adaptation [31]. There is diversity among *C. felis* strains, which differ in their virulence [5, 8].

Chlamydiae are characterized by a unique developmental cycle consisting of two phases: extracellular and intracellular. In the extracellular phase, the pathogen is represented by EBs, which are infectious. In the intracellular phase, the pathogen exists as non-infectious RBs [8, 26, 32]. Elementary bodies, responsible for infecting target cells, are protected by a three-layer envelope, which, together with their spherical shape, provides resistance to physical and chemical factors in the extracellular environment. Reticulate bodies are also spherical but have a reticulate structure, making them polymorphic and possessing a thin envelope [14, 17, 32]. Briefly, the reproduction cycle consists of sequential stages: 1) adsorption onto host cell receptors; 2) penetration of EBs into the cell via endocytosis and residence in a phagosome; 3) suppression of phagosome-lysosome fusion; 4) differentiation of EBs into RBs; 5) multiplication of RBs by binary fission; 6) differentiation of RBs into EBs; 7) release of EBs from the cell via lysis [44].

The cycle initiates when a susceptible host cell encounters the pathogen, the EBs first adhere to sialic acid on the cell membrane and then penetrate into the cytoplasm. Once inside, they form intracytoplasmic inclusions near the host cell nucleus, Golgi apparatus and endoplasmic reticulum. The metabolic activity of EBs, constrained by a limited set of enzymes, is sustained by the host cell's reserves. From these reserves, EBs obtain the necessary metabolites and adenosine triphosphate (ATP) for their replication. Inside the inclusions, EBs transform into RBs, which then replicate via binary fission. Following replication, RBs differentiate back into infectious EBs. These new EBs are released from the host cell either by lysis or extrusion, enabling the infection to spread. The entire cycle is completed within 48 to 72 hours [8, 17, 30, 31, 32, 33, 45].

Currently, another stage of the chlamydial developmental cycle is recognized: the persistence stage. This latent form, also known as non-infectious aberrant bodies (ABs), is induced under stressful conditions, such as exposure to *β*-lactam antibiotics (e.g., penicillins, cephalosporins, carbapenems). In this state, the bacteria do not transform into EBs and become a non-replicating intracellular form; ABs are characterized by the absence of MOMP and the overproduction of stress proteins, notably Chsp60 (chlamydial heat shock protein 60, 60 kDa heat shock proteins). This response allows the infection to persist, contributing to chronic inflammation. The latent form is reversible if the stressor is removed: ABs will revert to the RB form, begin the replication phase, and continue development, transforming into EBs [31, 45]. Reticulate bodies differ from EBs in that they are dedicated to nutrient uptake and replication, a process that can lead to their asynchronous differentiation back into EBs. The latter can persist for long periods in the intercellular space without being phagocytized. There is a constant resilience between the host and the pathogen, and some clinical chlamydial infections can be asymptomatic for several months [17, 31, 33].

Chlamydiae exhibit epitheliotropism. Chlamydial replication in epithelial cells leads to the destruction of the epithelial layer with the formation of scars and adhesions. During prolonged persistence in epithelial cells, the pathogen can enter the bloodstream and hematogenously spread to parenchymal organs and lymphoid tissues. *C. felis* affects the eyes and upper respiratory tract (nose or throat) of cats [8, 33]. The pathogen is most commonly found in multi-cat households, shelters, and catteries. The main carriers of the pathogen are diseased cats, however, dogs can also serve as a reservoir for *C. felis*. Transmission occurs mainly through contact with infected material, such as ocular discharge [46]. There is evidence that animals can become infected through sexual contact. In female cats, the pathogen localizes in the cervix, while in male cats, the testes are infected and the pathogen is shed during ejaculation [47]. It was later proven that *C. felis* can be isolated from rectal and vaginal swabs taken from cats with natural and experimental chlamydial conjunctivitis, but the possibility of sexual transmission is unclear; likely, the intestine and reproductive organs may be sites of persistent infection [8, 35]. Experimental ocular infection of cats led to the isolation of *Chlamydiae* from the vagina and rectum in 50% and 40% of cases, respectively, demonstrating that *C. felis* is not limited to the conjunctival mucosa [35].

PATHOGENESIS AND CLINICAL SIGNS OF CHLAMYDIOSIS

The incubation period ranges from 5 to 15 days [32]. Infection caused by *C. felis* leads to the development of acute or chronic conjunctivitis in cats. The infection typically begins in one eye and may subsequently spread to the other, resulting in bilateral conjunctivitis. Other clinical manifestations include unilateral or bilateral epiphora, hyperemia of the nictitating membrane, blepharospasm, mucoid/mucous/serous/seropurulent discharge, inflammation and chemosis of the conjunctival membranes. These symptoms persist for 22–45 days. Meanwhile, a mild form of conjunctivitis can last for several months [9, 35, 46, 48, 49]. Clinical signs from the respiratory system are usually minimal [48, 49]. Some cats may experience weight loss. Fever, decreased or loss of appetite, lethargy, sneezing, serous nasal discharge, and enlargement of the submandibular lymph nodes are also possible [8, 32, 35]. The condition of most cats remains satisfactory, but a small number of animals develop serious respiratory distress, severe wheezing, which, if left untreated, can lead to death from pulmonary edema or asphyxia due to poorly developed mouth breathing. In the case of systemic infection, which is rare, the pathogen spreads through the bloodstream to almost all organs, joints, the brain and spinal cord, which is highly likely to be fatal [8, 17, 32]. Most untreated cats develop chronic conjunctivitis, which is characterized by mild conjunctival hyperemia and scant ocular discharge, or they may develop a follicular form of the condition [32, 35]. Ocular discharge resolves within 60 days, but the absence of clinical symptoms does not guarantee recovery; chlamydial infection can persist asymptomatically for several months [17, 35]. Co-infection caused by a combination of *C. felis* with other pathogens (feline calicivirus, feline herpesvirus type 1, mycoplasmas) increases the severity of the disease and the duration of chlamydial shedding into the environment. Chlamydial infection can manifest as peritonitis [8, 50]. In experimental infection of cats, chlamydiae were detected in conjunctival swabs for up to 8 months after infection, suggesting a long period of asymptomatic carriage. Aerosol and oral administration of the pathogen to cats led to the development of upper respiratory tract pathology and mild gastritis. An association between chlamydial infection and lameness in sick cats has also been identified, manifesting two weeks after the onset of conjunctivitis in 10 out of 19 infected animals, although this fact requires more detailed study [8, 17, 35]. Furthermore, experimental inoculation of the pathogen into the genital tract resulted in chronic salpingitis with subsequent spread to the oviduct [46]. Literature data are conflicting regarding reproductive disorders; it has been suggested that the pathogen is a cause of abortions or missed pregnancy in cats. Some authors have indirect evidence that *C. felis* can cause abortion, neonatal mortality, and infertility, but definite cause-and-effect relationships have not been established [8, 10]. Infertility and abortions are the most serious consequences of the chronic form of chlamydiosis [40]. It is believed that some metabolites (isoleucine) may act as inhibitors of chlamydial growth and contribute to the latent course of chlamydial infection [32]. Although chlamydiosis can be asymptomatic and usually not fatal, the infected animal remains a carrier capable of transmitting the pathogen [51].

PATHOLOGICAL CHANGES IN CHLAMYDIOSIS OF CATS

Post-mortem examination reveals the following pathological changes: hyperemia and enlargement of the liver and spleen, lymph nodes; signs of pneumonia and pericarditis; hemorrhages on serous membranes and under the renal capsule; lesions of the digestive tract. The most characteristic changes are observed in lung tissues, mediastinal and bronchial lymph nodes [32, 47]. At the macroscopic level, the following phenomena are visible: serous conjunctivitis, hyperplasia of splenic lymphoid nodules, catarrhal bronchitis, subtotal and total interstitial pneumonia and pneumosclerosis, hyperplasia and serous lymphadenitis of regional lymph nodes. Histological examination reveals the following morphological criteria of chlamydial infection at the microscopic level: interstitial pneumonia, pneumosclerosis, fibrinous-purulent pleuropneumonia, interstitial bronchopneumonia (thickening of interstitial alveolar septa due to infiltration of inflammatory cells and exudate in bronchial tissue), hyperplasia and serous lymphadenitis of mediastinal and bronchial lymph nodes, hyperplasia of lymphoid nodules of the spleen and intestinal mucosa, venous hyperemia of the liver and kidneys, fatty and granular dystrophy of the liver. There is also marked congestion of organs with diapedesis phenomena around capillaries and hyperplasia of the islet regions secreting insulin in the pancreas. Mild external clinical symptoms may be combined with serious degenerative, destructive changes in internal organs and tissues [17, 41, 52].

LABORATORY DIAGNOSIS OF CHLAMYDIOSIS

Laboratory diagnosis is based on the direct detection of the pathogen in the material and the determination of anti-chlamydial antibodies through serological reactions (enzyme-linked immunosorbent assay, complement fixation test, long-term complement fixation test). Serological reactions in the diagnosis of chlamydiosis are screening tests requiring further confirmation by other methods. There are laboratory methods for detecting RBs and EBs. These include cytological (this analysis does not require special equipment, but its sensitivity and specificity are quite low), immunological (direct and indirect immunofluorescence assay, enzyme immunoassay), cultural (considered the gold standard but labor-intensive and time-consuming, used in scientific practice), and molecular biological (polymerase chain reaction – the most sensitive, rapid, and reliable) methods [33, 53, 54]. Molecular analysis is the most reliable for making a definitive diagnosis of "feline chlamydiosis", as the disease can have various clinical manifestations and occur in a subclinical form (bacterial carriage) [51].

Due to the inability of *Chlamydia* to grow on artificial nutrient media, 6–7-day-old specific pathogen-free chicken embryos are used for their cultivation, infected into the yolk sac. Embryo death within the first two days after infection is considered non-specific. The incubation period lasts up to 13 days, which is its main disadvantage. Cultivation in chicken embryos until 1965 was the only method for isolating and propagating *Chlamydia*; however, the subsequent use of cell cultures simplified this procedure [17, 55]. To reduce cultivation time to 48–72 hours, sensitive cell cultures are used; in particular, for growing *C. felis*, cell lines such as Vero (a continuous cell line from the kidney of the African green monkey *Chlorocebus aethiops*), McCoy (a hybrid line of human synovial cells and mouse fibroblasts), CrFK (a continuous cell culture of feline kidney), BHK-21 (a cell line from the kidney of a newborn Syrian hamster), HeLa (human cervical cancer cells), L929 (a mouse fibroblast cell line) are used [17, 42, 50]. To increase the adsorption and penetration of *Chlamydia* into the cell, centrifugation or physicochemical methods to reduce the resistance of cell cultures are used. For example, polycations (DEAE-dextran – diethylaminoethyl-dextran) are used to treat the monolayer before infecting cell cultures; they neutralize the anionic surface of *Chlamydia*, creating conditions for contact. After contact, antimetabolites are added to the nutrient medium, which slow down the metabolism of the cells but do not affect *Chlamydia*, indirectly stimulating their reproduction. Most often, *Chlamydia* are cultured on cell cultures treated with cycloheximide or analogs (L-cysteine hydrochloride, hydrocortisone, colchicine) [56]. Conjunctival, rectal, and vaginal washes are used to isolate the bacteria, placed in a special transport medium; the biological material must be examined no later than 24 hours after collection, as freezing and thawing can be detrimental to *C. felis* [50].

TREATMENT AND PROPHYLAXIS OF CHLAMYDIOSIS

Due to the systemic course of chlamydiosis, local therapy is ineffective [8]. Antibiotics such as doxycycline, azithromycin, and amoxicillin are used in long courses, supplemented by local treatment. For prophylaxis, melatonin, serotonin, or their derivatives can be used. According to some data, melatonin inhibits the initial progression of the chlamydial developmental cycle, preventing the occurrence of intracellular infection and the transformation of EBs into RBs [57]. Chlamydial bacteriophages can also be used as an alternative to antibiotics, thereby addressing the problem of antibiotic resistance. In particular, antibiotics are unable to suppress chlamydial EBs, which are metabolically inert and difficult to eradicate [42]. Chlamydiaphages cause inhibition of the developmental cycle and delay the transition of RBs to EBs, thereby reducing the possibility of infecting other cells. Chlamydial bacteriophages are found in only six species, including *C. felis* [43].

Vaccination for the prevention of chlamydial infection usually begins at 8–9 weeks of age, followed by revaccination after 2–4 weeks and then annually. It should be noted that information on the duration of immunity is limited. Live attenuated and inactivated adjuvanted vaccines are available on the market. There are no reliable data to compare their effectiveness. Vaccines are effective in protecting against clinical symptoms of the disease but not against the occurrence of infection. They reduce chlamydial replication in the body and lessen the clinical manifestations of infection. According to World Small Animal Veterinary Association recommendations, vaccination against chlamydiosis caused by *C. felis* is non-core and is recommended in cases where there is a risk of infection (outdoor access), as part of disease control measures, in crowded conditions, shelters, and for animals participating in shows and breeding [30, 50, 58]. No strong and convincing correlation has been found between the level of specific antibodies and the resistance of vaccinated cats to *C. felis* infection [50].

Immunity against the chlamydial pathogen is weak or short-term and does not protect against reinfection. Cats develop resistance to infection with age. Both humoral and cellular immunity are involved in response to chlamydial infection [8]. Cellular immunity is considered to play a decisive role in protecting the body against this pathogen. MOMP and POMP proteins are targets for protective immune responses of the body. Kittens are initially protected by antibodies for 9–12 weeks when receiving colostrum from recovered queens [8, 30].

At the initial stage of the infectious process, polymorphonuclear lymphocytes are involved in the immune response. Polyclonal activation of B-lymphocytes is of primary importance in protection against chlamydiosis. Nevertheless, the leading role in immune defense against chlamydiosis belongs to T-helpers, which activate the phagocytic activity of macrophages [59].

One of the key virulence factors of *Chlamydia* is the proteasomal protein CPAF (chlamydial protease / proteasome-like activity factor), which suppresses neutrophil activation. In the presence of CPAF, the expression of the anti-apoptotic protein myeloid cell leukemia 1 (Mcl-1) is induced, which promotes the degradation of pro-apoptotic molecules such as BCL-2-like protein 11 (Bim). Thus, *Chlamydia* prevents apoptosis of host cells, leading to a prolonged period of persistence, i.e., replication inside host cells. CPAF degrades the major histocompatibility complex (MHC), hindering antigen presentation to T-cells. Furthermore, members of the genus *Chlamydia* increase the expression of PD-L1 (Programmed Death-Ligand 1) in host cells. The binding of PD-L1 to the PD-1 (programmed cell death protein 1) receptor on the surface of T-cells represents a negative signal that suppresses T-cell receptor (TCR) activation [60]. Thus, *Chlamydia* is a bacterium well-adapted to many of the host's protective mechanisms, which complicates the process of its elimination and confirms the need for developing effective prevention methods.

CONCLUSION

Analysis of modern scientific data on feline chlamydiosis allows us to conclude that information on the epizootic situation does not reflect the true prevalence of this disease. *C. felis* is a pathogen capable of infecting not only cats but also other animals and humans.

The complex developmental cycle, the ability to overcome host immunity, and long-term persistence in the body complicate its eradication. Diagnosis of chlamydiosis is difficult due to the low stability of the pathogen outside the body; the most sensitive method is PCR. The long course of treatment, transition to a chronic course, and regular relapses reduce the quality of life of companion animals, and therapy requires high economic costs.

Prevention involves timely vaccination; however, immunity against the chlamydial pathogen is weak or short-lived and does not protect against reinfection. Moreover, cellular immunity is more important than humoral immunity. Hence, the need for preventive measures to protect animals from the disease arises.

For the purpose of developing successful treatment and prevention regimens for feline chlamydiosis, a more detailed study of *C. felis* is required.

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FMD under control: enhanced FMD surveillance in the Russian Federation results in the WOAH Official Recognition of Zone Western Siberia – Urals as FMD-free

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ABSTRACT

Introduction. Foot-and-mouth disease is one of the key threats to global animal welfare and international economic relations. Like any other transboundary animal disease, it shall be notified to the World Organisation for Animal Health (WOAH) in accordance with the relevant international standards. By 2016, the largest part of the territory of the Russian Federation (i.e. 50 subjects and 2 federal cities) had been recognized by the WOAH as an FMD-free zone without vaccination. From 2021 to 2023, 4 more zones of the country were granted the status of freedom from foot-and-mouth disease with vaccination. At the end of 2024, only 10 subjects of the Russian Federation, all located within zone Western Siberia – Urals, lacked official recognition.

Objective. Descriptive analysis of the animal health situation in the Russian Federation from 2021 to 2024: substantiating success of the Rosselkhoznadzor's systematic approach to regionalization in accordance with the WOAH Terrestrial Animal Health Code.

Materials and methods. Various information sources were used to collect and analyze materials on the animal health situation in Russia, including the USSR archives, veterinary reports, and the WOAH statistics.

Results. FMD situation was analyzed in 10 subjects of the Russian Federation bordering on the Republic of Kazakhstan, with an emphasis on FMD control measures, regionalization and zoning. This section focuses on distribution of statuses across Russia's administrative subjects and the historical records on the disease outbreaks. It also outlines regulatory and surveillance measures implemented by the Federal Service for Veterinary and Phytosanitary Surveillance (the Rosselkhoznadzor) to manage the FMD status in various regions, taking into account the prophylactic measures implemented in place. The paper describes these measures and their results step-by-step, showing a dynamic improvement of the FMD surveillance system.

Conclusion. On 29 May 2025, at the 92nd WOAH General Session of the World Assembly of Delegates, Zone Western Siberia – Urals was officially recognized as a foot-and-mouth disease-free zone with vaccination. This decision completes the process of structuring the territory of the Russian Federation into 6 zones. The WOAH-granted disease-freedom statuses confirm efficacy of the preventive and surveillance measures, which is crucial to ensure global epizootic stability. These accomplishments result from the efforts jointly taken by the Rosselkhoznadzor and its subordinate institution the Federal Centre for Animal Health.

Keywords: foot-and-mouth disease, surveillance, regionalization, zoning, WOAH, analysis, FMD-free status

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Ящур под контролем: официальное признание ВОЗЖ статуса благополучия зоны «Западная Сибирь – Урал» как итог совершенствования мер по надзору за заболеванием на всей территории Российской Федерации

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РЕЗЮМЕ

Введение. К числу основополагающих угроз, оказывающих влияние на состояние мировой эпизоотической обстановки и на межгосударственные экономические взаимоотношения, относится ящур – заболевание, подлежащее обязательной нотификации во Всемирную организацию здравоохранения животных (ВОЗЖ).

Большая часть территории Российской Федерации (50 субъектов и 2 города федерального значения) в 2016 г. была признана ВОЗЖ зоной, свободной от ящура без вакцинации. Еще 4 зонам нашей страны в период с 2021 по 2023 г. присвоен статус свободы от ящура с вакцинацией. На конец 2024 г. официальное признание данного статуса отсутствовало лишь для 10 субъектов Российской Федерации, входящих в зону «Западная Сибирь – Урал».

Цель исследования. Описательный анализ эпизоотической обстановки по ящуру в Российской Федерации в период с 2021 по 2024 г.; аргументация успешности системного подхода Россельхознадзора к регионализации в соответствии с положениями Кодекса здоровья наземных животных ВОЗЖ.

Материалы и методы. Для сбора и последующего анализа информационных материалов по эпизоотической ситуации по ящуру в России использованы различные источники, в том числе архивные материалы СССР, ветеринарная отчетность и статистические данные ВОЗЖ.

Результаты. Проведен анализ эпизоотической ситуации по ящуру в 10 субъектах Российской Федерации, граничащих с Республикой Казахстан, с акцентом на меры контроля, регионализации и зонирования по ящуру. Рассматривается статусное распределение административных субъектов Российской Федерации и исторические данные о вспышках заболевания. Описаны нормативные действия и регламентирующие меры, реализуемые Федеральной службой по ветеринарному и фитосанитарному надзору (Россельхознадзор) в отношении регулирования зоосанитарного статуса по ящуру в регионах с учетом проводимых профилактических мероприятий. Изучена последовательность этих мероприятий и их результаты в динамике совершенствования надзорных мер по ящуру.

Заключение. 29 мая 2025 г. на 92-й Генеральной сессии Всемирной ассамблеи делегатов ВОЗЖ зона «Западная Сибирь – Урал» официально признана зоной, свободной от ящура с вакцинацией. Это решение завершает структурирование регионов Российской Федерации на шесть зон. Статусы благополучия ВОЗЖ подтверждают эффективность профилактических и надзорных мер, что важно для глобальной эпизоотической стабильности. Эти достижения – результат совместной работы Россельхознадзора и подведомственного Федерального центра охраны здоровья животных.

Ключевые слова: ящур, надзор, регионализация, зонирование, ВОЗЖ, анализ, статус благополучия

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INTRODUCTION

In view of the recent world events, the Russian Federation is actively building new foreign economic ties. Recently, there has been a significant increase in import and export of meat and dairy products with many countries, including, inter alia, China, Kazakhstan and Mongolia [1, 2]. International trade dynamics is boosting the variety and volumes of the traded animal products, however, simultaneously is elevating the risk of introducing highly dangerous animal diseases [3, 4, 5]. Foot-and-mouth disease (FMD) is considered to be one of the essential threats to global epizootic stability and international economic relations. As a transboundary animal disease, it shall be notified to the World Organisation for Animal Health (WOAH) in accordance with the international standards [6, 7, 8].

In addition to the risks associated with import of live animals and livestock products, wild ungulates in Mongolia, Kazakhstan, and Turkey pose an extra risk [9, 10, 11, 12], because they are a natural reservoir of FMD virus (FMDV) in the wild, able of complicating the FMD situation in these regions [13].

As the WOAH data suggest, the FMD epizooty is still of great concern [14]. Consequently, enhanced control measures taken by the veterinary services are a crucial preventive component aimed at minimizing the risk of FMD introduction [15, 16, 17, 18].

By 2016, the greater part of the Russian Federation territory, i.e. 50 zone and 2 Federal Cities (Moscow and Saint Petersburg) had been recognized by the WOAH as an FMD-free zone without vaccination [19].

Between 2020 and 2024, the Russian Federation kept submitting dossier materials to the WOAH for reviewing FMD statuses in the zones recognized as FMD-free with vaccination.

Coordinated efforts of the Rosselkhoznadzor and the Federal Centre for Animal Health succeeded in getting the official WOAH FMD-free status with vaccination for the following zones:

– Zone I South, which includes 13 zone of the Southern and North Caucasian Federal Districts (2021);

– Zone III Eastern Siberia, which includes the Republics of Buryatia, Tyva and Kosh-Agachsky Raion of the Altai Krai (2022);

– Zone IV Sakhalin, which includes the Sakhalin Oblast and the Kuril Islands (2021);

– Zone V Far East, which includes the Amur Oblast, the Jewish Autonomous Oblast, the Zabaikalsky, Primorsky and Khabarovsk Krais (2023).

At the end of 2024, only 10 zone of the Russian Federation lacked the officially recognised status of FMD freedom. This territory included the Russian Federation zone bordering on the Republic of Kazakhstan and belonging to zone II Western Siberia – Urals with vaccination (Fig.).

MATERIALS AND METHODS

Various information sources were used to assess the FMD situation in the RF. In particular, the USSR archives – documents of the established Form No. 3-Vet "Logbook for Documenting District (City) Epizootic Status"; official veterinary records – information on registered FMD cases in certain regions of the RF, as well as information on the FMD-vaccination campaigns. The country's FMD statistics, collected and verified through the official reports published on the WOAH platform, were accordingly analysed.

Data on the infection sources in the Russian Federation zone included into zone II Western Siberia – Urals were collected as a follow-up to the analysis of the archives and official reports. Changes in the administrative statuses of the Russian Federation zone in zone II Western Siberia – Urals were reviewed pursuant to the decisions made by the Rosselkhoznadzor; effectiveness of pathogen monitoring and control measures were accordingly analyzed.

The WOAH Terrestrial Animal Health Code, 2019 and 2024 editions (hereinafter referred to as the WOAH Code), was used as a basic reference to define the criteria for the disease-freedom status and to assess compliance with the veterinary regulatory framework.

RESULTS AND DISCUSSION

As the USSR archives and official veterinary records of the Russian Federation show, the last FMD outbreaks in zone II Western Siberia – Urals were registered in the following subjects:

the Tyumen Oblast – in 1949;

the Altai Republic (Ust-Kansky, Ust-Koksinsky, Shebalinsky, Ongudaysky, Chemalsky, Maiminsky, Choysky, Ulagan and Turochak Raions) – in 1966;

the Samara Oblast – in 1970;

the Omsk Oblast – in 1972;

the Chelyabinsk and Novosibirsk Oblasts – in 1973;

the Kurgan Oblast – in 1974;

the Altai Krai – in 1974;

the Saratov Oblast – in 1984;

the Orenburg Oblast – in 2021.

The principal approach used in the USSR to eradicate FMD included regular preventive measures, i.e. vaccination, and stamping-out of FMD-susceptible animals in the outbreak.

Concurrently, ring vaccination was implemented together with stringent restrictions on movement of animals and animal products, thus, mitigating the risk of the disease spread.

The measures were taken in accordance with the "Instruction on FMD prevention and eradication", developed on the basis of a detailed analysis of epizootological data, as well as on the practical experience, which provided a scientific substantiation for the measures and techniques used.

To minimize the risk of FMD introduction into the RF, some Russian Federation subjects – namely, the Chelyabinsk, Kurgan, Tyumen, Omsk, and Novosibirsk Oblasts, the Altai Krai, and the Altai Republic – were designated as part of the zone with annual FMD vaccination. This

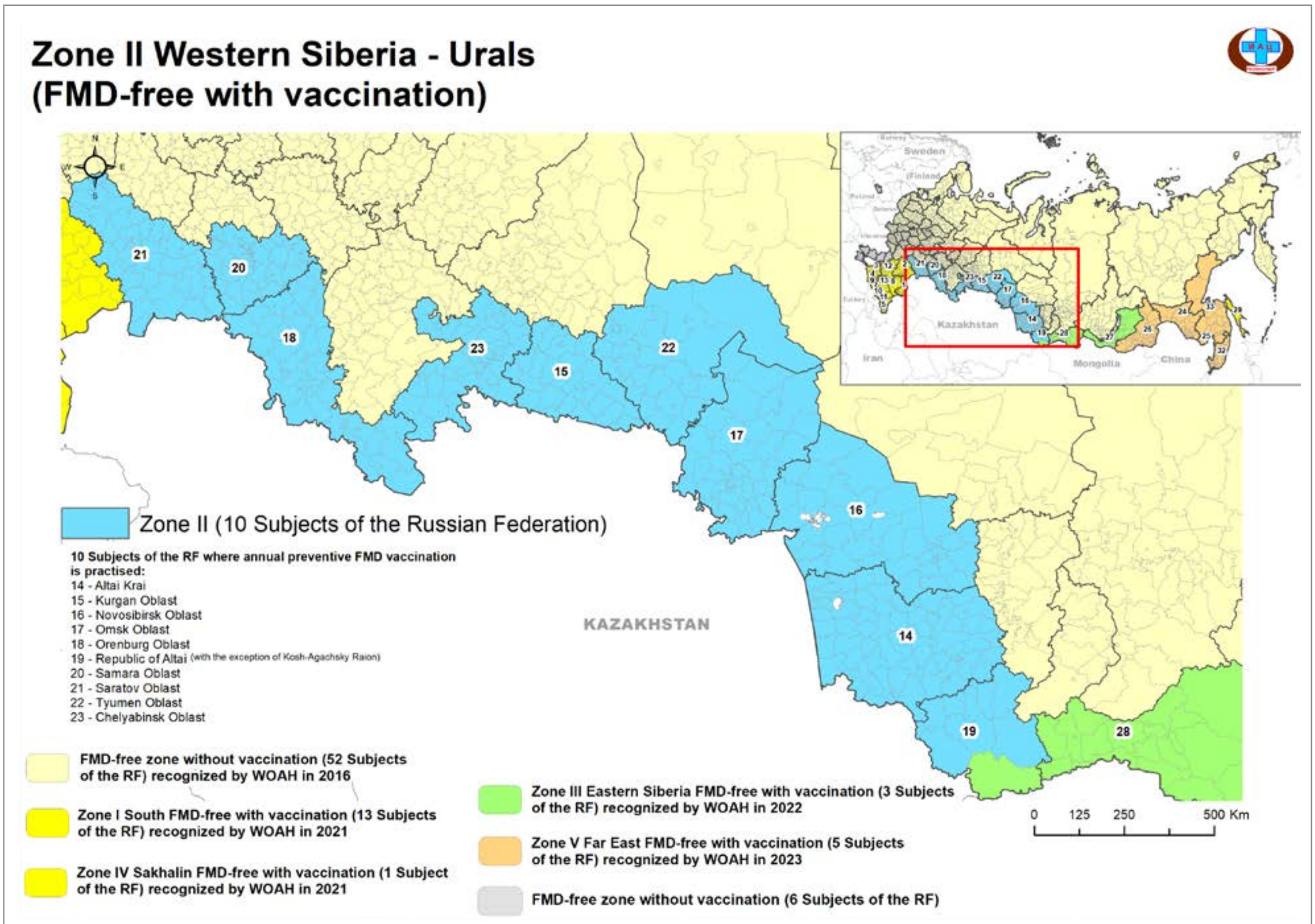

Fig. Map of zone II Western Siberia – Urals administrative devision (provided by the Federal Centre for Animal Health Information and Analysis Center) decision enabled to timely implement required preventive measures, thereby reducing the likelihood of outbreaks by establishing a framework for rapid response and control of FMD. Thus, this comprehensive approach based on preventive measures and strict sanitary control, has become a cornerstone for ensuring safety of the livestock industry and maintaining epizootic stability in the region.

FMD vaccination campaigns in these areas were implemented up to and including 2019. In 2019, in compliance with the Order of the Minister of Agriculture of the Russian Federation and as part of implementation of the national project "International Cooperation and Export", anti-FMD vaccination of susceptible animals was officially ceased and this decision aligned with the changes in the veterinary control regulations.

From 2017 to 2021, the aforementioned 10 Russian Federation subjects shared borders with the WOAH-officially recognized zones of the Republic of Kazakhstan that were FMD-free without vaccination.

Therefore, in 2020, those 10 Russian Federation zone were incorporated into the FMD-free zone where, according to article 8.8.2 of the WOAH Code: "no FMD-specific vaccination had been practiced during the preceding 12 months; no outbreaks or evidence of infection had been detected during the preceding 12 months; no vaccinated livestock had been introduced since cessation of vaccination; disease and infection surveillance complied with the WOAH Code provisions; a regulatory framework was in place for FMD early detection, prevention, and control".

In August 2021, the "Dossier on FMD Control and Surveillance in the Russian Federation" (hereinafter, the Dossier) was submitted to the WOAH for zone II Urals – Western Siberia, considered as FMD-free without vaccination, with the objective to be officially recognized by the WOAH. However, in December 2021, this Dossier was withdrawn due to an outbreak caused by FMDV type O in the Orenburg Oblast. Following the FMD outbreak on 29 December 2021, restrictive measures were imposed by a Decree of the Governor of the Orenburg Oblast in the Karagachsky Selsoviet of the Belyaevsky Raion, the Orenburg Oblast [20]. The susceptible animals kept in the risk and protection zones were vaccinated with inactivated adsorbed monovalent FMD vaccine against type O.

Pursuant to Decree of the Governor of the Orenburg Oblast, the FMD-related restrictive measures were lifted on 14 February 2022.

Pursuant to the official letter from the Rosselkhoznadzor as of 28 December 2021, FMD vaccination for cattle and small ruminants was resumed in January 2022 in the 10 zone of the Russian Federation bordering on the Republic of Kazakhstan. This measure was implemented to mitigate the risk of FMD introduction into these regions and, in the event of an outbreak, to contain further FMD spread.

Field efficacy of the FMD vaccination is assessed, according to the WOAH recommendations, with the help of monitoring tests that show antibody titres in the vaccinated herds. According to the WOAH guidelines, the immunity response in cattle and small ruminants shall account for at least 80%.

The post-vaccination monitoring program involves a series of tests in the vaccinated animals aimed at objectively assessing their FMD immunity status, as well as at detecting hidden circulation of this pathogen in the susceptible livestock. These measures are implemented pursuant to the official Order of the Rosselkhoznadzor, which is annually updated and approved. Standardized laboratory methods are used by the Federal Centre for Animal Health for the research purposes and the enzyme-linked immunosorbent assay is the main tool, which is distinguished by its capacity to quantitatively assess post-vaccination immune response and antibody titres in animals.

In response to the FMD outbreak in the Orenburg Oblast and due to the initiation of the vaccination campaign in zone II Urals – Western Siberia, on 29 December 2021, the Rosselkhoznadzor issued a decision changing the status of the 10 zone within the zone to FMD-infected with vaccination.

In January and June 2022, the WOAH suspended the official status of FMD freedom without vaccination for the relevant zones in Kazakhstan (including those ones that border on the regions of the RF), following an FMD outbreak in the Karaganda Oblast and the subsequent vaccination [20]. Considering these circumstances and pursuant to the Rosselkhoznadzor's Decision of 22 December 2022 "On granting statuses to the Russian Federation regions for contagious animal diseases and specifying movement conditions for commodities subject to the state veterinary surveillance", 10 Russian regions bordering on Kazakhstan were grouped into three isolated zones based on the established regionalization requirements:

– Zone II Saratov – Samara: an FMD-free zone with vaccination, comprising 2 zone of the RF;

– Zone VI Urals – Western Siberia, which has the status of an FMD-free zone with vaccination and comprises 7 zone of the RF;

– Zone without the WOAH FMD-free status with vaccination, comprising 1 Russian Federation Subject (the Orenburg Oblast) established in accordance with Article 8.8.5 of the WOAH Code.

The isolated zone II Saratov – Samara and zone VI Urals – Western Siberia were established due to:

– the absence of recorded FMD outbreaks or evidence of FMDV for not less than the preceding 24 months;

– the absence of FMDV transmission events over the preceding 12-month period;

– the FMD surveillance conducted in accordance with Articles 8.8.40–8.8.42 of the WOAH Code;

– the routine anti-FMD vaccination of all susceptible livestock (cattle and small ruminants), using a vaccine compliant with the requirements of Chapter 3.1.8 of the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, since January 2022;

– compliance with the WOAH import requirements and adherence to regulations governing the movement of live animals and livestock products;

– the specific operational characteristics of livestock establishments in these districts (specifically, their self-sufficient and independent functioning in terms of both feed supply and processing).

In August 2023, two dossiers documenting FMD control and surveillance results in the Russian Federation were submitted to the WOAH for zone II Saratov – Samara and zone VI Ural – Western Siberia – both recognized as FMD-free with vaccination – with the aim of obtaining an official WOAH status.

After the dossier review, the Scientific Commission concluded that the application does not fully comply with the provisions outlined in Chapter 8.8 of the WOAH Code.

Before resubmitting the application, it was necessary to implement a number of recommendations put forward by the Scientific Commission:

– provide updates on the adopted and implemented national legislation aimed at expanding the FMD case definition;

– submit data, including quantitative indicators, to objectively assess effectiveness of the implemented legislative framework;

– ensure continuous monitoring of the herd immunity status across all the vaccinated animal species. Special attention shall be paid to the territories with livestock immunity levels below 80%, as well as to regions characterized by a high risk of FMD introduction. In this context, it is recommended that systematic data collection and analysis be conducted to evaluate trends in immunity indicators, thereby enabling development of corrective measures, if required;

– include the 6–12-month-old group into post-vaccination monitoring tests. This age group is characterized by a lower number of prior immunizations, making it a key indicator for assessing effectiveness of the implemented vaccination strategy. The data obtained shall be stratified by age group. This will help to get reliable and detailed immune response indicators and will help to identify those groups that require additional protection from the standpoint of adequate immunity;

– review the existing scheme of serological tests. Specifically, it is essential to ensure that test parameters, including sensitivity and specificity, are based on detection of antibodies to non-structural proteins (NSPs) in the vaccinated animals. This methodological adjustment is crucial for securing diagnostic reliability and population-wide sample representativeness, ultimately improving precision of the epizootic situation surveillance [21];

– conduct an investigation into detection of animals showing a serological response to FMD NSPs. As part of this investigation, sample collection for serological analysis shall be conducted to enable subsequent animal monitoring, as stipulated in Article 8.8.42 of the WOAH Code;

– pay attention to segregation of livestock and control movement of both animals and products across the zones with differing sanitary and vaccination statuses. Such a measure will reduce the risk of FMD spread and ensure country's biosafety.

In 2024, following implementation of the recommendations from the WOAH Scientific Commission for Animal Diseases regarding FMD surveillance criteria, and based on the analysis of data submitted by 10 zone of the Russian Federation, on 6 May 2024, the Rosselkhoznadzor issued Decision "On granting statuses to the Russian Federation regions for contagious animal diseases and specifying movement conditions for commodities subject to state veterinary surveillance", which established a unified zone II Western Siberia – Urals with an FMD-free status with vaccination. The zone comprised 10 Russian zone bordering on Kazakhstan: the Saratov, Samara, Orenburg, Chelyabinsk, Kurgan, Novosibirsk, Omsk, Tyumen Oblasts, the Altai Krai, and the Altai Republic (with the exception for the Kosh-Agach Raion).

This decision was made pursuant to Article 8.8.4 of the WOAH Code and was based on several preliminary criteria:

– no recorded FMD outbreaks or evidence of FMDV infection for a minimum of the preceding 24 months;

– no FMDV transmission events over the preceding 24-month period;

– FMD surveillance conducted in compliance with Articles 8.8.43–8.8.45 of the WOAH Code over the preceding 24 months;

– mandatory systematic anti-FMD immunization in the target animal population since January 2022 to achieve adequate immunization rate and herd immunity;

– compliance with the WOAH Code import requirements and adherence to regulations governing movement of live animals and livestock products into the country or zone;

– compliance with the relevant provisions of paragraph 2 of Article 1.4.6 of the WOAH Code.

Consequently, these measures regulating the status modification demonstrate a systematic approach to control over contagious animal diseases in the Russian Federation. The decisions taken by the Rosselkhoznadzor in 2022–2024 on regionalization are based on the WOAH Code criteria, thus, ensuring compliance with the international standards on the veterinary surveillance and disease control, as well as contributing to preventive measures at both national and regional levels.

CONCLUSION

On 29 May 2025, during the 92nd WOAH General Session of the World Assembly of Delegates, a decision was made to grant the Russian Federation's zone II Western Siberia – Ural the official status of an FMD-free zone with vaccination.

This decision was made within the overall classification of the Russian Federation's regions into six separate zones. Five of the aforementioned zones had already been granted official WOAH FMD-free statuses.

The official FMD-free status granted to zone II Western Siberia – Urals, stretching along the border with Kazakhstan, is considered as an international recognition of the fact that the Russian Federation's territory is safe and disease-free, which fully complies with the standards of the WOAH Code. This decision confirms adherence to the most rigorous disease control protocols, which in turn demonstrate effectiveness of both preventive measures and well-organized surveillance within the country.

From 2013 to 2025, the Rosselkhoznadzor kept taking all the required measures for the whole country to be officially recognized as FMD-free. This result was achieved due to comprehensive prevention, coupled with the consistent activities mandated by the Rosselkhoznadzor's Decision "On granting statuses to the Russian Federation regions for contagious animal diseases and specifying movement conditions for commodities subject to the state veterinary surveillance". Collectively, these efforts ensured a high degree of disease control, as formally confirmed by the official statuses, which reflect successful implementation of the preventive strategies.

The granted FMD-free statuses objectively demonstrate that significant progress has been achieved in control of the infectious disease. Furthermore, they demonstrate efficacy of the preventive and organizational measures that facilitate safe trade in livestock products.

These outcomes are critical for maintaining global animal health stability and for mitigating the risk of new disease outbreaks at both regional and international levels.

The WOAH-recognized FMD-free zones in the Russian Federation significantly enhance export potential of the business operators located within them. Therefore, relevant measures have been implemented both to achieve regulatory statuses and to enhance competitiveness of the national commodities worldwide.

Official recognition from the WOAH for FMD-free zones, both with and without vaccination, is the result of a long-term and intensive collaboration between the Rosselkhoznadzor and the Federal Centre for Animal Health. The implemented measures have enabled to annually reconfirm the WOAH FMD-free status for the entire territory of the Russian Federation, which is substantiated by statistical reviews and findings from epizootiological monitoring.

Consistent measures implemented to enhance infection control have significantly improved animal health situation.

The WOAH status granted to zone II Western Siberia – Urals points to both efficacy of the applied preventive measures and to a high degree of international recognition for the properly organized veterinary services and research activities.

Consequently, the measures taken to obtain the WOAH status bolster Russia's epizootic stability and help Russia to effectively compete on the global livestock markets.

This experience can be a good example to follow in order to further enhance preventive programs ensuring high-level protection against infectious diseases that aligns with the international standards and the WOAH recommendations.

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Spread dynamics of bovine leukosis on breeding farms in the Republic of Dagestan

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ABSTRACT

Introduction. The problem of bovine leukosis on breeding farms in the Republic of Dagestan has been a pressing issue since the mid-1960s. Due to the fact that the coverage of planned serological testing did not exceed 1–2% of the existing population of susceptible animals, there was no clear understanding of the scale of leukosis spread.

Objective. Analysis of the current situation regarding the spread of bovine leukosis on breeding farms in the Republic of Dagestan.

Materials and methods. Animals infected with the bovine leukemia virus were identified using the agar gel immunodiffusion test (AGID). Animal disease control measures were assessed in accordance with the new "Veterinary Rules for the Implementation of Preventive, Diagnostic, Restrictive and Other Measures as well as for the Imposition and Release of Quarantine and Other Restrictions Aimed at Containing Bovine Leukosis as well as at Eradicating its Outbreaks" approved by Order No. 156 of the Ministry of Agriculture of Russia of March 24, 2021.

Results. The bovine leukemia virus infection rate in animals in the period 2009–2017 ranged from 0.1 to 77.3%. With the adoption of the subprogram "Prevention and Eradication of Bovine Leukosis on Farms in the Republic of Dagestan" (2018–2020) under the republican target program, serological testing coverage has increased by more than 5.7 times over the past seven years, and the detection rate of new seropositive animals has decreased from 23.6 to 0.1% in 2024.

Conclusion. Epizootological analysis revealed a heterogeneous structure and dynamics of the bovine leukosis spread in cattle. The system of measures aimed at prevention and eradication of bovine leukosis in cattle implemented in the Republic of Dagestan has led to sustainable stabilization of the disease situation and a reduction in the infection rate in animals on breeding farms. Owing to the veterinary service's systematic efforts to eradicate the viral infection, breeding farms are now completely free from bovine leukosis. Health improvement work, including the use of serological diagnostics and immediate culling of AGID-positive animals, continues.

Keywords: bovine leukosis (BL), bovine leukemia virus (BLV), spread, breeding farms, serological and hematological testing, health improvement measures, Republic of Dagestan

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Динамика распространения лейкоза крупного рогатого скота в племенных хозяйствах Республики Дагестан

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РЕЗЮМЕ

Введение. Проблема лейкоза крупного рогатого скота в племхозах Республики Дагестан стала актуальной еще с середины 60-х годов XX века. В связи с тем, что в те годыохват плановыми серологическими исследованиями не превышал 1–2% имеющегося поголовья восприимчивых животных, ясного представления о масштабах распространения лейкоза не было.

Цель исследования. Анализ современной ситуации по распространению лейкоза крупного рогатого скота в племенных хозяйствах Республики Дагестан.

Материалы и методы. Инфицированных вирусом лейкоза животных выявляли с помощью реакции иммунной диффузии в агаровом геле (РИД). Противоэпизоотические мероприятия оценивали с учетом новых «Ветеринарных правил осуществления профилактических, диагностических, ограничительных и иных мероприятий, установления и отмены карантина и иных ограничений, направленных на предотвращение распространения и ликвидацию очагов лейкоза крупного рогатого скота», утвержденных приказом Минсельхоза России от 24 марта 2021 г. № 156.

Результаты. Зараженность животных вирусом лейкоза крупного рогатого скота в ретроспективе за 2009–2017 гг. варьировала от 0,1 до 77,3%. С принятием подпрограммы «Профилактика и ликвидация лейкоза крупного рогатого скота в хозяйствах Республики Дагестан» (2018–2020 гг.) в рамках республиканской целевой программы за последние семь лет увеличился охват поголовья серологическими исследованиями более чем в 5,7 раза, частота выявления новых случаев серопозитивных животных снизилась с 23,6 до 0,1% в 2024 г.

Заключение. Эпизоотологический анализ показал неоднородную структуру и динамику распространения лейкозного процесса среди крупного рогатого скота. Реализуемая в условиях Республики Дагестан система мер по предупреждению и ликвидации заболевания крупного рогатого скота лейкозом позволила добиться устойчивой стабилизации эпизоотической обстановки и сократить уровень зараженности животных в племенных хозяйствах. Благодаря проводимой ветеринарной службой планомерной работе по ликвидации вирусной инфекции племхозы сегодня полностью благополучны по лейкозу. Оздоровительная работа, включающая применение серологической диагностики и немедленной выбраковки РИД-позитивных животных, продолжается.

Ключевые слова: лейкоз, вирус лейкоза крупного рогатого скота, распространение, племенные хозяйства, серологические и гематологические исследования, оздоровительные мероприятия, Республика Дагестан

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INTRODUCTION

Bovine leukosis (BL) is a chronic infectious disease caused by an RNA-containing tumorigenic virus of the family *Retroviridae*, genus *Deltaretrovirus* [1, 2, 3, 4]. Diseased animals and bovine leukemia virus (BLV) infected animals are considered to be the source of the disease [5]. Key factors contributing to its spread include delayed or untimely diagnosis, non-compliance with veterinary and sanitary requirements when purchasing livestock for breeding and production purposes, and the common housing of infected and healthy livestock [6, 7, 8, 9].

Bovine leukosis inflicts significant economic losses on agricultural establishments of various forms of ownership, primarily, on breeding farms. The financial impact is multifaceted, arising from: loss of milk and offspring due to the premature culling of BLV-infected cows, slaughter of stud bulls, destruction of carcasses of diseased animals, sale of breeding young stock from diseased dam cows for meat, reclassification of breeding animals into the commercial category if they are BLV-infected, culling of young BLV-infected stock, as well as substantial operational expenses for diagnostic, veterinary-sanitary and zootechnical measures required for herd health management (improvement) and BL outbreak control to be conducted on farms and locations affected by BL. Beyond these direct costs, BL negatively impacts overall livestock productivity and operational efficiency, constraining the economic potential of affected farms [10, 11, 12, 13].

The presence of BLV carriers on breeding farms, which concentrate valuable cattle gene pools, poses a significant risk of spreading the infection to disease-free farms through the sale of animals [14, 15, 16].

The problem of BL on breeding farms in the Republic of Dagestan emerged as early as the mid-1960s. Initial studies conducted by scientists from the Dagestan Research Veterinary Station, employing hematological and pathomorphological methods, detected the disease in 14.0–19.1% of affected cows. Furthermore, the frequent observation of carcasses with BL-characteristic lesions at meat-processing plants during that period confirmed the widespread of the disease [17].

Beginning in 1988, comprehensive lifetime diagnosis of BL was implemented on cattle farms using the agar gel immunodiffusion (AGID) test [18, 19, 20, 21], facilitating the development of optimized BLV prevention and eradication strategies. However, as planned serological testing in those years covered less than 1–2% of the susceptible cattle population, the true scale of BLV infection remained unclear.

This study aims to analyze the current epidemiological situation of BL on breeding farms in the Republic of Dagestan.

MATERIALS AND METHODS

The research was conducted in the Laboratory of Infectious Pathology of Farm Animals at the Caspian Zonal Research Veterinary Institute – Branch of Dagestan Agriculture Science Center.

Data reported by the Committee on Veterinary Medicine of the Republic of Dagestan and republican and district veterinary laboratories were analyzed. This data was obtained from BL monitoring on breeding farms during 2002 and the period from 2009 to 2024, and was subjected to statistical processing.

Serological and hematological tests were conducted in veterinary laboratories in accordance with

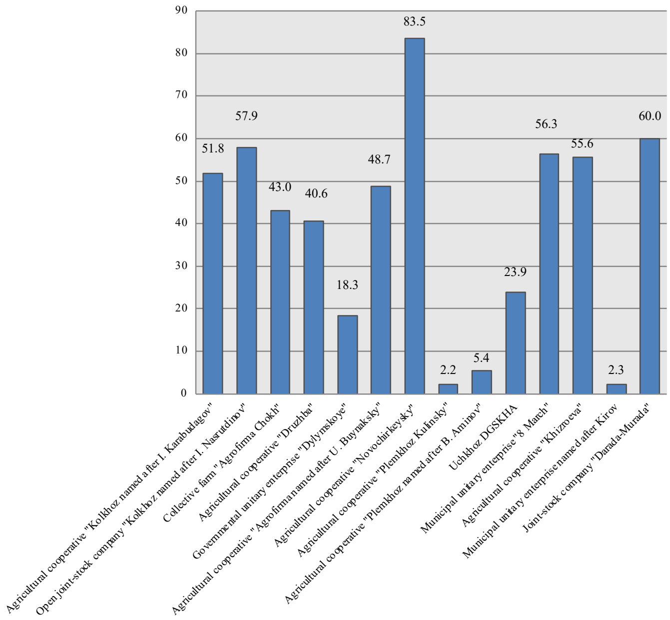

Fig. 1. The BLV infection rate on the breeding farms in the Republic of Dagestan in 2002 (%)

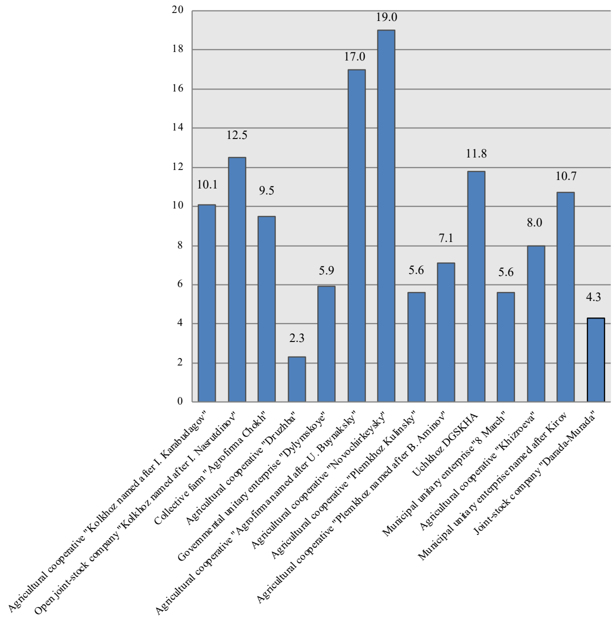

Fig. 2. The BL incidence rate on the breeding farms in the Republic of Dagestan in 2002 (%)

the "Methodological Guidelines for the Diagnosis of BL in Cattle"[1], epizootological tests were conducted in accordance with the "Methodological Recommendations for Epizootological Investigation of BL in Cattle"[2].

The effectiveness of preventive and disease control measures was evaluated based on the new "Veterinary Rules for the Implementation of Preventive, Diagnostic, Restrictive and Other Measures the Establishment and Cancellation of Quarantine and Other Restrictions Aimed at Containing BL as well as at Eradicating its Outbreaks", approved by Order No. 156 of the Ministry of Agriculture of Russia of March 24, 2021[3].

RESULTS AND DISCUSSION

A preliminary assessment of the BL situation on Dagestan's breeding farms was conducted in 2002. Official statistics from January 1, 2002, reported 14 breeding farms in the region, maintaining a total cattle population of 13,411, which included 4,955 cows. The findings on the spread of BL and BLV on these farms are presented in Figures 1 and 2.

Our analysis established a widespread distribution of BL at the start of the study period. Among Dagestan's breeding farms, the BLV infection rate in susceptible animals varied considerably, ranging from 2.2% at agricultural cooperative "Plemkhoz Kulinsky" to 83.5% at agricultural cooperative "Novochirkeysky". Similarly, BLV incidence rates ranged from 2.3% (agricultural cooperative "Druzhba") to 19.0% (agricultural cooperative "Novochirkeysky"), with average rates of 32.2 and 10.4%, respectively.

Serological testing coverage for BL was 37.9%, while hematological testing covered 25.8% of the total cattle subjected to diagnostic screening.

[1] https://docs.cntd.ru/document/1200118749

[2] https://elibrary.ru/ucvzwj

[3] https://docs.cntd.ru/document/603433105

A comparative epizootological assessment revealed a more intense epizootic process caused by BLV on breeding farms compared to commercial and backyard farms, with infection rates of 29.7, 24.7, and 7.9%, respectively [22].

Thus, determining the BLV prevalence rate in cattle and clinical severity of BL on breeding farms through systematic diagnostic testing became imperative. To this end, the dynamics of the BLV infection rate were assessed annually from 2009 to 2017 across an average of 11–19 farms (Table 1).

Over the nine-year period, 33,838 animals were tested using the AGID-test, of which 7,977 (23.6%) were seropositive for BLV. Furthermore, hematological examination of 1,950 cows confirmed a BL diagnosis in 606 individuals (31.1%).

BLV infection rates on breeding farms remained persistently high. The lowest number of virus-carriers in animals was reported in 2012 – 7.2%, in other years it ranged from 10.1 to 37.1%. Similarly, the proportion of animals with BL, as determined by hematological tests, remained elevated, fluctuating between 15.9 and 67.5%.

It was established that breeding farms company "Vympel-1", agricultural cooperative "Agrofirma Sivukh", agricultural cooperative "Named after A. Daniyarov" were free from BL; on family operated farm "Boztorgay", company "Kurbanservice", municipal unitary enterprise named after Kirov, Agricultural cooperative plemkhoz "Urkarakhsky", agricultural cooperative "Plemkhoz Kulinsky", agricultural cooperative "Plemkhoz named after B. Aminov", governmental unitary enterprise "Dylymskoye", the infection rate did not exceed 10%; on agricultural cooperative "Druzhba", company "Agrofirma "Molochnik", agricultural cooperative "Novaya Zhizn", company "Plempredpriyatiye Elita", scientific production association "Plemservice" the infection rate ranged

Table 1
Dynamics of BLV infection on the breeding farms in the Republic of Dagestan in 2009–2017

Breeding farms	Number of BLV-carriers identified, %									Total number for 9 years, %
	2009	2010	2011	2012	2013	2014	2015	2016	2017	
Joint-stock company "Kizlyaragrocomplex"	47.6	37.9	0	–	–	–	0	12.6	44.1	39.9
Company "Plempredpriyatiye Elita"	–	54.1	–	–	–	–	–	11.5	–	27.0
Company "Averyanovka"	–	51.4	48.2	–	–	–	–	–	–	50.8
Company "Agrofirma "Molochnik"	22.5	–	–	–	–	–	–	–	–	22.5
Open joint-stock company "Marenevka"	–	64.2	–	–	–	–	–	–	–	64.2
Agricultural cooperative "Khizroeva"	60.7	60.8	81.5	–	–	70.0	–	90.8	0	62.2
Agricultural cooperative "Kolkhoz Krasny Partizan"	76.1	90.5	92.3	0	94.2	0	69.7	93.0	0	77.3
Scientific production association "Plemservice"	–	–	–	–	–	–	–	76.7	0	27.1
Agricultural cooperative plemkhoz "Urkarakhsky"	2.0	0.5	0	0	0	0	15.8	7.8	7.6	1.8
Collective farm "Agrofirma Chokh"	38.4	13.6	52.8	–	38.9	48.0	64.7	61.3	64.9	48.0
Agrofirma "Sogratl"	–	–	–	–	–	–	–	45.4	36.9	40.9
Agricultural cooperative "Agrofirma named after U. Buynaksky"	34.5	–	0	–	–	0	24.9	55.8	26.9	35.3
Agricultural cooperative "Novochirkeysky"	58.3	–	–	–	–	–	–	–	40.0	53.1
Agricultural cooperative "Plemkhoz Kulinsky"	11.7	14.0	–	21.4	14.7	–	1.9	0	0	6.5
Agricultural cooperative "Plemkhoz named after B. Aminov"	–	14.3	–	8.2	–	4.8	2.0	1.9	2.2	6.8
Joint-stock company "Darada-Murada"	52.7	79.1	58.5	0	20.0	–	62.4	–	–	51.6
Worker cooperative "Murad"	–	68.5	–	34.2	–	–	35.3	8.2	24.0	41.2
Municipal unitary enterprise named after Kirov	0	0	0.6	–	–	–	–	–	–	0.2
Agricultural cooperative "Druzhba"	12.7	49.5	32.1	–	2.2	3.6	0	13.0	9.4	15.5
Agricultural cooperative "Novaya Zhizn"	–	25.4	21.9	22.0	18.9	21.6	21.8	16.3	32,.8	23.1
Governmental unitary enterprise "Dylymskoye"	0	40.3	14.8	0.3	6.9	1.4	0	1.7	0	8.5
Company "Vympel-1"	–	–	–	–	–	–	–	–	0	0
Agricultural cooperative "Agrofirma Sivukh"	–	0	0	0	–	0	–	–	–	0
Agricultural cooperative "Named after A. Daniyarov"	0	0	0	–	0	–	0	–	0	0
Municipal unitary agricultural enterprise "Talovka"	0	84.8	38.3	–	–	–	–	–	–	42.2
Family operated farm "Boztorgay"	–	–	–	0	–	0	0.6	–	0	0.1
Company "Kurbanservice"	–	–	–	–	0.7	–	0	0	0	0.1
Company "OORKh «Dagestanskoye"	–	–	72.9	–	81.8	–	0	–	–	46.1
Total for the year, %	29.0	37.1	35.1	7.2	18.3	10.1	22.6	23.2	13.5	23.6

"–" – there was no data on the status of the breeding farm.

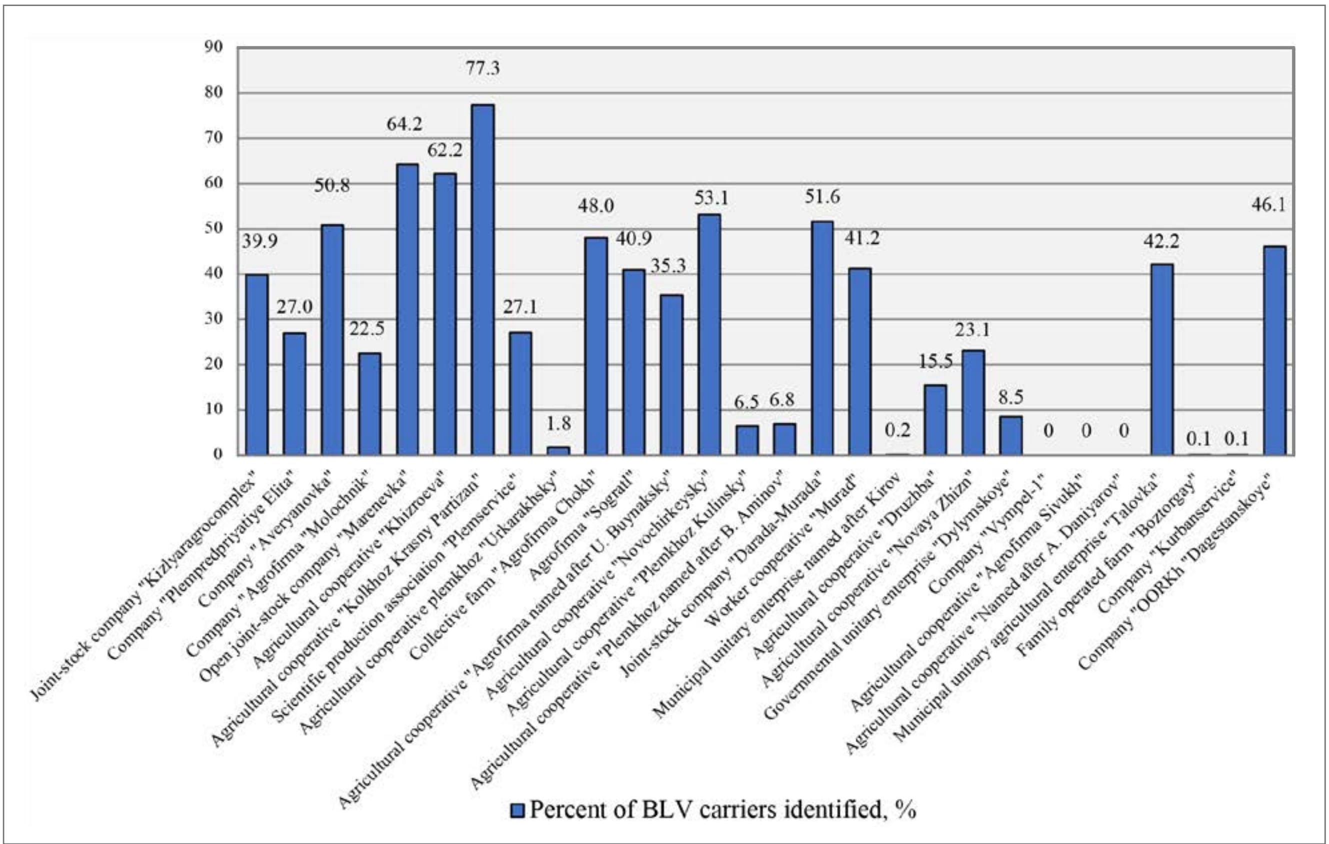

Fig. 3. The BLV infection rate on the breeding farms in the Republic of Dagestan in 2009–2017 (%)

from 10 to 30%, on the remaining breeding farms the infection rate ranged from 35.3 to 77.3% (Fig. 3).

In 2009, BL restrictions were officially imposed on 17 farms, 9 of which were breeding farms.

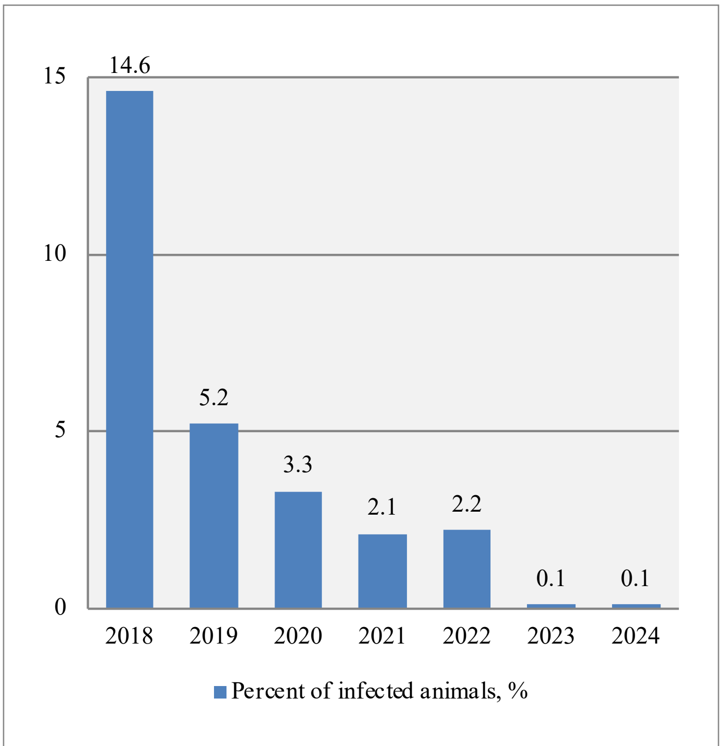

Fig. 4. Dynamics of BLV infection on the breeding farms in the Republic of Dagestan in 2018–2014

By 2013, one additional breeding farm was identified as affected, while health status of animals on 2 farms was improved. However, for a prolonged period (as of January 1, 2019), a significant number of breeding farms remained BLV-positive (agricultural cooperative "Agrofirma named after U. Buynaksky", agricultural cooperative "Kolkhoz Krasny Partizan", scientific production association "Plemservice", agricultural cooperative "Plemkhoz Kulinsky", agricultural cooperative "Plemkhoz named after B. Aminov", agricultural cooperative "Druzhba", governmental unitary enterprise "Dylymskoye", agricultural cooperative "Novaya Zhizn") [23, 24, 25].

When assessing the disease situation, it is important to note that leukosis intensity varied significantly across breeding farms in the Republic of Dagestan. The disease's widespread persistence is attributed to several key factors: the long-standing infection on these farms, a lack of comprehensive control measures, and insufficient diagnostic coverage. From 2009 to 2017, serological testing rates remained critically low, at only 17.5–21.6%.

It should be noted that the number of breeding farms in the region often varied depending on the disease situation. Due to BL restrictive measures, some breeding farms were converted to commercial farms.

Thus, a tense situation regarding BLV persisted in the region's breeding farms until 2017. The presence of hematologically BL-diseased animals and BLV-infected animals was confirmed in almost all breeding herds. In fact, with the exception of agricultural cooperatives "Plemkhoz Kulinsky" and "Plemkhoz

Table 2
Serological testing of cattle for BL on the breeding farms in the Republic of Dagestan in 2018–2024

No. item	2018			2019			2020			2021			2022			2023			2024		
	tests	+	%	tests	+	%	tests	+	%	tests	+	%	tests	+	%	tests	+	%	tests	+	%
1	2,474	705	28.5	6,633	488	7.4	6,062	128	2.1	6,192	98	1.6	2,449	216	8.8	470	13	2.8	619	11	1.8
2	51	34	66.7	851	29	3.4	1,221	255	20.9	813	78	9.6	2,264	23	1.0	1,548	0	0	–	–	–
3	–	–	–	150	91	60.7	533	1	0.2	807	70	8.7	1,223	37	3.0	1,457	0	0	–	–	–
4	422	18	4.3	1,927	96	5.0	1,505	83	5.5	2,017	63	3.1	1,383	20	1.4	1,295	0	0	1,377	0	0
5	511	102	20.0	1,067	83	7.8	1,273	51	4.0	1,751	77	4.4	831	46	5.5	897	0	0	1,457	0	0
6	144	5	3.5	785	4	0.5	415	8	1.9	492	4	0.8	520	11	2.1	660	0	0	510	0	0
7	58	1	1.7	130	1	0.8	158	3	1.9	232	3	1.3	130	0	0	623	0	0	–	–	–
8	67	22	32.8	197	0	0	365	0	0	526	0	0	778	20	2.6	–	–	–	350	0	0
9	640	28	4.4	1,131	83	7.3	1,376	0	0	1,115	9	0.8	1,534	0	0	1,553	0	0	1,060	0	0
10	361	7	1.9	630	5	0.8	700	9	1.3	315	3	1.0	553	0	0	553	0	0	580	0	0
11	1,009	71	7.0	2,028	18	0.9	1,167	0	0	1,192	0	0	1,171	0	0	1,643	0	0	1,646	0	0
12	1,010	0	0	890	0	0	1,185	0	0	1,368	0	0	1,667	0	0	1,809	0	0	2,005	0	0
13	221	44	19.9	306	50	16.3	363	57	15.7	172	0	0	–	–	–	–	–	–	–	–	–
14	161	5	3.1	395	2	0.5	196	5	2.6	180	0	0	–	–	–	–	–	–	–	–	–
15	250	31	12.4	317	50	15.8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
16	–	–	–	942	4	0.4	905	0	0	875	0	0	1,002	0	0	1,045	0	0	1296	0	0
17	199	49	24.6	506	49	9.7	403	0	0	65	0	0	–	–	–	–	–	–	–	–	–
18	198	38	19.2	565	2	0.4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
19	114	9	7.9	243	2	0.8	–	–	–	–	–	–	–	–	–	267	0	0	271	0	0
20	168	5	3.0	830	6	0.7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
21	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	505	0	0	1054	0	0
22	–	–	–	–	–	–	86	0	0	270	0	0	114	0	0	126	0	0	140	0	0
23	–	–	–	–	–	–	353	1	0.3	555	2	0.4	767	0	0	1,768	0	0	832	0	0
24	–	–	–	–	–	–	–	–	–	–	–	–	280	0	0	344	0	0	419	0	0
25	–	–	–	–	–	–	–	–	–	–	–	–	261	0	0	320	0	0	–	–	–
26	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	253	0	0
27	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	756	0	0
28	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	230	0	0
Total number	8,058	1,174	14.6	20,523	1,063	5.2	18,266	601	3.3	18,937	407	2.1	16,927	373	2.2	16,883	13	0.1	14,855	11	0.1

* No. (breeding farms): 1 – joint-stock company "Kizlyaragrocomplex", 2 – joint-stock company "Darada-Murada", 3 – worker cooperative "Murad", 4 – collective farm "Agrofirma Chokh", 5 – agrofirma "Sogratl", 6 – agricultural cooperative "Kolkhoz Krasny Partizan", 7 – agricultural cooperative "Alkhas Kuli", 8 – scientific production association "Plemservice", 9 – agricultural cooperative "Plemkhoz Kulinsky", 10 – agricultural cooperative "Plemkhoz named after B. Aminov", 11 – agricultural cooperative "Agrofirma named after U. Buynaksky", 12 – company "Kurbanservice", 13 – agricultural cooperative "Novaya Zhizn", 14 – governmental unitary enterprise "Dylymskoye", 15 – agricultural cooperative "Druzhba", 16 – company "Vympel-1", 17 – company "Averyanovka", 18 – agricultural cooperative "Novochirkeysky", 19 – agricultural cooperative plemkhoz "Urkarakhsky", 20 – agricultural cooperative "Khizroeva", 21 – family operated farm "Iman", 22 – agricultural cooperative "Ulluchai", 23 – agricultural cooperative "Mesed", 24 – agricultural artel "Otgonnik", 25 – family operated farm "Kosulya", 26 – agricultural cooperative "Agrofirma-Tsovkra-2", 27 – company "Chirkeysky ecoproduct", 28 – company "Chokh-Agroproduct";
"+" – number of animals infected with BLV; "–" – no data on the status of the breeding farm.

named after B. Aminov" in the highland zone, all other breeding farms were affected by leukosis.

The most valuable cattle gene pool in Dagestan is concentrated on breeding farms where leukosis infection was also was also widespread. Therefore, in the following years, the primary task for the regional veterinary service became the health improvement on breeding farms from leukosis infection.

This was particularly critical as these farms concentrated Dagestan's most valuable cattle gene pool. Consequently, the regional veterinary service prioritized the improvement of animal health on these breeding farms from leukosis.

A large-scale, systematic effort began with the adoption of the "Bovine Leukosis Prevention and Control Action Plan in the Republic of Dagestan"[4] and the subprogram "Prevention and Eradication of Bovine Leukosis on Farms in the Republic of Dagestan" under a republican target program[5], which has been extended through the current year. The health improvement strategy on BL-affected farms (herds) involves culling and sending for slaughter of all seropositive animals identified during routine laboratory testing.

It should be noted that since 2019, serological testing has covered nearly the entire susceptible cattle population on breeding farms. The resulting trend of reduced BLV infection from 2018 to 2024 is presented in Table 2 and Figure 4.

Over the seven-year period, state veterinary service laboratories tested 114,449 blood serum samples using the AGID test obtained from 28 regional operational breeding farms, revealing an average infection rate of 3.2%. Hematological testing for BLV-infected animals has been discontinued, as all BLV-infected animals are now sent for immediate slaughter without being held over.

Analysis of infection dynamics confirms a consistent downward trend in BLV infection rate among susceptible animals (Fig. 4). Specifically, the infection rate (carrier state) among the tested livestock dropped from 14.6% in 2018 to 5.2% in 2019, 3.3% in 2020, 2.1% in 2021, 2.2% in 2022, and 0.1% in 2023. This progress was sustained in 2024, with only 11 AGID-positive animals (0.1%) detected out of 14,855 tested, underscoring the stability and efficacy of the control measures implemented on the breeding farms in the Republic of Dagestan. The BLV-infected animals were traced to a single source: company "Plempredpriyatiye Elita", a subsidiary of joint-stock company "Kizlyaragrocomplex".

CONCLUSION

Epizootological analysis confirmed a heterogeneous pattern in the spread and progression of BL. The comprehensive system of measures implemented in the Republic of Dagestan aimed at prevention and eradication of BL has successfully stabilized the disease situation and significantly reduced the level of BLV infection on breeding farms. As a result of these systematic veterinary efforts, the breeding farms are now recognized as leukosis-free. Ongoing health improvement work, based on serological testing and the immediate culling of AGID-positive reactors, remains in place to sustain this status.

[4] https://docs.cntd.ru/document/450340001

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Immunological control of aerosol phytotherapy of acute catarrhal bronchopneumonia for its effectiveness in calves

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ABSTRACT

Introduction. Respiratory diseases are widespread on livestock farms, especially in high-yielding animals, and they are particularly severe in young animals. Non-specific bronchopneumonia in calves is caused by a combination of factors including opportunistic respiratory microbiota, which can become pathogenic under unfavorable conditions, overcrowding, nutritional imbalances, stress, drafts, noise, other environmental stressors as well as compromised immunity in newborn animals.

Objective. Immunological control of aerosol phytotherapy of acute catarrhal bronchopneumonia for its effectiveness in calves.

Materials and methods. One – three month-old calves with acute catarrhal bronchopneumonia ($n = 60$) were used for the study. The calves were divided into three test groups, 20 calves per group. Blood samples were collected from the diseased animals before the start of treatment, as well as on day 7 and 12 after treatment and used for immunological tests.

Results. Aerosol administration of *Hypericum perforatum* extract, herbal product, in the complex treatment of calves with acute catarrhal bronchopneumonia demonstrated high efficacy compared to two other treatment regimens. In the test group receiving phytotherapy overall clinical improvement was observed as early as on (4.90 ± 0.64) day, which was 47.0% faster than in the group where animals were treated according to the treatment regime routinely used on the farm. Furthermore, the calves in this group demonstrated a faster recovery of appetite, consumed feed more readily, their coats became smooth and shiny, and their cellular and humoral immunity levels, as well as their pro-inflammatory cytokine levels reached the reference levels of clinically healthy animals by day 12 and day 7, respectively.

Conclusion. While all three regimens for acute catarrhal bronchopneumonia were effective, the aerosolized *Hypericum perforatum* extract produced the best results. Calves receiving this treatment showed the most significant improvements in cellular and humoral immunity, along with the reduction in pro-inflammatory cytokine levels.

Keywords: bronchopneumonia, therapy, aerosol treatment, herbal products, *Hypericum perforatum,* calves

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Иммунологический контроль эффективности аэрозольной фитотерапии острой катаральной бронхопневмонии у телят

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РЕЗЮМЕ

Введение. Респираторные заболевания широко распространены в животноводческих хозяйствах, особенно среди высокопродуктивных животных, при этом достаточно тяжело они протекают у молодняка. Возникновение неспецифической бронхопневмонии у телят связано с комплексом причин, включающим прежде всего условно-патогенную микробиоту дыхательных путей, которая при неблагоприятных условиях может стать патогенной, а также скученность содержания, несбалансированное кормление, стресс, сквозняки, шум, влияние негативных факторов окружающей среды, а также снижение резистентности и иммунной реактивности новорожденных животных.

Цель исследования. Проведение иммунологического контроля эффективности аэрозольной фитотерапии острой катаральной бронхопневмонии телят.

Материалы и методы. Исследование провели на 1–3-месячных телятах, больных острой катаральной бронхопневмонией (*n* = 60). Было сформировано три опытные группы по 20 особей в каждой. У больных животных до начала терапии, а также на 7-е и 12-е сут после лечения отбирали кровь для проведения иммунологических исследований.

Результаты. Установлено, что аэрозольное применение фитопрепарата «Экстракт зверобоя продырявленного» в комплексном лечении телят с острой катаральной бронхопневмонией продемонстрировало более высокую эффективность по сравнению с остальными двумя схемами. В опытной группе, где использовали фитотерапию, общее клиническое улучшение наблюдали уже на (4,90 ± 0,64) сут, что на 47,0% быстрее, чем в группе, где лечение животных проводили по общепринятой в хозяйстве схеме. При этом у телят указанной группы аппетит восстанавливался быстрее, они лучше поедали корм, шерсть становилась гладкой и блестящей, показатели клеточного и гуморального иммунитета на 12-е сут, а уровень провоспалительных цитокинов уже на 7-е сут приближались к референсным показателям клинически здоровых животных.

Заключение. Все три терапевтические схемы при борьбе с острой катаральной бронхопневмонией показали относительную эффективность, однако аэрозольное применение фитопрепарата «Экстракт зверобоя продырявленного» в комплексном лечении больных телят продемонстрировало наилучшие результаты, о чем свидетельствуют значительные позитивные сдвиги в клеточном и гуморальном звене иммунитета, а также снижение уровня провоспалительных цитокинов.

Ключевые слова: бронхопневмония, терапия, аэрозольная обработка, фитопрепараты, зверобой продырявленный, телята

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INTRODUCTION

Currently, respiratory diseases are widespread on livestock farms, especially among high-yielding animals, while they are quite severe in young animals [1, 2, 3, 4]. Respiratory diseases in livestock cause significant economic losses through animal mortality, reduced performance, stunted growth and development in survivors, and the costs of treatment and disease prevention [5, 6, 7]. Bronchopneumonia is reported in calves in almost all regions of the country and ranks second among all pathologies on farms, second only to gastrointestinal diseases, while reaching 30% of cases in the nosological profile of all pathologies [8, 9]. The etiological factors of nonspecific bronchopneumonia in calves represent a complex of causes, including, first of all, the opportunistic respiratory microbiota, which under unfavourable conditions can become pathogenic, overcrowding, imbalanced nutrition, stress, draughts, noise, other environmental stressors, as well as a compromised resistance and immune response of new-born animals [10, 11, 12, 13].

Under production conditions, antibiotics are widely used as antimicrobials for the prevention and control of factor diseases, including bronchopneumonia in calves, and are most often prescribed empirically [14, 15, 16]. Therewith, empirical and uncontrolled use of antibiotics promotes mutations as well as microbial resistance, it also leads to their accumulation in animal tissues and products, thereby contributing to the development of antibiotic-resistant microbiota in humans consuming such animal products. In addition, antibiotic use can result in systemic toxicity, which may culminate in multiple organ failure in animals. Therefore, the search for alternative tools for combating multidrug-resistant pathogenic bacteria is of current importance [17, 18, 19].

Studies on the immune system of calves with respiratory diseases often yield conflicting results, though they consistently indicate immune dysfunction and a reduced immune response [20, 21]. Therewith, the immunobiological responsiveness plays one of the key roles in the formation and progression of various infectious diseases, including acute catarrhal bronchopneumonia in calves. Therefore, immunological screening during the treatment of respiratory diseases in animals provides a critical method for monitoring therapeutic efficacy, representing, in our opinion, an urgent and promising area for further research.

The study was aimed at immunological control of the aerosol phytotherapy of acute catarrhal bronchopneumonia for its effectiveness in calves kept on livestock farms.

MATERIALS AND METHODS

The study was funded by grant of the Russian Science Foundation No. 24-26-00091 (https://rscf.ru/project/24-26-00091) and carried out on the "Babayevo" livestock farm located in the Sobinsky Raion of the Vladimir Oblast and "Delta-F" livestock farm located in the Sergiyev Posad municipality of the Moscow Oblast having the same animal keeping and feeding practices.

One-three month-old calves with acute catarrhal bronchopneumonia (*n* = 60) were used for the study. As the calves became diseased, they were randomly assigned to test groups, placed in separate isolated rooms, and treated according to the presented treatment regimes. Three test groups of animals ($_1$T, $_2$T and $_3$T) were formed, 20 calves with bronchopneumonia per group. The animals of test group 1 ($_1$T) were treated according to the scheme commonly used on farms: indoor aerosol treatment of animals with iodoethylene glycol solution (3 mL/m^3 the room + 10% glycerine, v/v) using industrial "Hi-Fog" cold mist generator for 30 minutes once a day during 7 days. As an antibacterial therapy, "Penstrep 400" (1 mL/10 kg) was administered intramuscularly to calves of $_1$T group once a day for three days. The calves of test group 2 ($_2$T) were also subjected to indoor aerosol treatment with iodoethylene glycol solution (3 mL/m^3 the room + 10% glycerine, v/v) using industrial "Hi-Fog" cold mist generator for 30 minutes once a day during 7 days. "Marbofloxacin" (fluoroquinolone), 10% solution, administered at a dose of 8 mg/kg, once a day, three times, was used for antibacterial therapy based on the results of microbiological tests and tests of the isolated pathogens for their susceptibility to antibiotics performed earlier. The animals of test group 3 ($_3$T) were subjected to indoor aerosol treatment with *Hypericum perforatum* extract, herbal product, demonstrating high antibacterial activity against the bronchopneumonia initiators in calves that was experimentally selected previously [22]. In addition, 10% "Marbofloxacin" (fluoroquinolone) solution was also administered at a dose of 8 mg/kg once a day, three times.

Blood samples were collected from diseased animals (10 animals from each group) before treatment and on day 7 and day 12 after treatment for immunological tests. The total number of T-lymphocytes was determined by spontaneous rosette formation using common methods. Therewith, T cells were tested for their susceptibility and resistance to theophylline. Number of T-suppressors was calculated as the difference between the total number of T-lymphocytes and the number of T-helpers. The immunoregulatory index (IRI) was calculated by dividing the number of T-helper cells by the number of T-suppressor cells. The number of natural killer (NK) cells was calculated by subtracting the total count of T-lymphocytes and B-lymphocytes from the total number of lymphocytes, using a complementary rosette formation method to distinguish and quantify these cell types. The structure of circulating immune complexes (CICs) was determined by molecular weight. The levels of pro-inflammatory IL-1α, IL-6, IL-8 interleukins and TNF-1α (tumour necrosis factor) were determined with enzyme-linked immunosorbent assay. Blood collected from clinically healthy calves (n = 10) once on day 1 of the study was used as a control.

The animals were handled in accordance with the European Convention (ETS No. 123).

The results were statistically processed using the STATISTICA 7.0 program (StatSoft, USA). Before the study, the Shapiro – Wilk test was used to check the dataset for normal distribution. With a normal distribution of quantitative variables, an ANOVA test was used to compare the two groups. Mann – Whitney test was used for assessment of significance of differences between the indicators before and after treatment of animals (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

RESULTS AND DISCUSSION

Optimal treatment outcomes are achieved through dynamic monitoring of the animals' state and timely adjustment of treatment regimens. In this context, comparative assessment of three different treatment regimens for acute catarrhal bronchopneumonia in calves for their effectiveness was carried out including monitoring the animals' state.

It should be noted that mean time to clinical improvement in calves in test group $_1$T was (9.25 ± 0.91) days, complications were reported in six cases during the treatment course, the final recovery rate was 90.0% (18/20), 2 calves (10.0%) died. In test group $_2$T overall clinical improvement was observed as early as on day (7.20 ± 0.61), and the final recovery rate was 100.0% (20 animals). Therapeutic monitoring in test group $_3$T showed a mean time to clinical improvement of (4.90 ± 0.64) days. This was 47.0% faster than in group $_1$T, and all 20 calves (100.0%) recovered.

The clinical outcome of an infectious process – encompassing both disease severity and animal convalescence – depends as much on the virulence of the infecting microflora as well as on the resistance and immunological competence of the host organism. Cellular immunity indicators in calves with acute catarrhal bronchopneumonia during the treatment are given in Table 1.

The data show that significant increase in T-helper cells by 29.27%: from (12.30 ± 0.89) to (15.90 ± 0.94)% (*↑) was recorded on day 7 when the calves were subjected to the treatment routinely used on tested farms (test group $_1$T). More significant shifts in the cellular immunity: 1.87-fold increase in the level of T-helper cells, from (11.90 ± 0.69) to (22.30 ± 1.31)% (***↑), and 1.30-fold and 1.19-fold decrease in the level of T-suppressors (**↓) and NK cells (**↓), respectively, was observed in the treated calves of test group $_2$T on day 7. It should be noted that the most significant positive changes in cellular immunity were recorded in calves of test group $_3$T: an increase in total T cells by 18.41%, from (39.10 ± 1.04) to (46.30 ± 1.34)% (***↑), and increase in T-helper cells by 111.72%, from (12.80 ± 0.87) to (27.10 ± 1.36)% (***↑), and significant decrease in serum T-suppressor levels by 1.37 times, from (26.30 ± 1.36) to (19.20 ± 1.15)%, and decrease in NK cell levels by 1.45 times, from (42.00 ± 1.81) to (29.00 ± 2.03)%, as compared to the indicators for the calves before the treatment was started. Further favourable changes in cellular immunity indicators were recorded in all test groups on day 12 after the start of treatment, but only in calves of test group $_3$T these indicators approached the reference ones of healthy animals. Thus, a highly significant increase in total T-lymphocytes and T-helper cells by 29.92% and by 188.28%, respectively, and a significant decrease in T-suppressors and NK cells by 1.79 and 1.68 times, respectively, were observed in calves of test group $_3$T.

Table 1
Cellular immunity indicators in calves with acute catarrhal bronchopneumonia during the treatment

Indicators	Healthy calves (*n* = 10)	Groups	Calves with bronchopneumonia		
			before treatment (*n* = 10)	day 7 (*n* = 10)	day 12 (*n* = 10)
T-lymphocytes, total number, %	50.40 ± 1.49	$_1$T	39.80 ± 1.31	40.50 ± 1.20	43.60 ± 1.21*↑
		$_2$T	39.30 ± 1,.49	43.40 ± 1.35	48.20 ± 1.33***↑
		$_3$T	39.10 ± 1.04	46.30 ± 1.34***↑	50.80 ± 1.58***↑
T-helpers,%	35.50 ± 0.98	$_1$T	12.30 ± 0.89	15.90 ± 0.94*↑	22.60 ± 1.21***↑
		$_2$T	11.90 ± 0.69	22.30 ± 1.31***↑	32.80 ± 1.03***↑
		$_3$T	12.80 ± 0.87	27.10 ± 1.36***↑	36.90 ± 1.71***↑
T-suppressors, %	14.90 ± 1.01	$_1$T	27.50 ± 1.12	24.60 ± 1.08	21.00 ± 1.94**↓
		$_2$T	27.40 ± 1.37	21.10 ± 1.58**↓	15.40 ± 0.79***↓
		$_3$T	26.30 ± 1.36	19.20 ± 1.15***↓	14.70 ± 1.27***↓
NK cells,%	25.10 ± 1.41	$_1$T	41.50 ± 1.81	40.30 ± 1.69	35.80 ± 1.34*↓
		$_2$T	42.50 ± 1.51	35.60 ± 1.38**↓	28.10 ± 1.59***↓
		$_3$T	42.00 ± 1.81	29.00 ± 2.03***↓	25.00 ± 1.50***↓

↑ – significant increase in indicators; ↓ – significant decrease in indicators; * $p < 0,05$; ** $p < 0,01$; *** $p < 0,001$ as compared to the indicators before treatment.

The immunoregulatory index is one of the main laboratory indicators of the satisfactory immune state, which makes it possible to give an objective assessment of immune response intensity, IRI dynamics in calves with acute catarrhal bronchopneumonia is given in the Figure.

It was detected that clinical manifestations of acute catarrhal bronchopneumonia in animals of all test groups were accompanied by a significant IRI decrease. However, it should be noted that on day 7 the IRI increased by 44.44% (*↑) in calves of the test group $_1$T, by 143.73% (***↑) in calves of test group $_2$T and by 188.24% (***↑) in calves of test group $_3$T (the most significant IRI increase as compared to the initial data). On day 12, highly significant (***↑) increase in IRI was reported in calves of all test groups as follows: 2.71 times increase in calves of test group $_1$T, from (0.45 ± 0.04) to (1.22 ± 0.18) conventional units (CU); 4.98 times increase in calves of test group $_2$T, from (0.44 ± 0.03) to (2.19 ± 0.16) CU; 5.35 times increase in calves of group $_3$T, from (0.51 ± 0.05) to (2.73 ± 0.32) CU, which approached the reference levels.

The data shown in Table 2 clearly demonstrate changes in the humoral immunity of calves with acute catarrhal bronchopneumonia during the treatment.

Acute catarrhal bronchopneumonia in calves was shown to trigger a major shift in humoral immunity characterized by decrease in B-lymphocyte counts and significant rise in pathogenic, medium- and small-molecular CICs. On day 7, significant positive changes were observed only in animals of test group $_3$T: increase in total B cells by 30.69%, from (18.90 ± 0.84) to (24.70 ± 0.88)% (***↑), and significant decrease in total CICs by 39.61%, from (481.40 ± 10.37) to (290.70 ± 11.26) AU (***↓), due to decrease in large-molecular CICs by 40.10%, from (60.10 ± 1.53) to (36.00 ± 0.73) AU (***↓), medium-molecular CICs by 22.49%, from (97.80 ± 3.36) to (75.80 ± 1.58) AU (***↓), and small-molecular CICs by 44.70%, from (323.50 ± 8.31) to (178.90 ± 10.94) AU (***↓) as compared to the initial data. It was found that on day 12, positive changes in the specified immunological indicators were recorded in calves of all test groups, but only in calves of test group $_3$T the humoral immunity indicators approached the reference indicators of clinically healthy calves: increase in total B-lymphocytes by 1.28 times, from (18.90 ± 0.84) to (24.20 ± 0.55)% (***↑), decrease in total CICs by 2.10 times, from (481.40 ± 10.37) to (228.80 ± 3.88) AU (***↓): large-molecular CICs – by 1.81 times, from (60.10 ± 1.53) to (33.20 ± 0.61) AU (***↓), medium-molecular CICs – by 1.43 times, from (97.80 ± 3.36) to (68.60 ± 1.68) AU (***↓), and small-molecular CICs – by 2.55 times, from (323.50 ± 8.31)

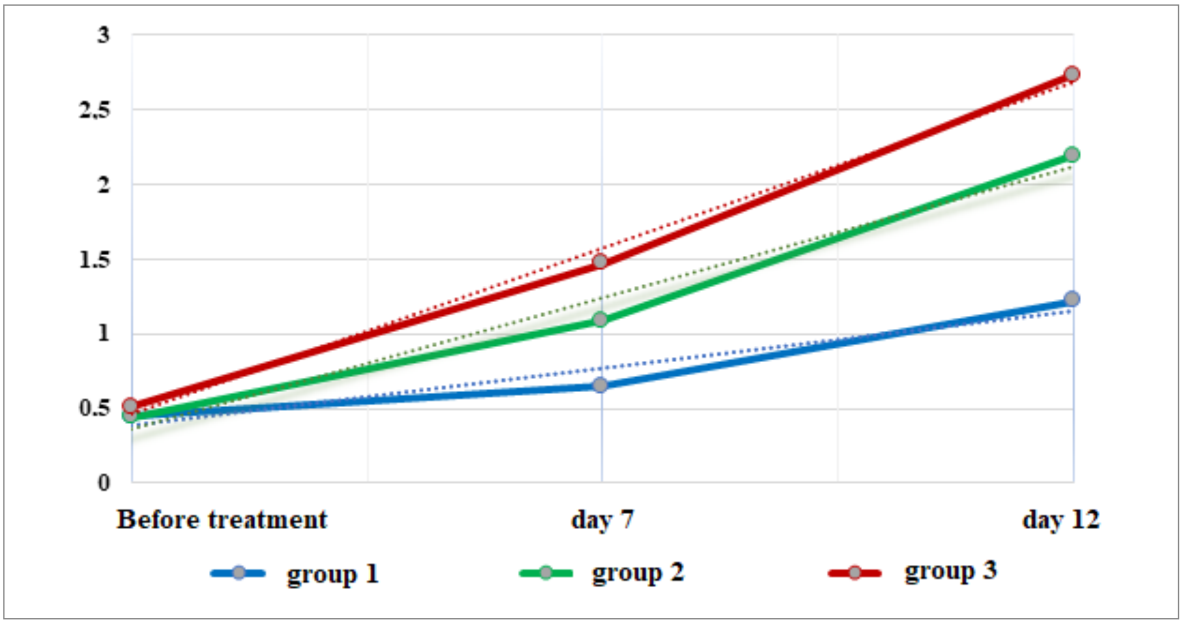

Fig. The immunoregulatory index in calves with acute catarrhal bronchopneumonia during the treatment

Table 2
Humoral immunity indicators in calves with acute catarrhal bronchopneumonia during the treatment

Indicators	Healthy calves (n = 10)	Groups	Calves with bronchopneumonia		
			before treatment (n = 10)	day 7 (n = 10)	day 12 (n = 10)
B cells, total count, %	24.50 ± 0.85	$_{1}T$	18.70 ± 0.66	19.20 ± 0.64	20.60 ± 0.52*↑
		$_{2}T$	18.20 ± 0.81	21.00 ± 0.64*↑	23.70 ± 0.77***↑
		$_{3}T$	18.90 ± 0.84	24.70 ± 0.88***↑	24.20 ± 0.55***↑
CICs total, AU	229.10 ± 4.34	$_{1}T$	464.60 ± 9.68	436.40 ± 8.63*↓	397.20 ± 7.70***↓
		$_{2}T$	459.70 ± 10.83	403.00 ± 8.03***↓	292.30 ± 6.38***↓
		$_{3}T$	481.40 ± 10.37	290.70 ± 11.26***↓	228.80 ± 3.88***↓
CICs large, AU	34.80 ± 0.81	$_{1}T$	51.50 ± 1.43	49.40 ± 1.62	43.10 ± 2.07**↓
		$_{2}T$	53.60 ± 1.17	47.30 ± 1.34**↓	34.80 ± 0.51***↓
		$_{3}T$	60.10 ± 1.53	36.00 ± 0.73***↓	33.20 ± 0.61***↓
CICs, medium, AU	67.10 ± 1.53	$_{1}T$	98.80 ± 3.24	93.10 ± 2.96	83.40 ± 2.05***↓
		$_{2}T$	97.10 ± 3.14	79.60 ± 2.33***↓	71.60 ± 2.17***↓
		$_{3}T$	97.80 ± 3.36	75.80 ± 1.58***↓	68.60 ± 1.68***↓
CICs small, AU	127.20 ± 3.83	$_{1}T$	314.30 ± 10.35	293.90 ± 9.61	270.70 ± 7.94**↓
		$_{2}T$	309.00 ± 8.58	276.10 ± 6.74**↓	185.90 ± 5.97***↓
		$_{3}T$	323.50 ± 8.31	178.90 ± 10.94***↓	127.00 ± 3.49***↓

↑ – significant increase in indicators; ↓ – significant decrease in indicators; * $p < 0,05$; ** $p < 0,01$; *** $p < 0,001$ as compared to the indicators before treatment.

Table 3
Pro-inflammatory cytokine levels in sera from the calves with acute catarrhal bronchopneumonia during the treatment

Indicators	Healthy calves (*n* = 10)	Groups	Calves with bronchopneumonia		
			before treatment (*n* = 10)	day 7 (*n* = 10)	day 12 (*n* = 10)
IL-1*α*, pg/mL	17.35 ± 0.41	$_1$T	37.50 ± 0.92	29.42 ± 0.61***↓	24.47 ± 0.74***↓
		$_2$T	35.97 ± 1.22	22.54 ± 0.51***↓	19.67 ± 0.29***↓
		$_3$T	36.55 ± 1.21	19.08 ± 0.31***↓	18.11 ± 0.27***↓
IL-6, pg/mL	13.14 ± 0.47	$_1$T	46.55 ± 1.14	37.51 ± 1.13***↓	25.03 ± 0.65***↓
		$_2$T	44.68 ± 1.36	26.78 ± 1.23***↓	17.74 ± 0.52***↓
		$_3$T	45.11 ± 1.31	15.39 ± 0.38***↓	13.48 ± 0.45***↓
IL-8, pg/mL	12.51 ± 0.50	$_1$T	30.67 ± 0.87	24.19 ± 1.23***↓	14.19 ± 0.47***↓
		$_2$T	30.04 ± 0.83	20.65 ± 0.48***↓	16.28 ± 0.40***↓
		$_3$T	30.67 ± 0.77	13.67 ± 0.25***↓	12.06 ± 0.33***↓
TNF-1*α*, pg/mL	44.55 ± 0.92	$_1$T	93.20 ± 2.05	82.43 ± 1.37***↓	55.18 ± 1.41***↓
		$_2$T	94.40 ± 1.96	58.35 ± 1.18***↓	44.04 ± 0.51***↓
		$_3$T	94.14 ± 1.68	46.23 ± 1.13***↓	41.66 ± 0.45***↓

↑ – significant increase in indicators); ↓ – significant decrease in indicators; * $p < 0{,}05$; ** $p < 0{,}01$; *** $p < 0{,}001$ as compared to the indicators before treatment.

to (127.00 ± 3.49) AU (***↓), when compared to indicators before the start of treatment.

Cytokines are known to be critical regulators of the host immune response during inflammation and infection. Cytokines are small proteins that facilitate intercellular communication and form an independent regulatory system that directs fundamental body functions, particularly in maintaining homeostasis following microbial challenge or tissue injury. Systemically, cytokines integrate the immune, nervous, endocrine, and hematopoietic systems, thereby organizing a complex defence against antigens and regulating protective reactions [20].

According to data given in Table 3, calves develop a cytokine storm accompanied with marked upregulation in pro-inflammatory cytokine synthesis during the clinical manifestation of acute respiratory pathology as compared to clinically healthy animals. Sharp decrease in interleukin levels was observed in animals of all test groups as early as on day 7, and in calves of test group $_3$T, these indicators approached the reference values. Thus, a significant decrease (***↓) of the following indicators were reported in tested sera on day 7 as follows: in calves of test group $_1$T: decrease in IL-1*α* – by 1.27 times, IL-6 – by 1.24 times, IL-8 – by 1.27 times, TNF-1*α* – by 1.13 times; in calves of test group $_2$T: decrease in IL-1*α* – by 1.60 times, IL-6 – by 1.67 times, IL-8 – by 1.45 times, TNF-1*α* – by 1.62 times; in calves of test group $_3$T the most significant positive changes were recorded: decrease in IL-1*α* – by 1.92 times, from (36.55 ± 1.21) to (19.08 ± 0.31) pg/mL, in IL-6 – by 2.93 times, from (45.11 ± 1.31) to (15.39 ± 0.38) pg/mL, in IL-8 – by 2.24 times, from (30.67 ± 0.77) to (13.67 ± 0.25) pg/mL and in TNF-1*α* increased by 2.04 times, from (94.14 ± 1.68) to (46.23 ± 1.13) pg/mL, when compared to indicators before the start of treatment. It should be noted that on day 12, the animals showed a further decrease in pro-inflammatory cytokine levels. In calves of test group $_1$T receiving standard treatment it was accompanied by decrease in IL-1*α* by 1.53 times only, in IL-6 – by 1.86 times, in IL-8 – by 2.16 times, and in TNF-1*α* – by 1.69 times. More significant interleukin shifts were detected in sera from calves of test groups $_2$T and $_3$T: IL-1*α* – by 1.83 and 2.02 times, IL-6 – by 2.52 and 3.35 times, IL-8 – by 1.85 and 2.54 times, TNF-1*α* – by 2.14 and 2.26 times, respectively, when compared with the initial data.

Thus, the treatment was shown to be effective for calves with acute catarrhal bronchopneumonia in all three test groups. However, the best results were achieved with the aerosol administration of *Hypericum perforatum* extract, herbal product, in combination with antibacterial treatment, as evidenced by significant positive changes in cellular and humoral immunity, as well as decrease in the of pro-inflammatory cytokine levels.

CONCLUSION

Aerosol administration of *Hypericum perforatum* extract, herbal product, within the complex treatment of calves with acute catarrhal bronchopneumonia demonstrated high efficacy compared to two other treatment regimens. Therewith, in test group $_3$T overall clinical improvement was observed as early as on (4.90 ± 0.64) day, which was 4.35 days faster than in calves of test group $_1$T; the calves in this group demonstrated a faster recovery of appetite, consumed feed more readily, their coats became smooth and shiny, and their serum immunological parameters reached the reference levels of clinically healthy animals by day 12. Calves receiving routine treatment (group $_1$T) demonstrated 29.27% increase in T-helper cells only on day 7. More significant shifts in the cellular immunity: a 1.87-fold increase in T-helper levels, and decrease in T-suppressor levels by 1.30 times and NK cells by 1.19 times were observed in calves of test group $_2$T on day 7. The most significant positive changes in cellular immunity were reported in calves of test group $_3$T: an increase in total T cells by 18.41% and T-helper cells by 111.72% and decrease in T-suppressor and NK cell levels by 1.37 and 1.45 times, respectively. Further positive changes in cellular immunity indicators were noted in all test groups on day 12, but only in calves of test group $_3$T they reached the reference values. Thus, increase in total T-lymphocytes and T-helper cells by 29.92% and by 188.28%, respectively, and decrease in T-suppressors and NK cells by 1.79 and 1.68 times, respectively, were observed in calves of test group $_3$T. On day 7 IRI increased in test group $_1$T by 44.44%, in test group $_2$T – by 143.73%, and test group $_3$T – by 188.24%. On day 12 calves of all test groups demonstrated highly significant increase in IRI: in calves of test group $_1$T – by 2.71 times, test group $_2$T – by 4.98 times; test group $_3$T – by 5.35 times (in $_3$T group it was reaching the reference values). Acute catarrhal bronchopneumonia in calves was shown to trigger a major shift in humoral immunity characterized by decrease in B-lymphocyte counts and significant rise in pathogenic medium- and small-molecular CICs. Clinically manifested acute respiratory pathology in calves can lead to a cytokine storm, where the immune system's overreaction causes an overproduction of pro-inflammatory cytokines. On day 7 a sharp drop in interleukin levels was observed in calves of all test groups, and in calves of test group $_3$T these indicators reached the reference values: IL-1*α* decreased by 1.92 times, IL-6 – by 2.93 times, IL-8 – by 2.24 times, and TNF-1*α* – by 2.04 times. It should be noted that on day 12, the animals showed a further decrease in pro-inflammatory cytokine levels. In calves of test group $_1$T receiving routine treatment it was accompanied by decrease in IL-1*α* by 1.53 times, in IL-6 – by 1.86 times, in IL-8 – by 2.16 times, and in TNF-1*α* – by 1.69 times only. More significant interleukin shifts were detected in sera from calves of test groups $_2$T and $_3$T: IL-1α – by 1.83 and 2.02 times, IL-6 – by 2.52 and 3.35 times, IL-8 – by 1.85 and 2.54 times, TNF-1*α* – by 2.14 and 2.26 times, respectively, when compared with the initial data.

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Seasonal and age-related dynamics of buffalo fascioliasis in the Dagestan lowlands

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ABSTRACT

Introduction. Fascioliasis is highly prevalent in the North Caucasus lowlands, infecting 37–46% of adult buffaloes; however, the critical drivers of infection – seasonality and host age – are poorly understood.

Objective. Investigating seasonal and age-related fascioliasis dynamics in buffaloes kept in the lowland zones of the Republic of Dagestan.

Materials and methods. To investigate the spread of fascioliasis in the lowland Babayurt region, a total of 240 fecal samples from buffaloes aged one year and older, 20 liver samples, and 1,428 pond snails (*Lymnaea palustris, Lymnaea stagnalis, Lymnaea auricularia,* and *Lymnaea truncatula*) were collected. Parasites were detected using both antemortem (coproscopy) and postmortem (helminthological autopsy of the liver and gallbladder) methods.

Results. A high prevalence of fascioliasis was established in adult buffaloes of the lowland zone, a phenomenon attributable to the cumulative nature of parasitic infection. Infection rates demonstrated significant seasonal variation, peaking in December (60%) and reaching a minimum in June (40%). A marked increase in both prevalence and intensity of infection was observed from August to November, likely driven by a seasonal rise in the population of infected intermediate hosts on pastures. Among the gastropods studied (*L. palustris, L. stagnalis, L. auricularia*, and *L. truncatula*), larval stages of fasciola were found exclusively in the dwarf and great pond snails, confirming their role as the key intermediate hosts in this region.

Conclusion. Understanding the regional epizootology of buffalo fascioliasis is crucial for developing effective control strategies against this zoonosis. Our findings, which elucidate the local dynamics of the parasite's life cycle, provide a foundation for targeted prevention measures tailored to the specific conditions of the area.

Keywords: Republic of Dagestan, lowlands, fasciolasis, *Fasciola hepatica*, buffalo, infestation rate, invasion prevalence and intensity, seasonality, age-related dynamics

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Сезонная и возрастная динамика фасциолеза буйволов в равнинной зоне Республики Дагестан

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РЕЗЮМЕ

Введение. В равнинных областях Северного Кавказа наблюдается высокая распространенность фасциолеза среди взрослого поголовья буйволов, показатели инвазированности варьируют от 37 до 46%. Сезонная и возрастная динамика зараженности буйволов фасциолами в данном регионе остается невыясненной.

Цель исследования. Изучение сезонной и возрастной динамики фасциолеза буйволов, содержащихся в равнинной зоне Республики Дагестан.

Материалы и методы. Материалом для исследования служили 240 проб фекалий буйволов (в возрасте от года и старше), 20 экземпляров печени и 1428 экземпляров моллюсков семейства прудовиков: *Lymnaea palustris, Lymnaea stagnalis, Lymnaea auricularia* и *Lymnaea truncatula*. С целью изучения распространения фасциолеза в равнинном Бабаюртовском районе проведены исследования с использованием прижизненных (копроовоскопия) и посмертных (гельминтологическое вскрытие печени и желчного пузыря) обнаружения паразитов.

Результаты. Установлена высокая степень инвазии взрослых буйволов в равнинной зоне республики. Данный факт объясняется кумулятивным характером заражения, то есть накоплением паразитов в организме животных. Сезонность оказывает значительное влияние на зараженность буйволов фасциолезом. Экстенсивность инвазии достигала пика в декабре (60%) и была минимальной в июне (40%). В период с августа по ноябрь наблюдался заметный рост экстенсивности и интенсивности инвазии, что, по-видимому, связано с увеличением численности промежуточных хозяев паразита на пастбищах в это время года. Взрослые буйволы, обитающие на равнинах, чаще всего и более интенсивно заражались фасциолами. В результате

проведенных исследований по инвазированности моллюсков *L. palustris, L. stagnalis, L. auricularia* и *L. truncatula* установлено, что только малый и обыкновенный прудовики были заражены личинками фасциол, в то время как другие виды лимнеид оказались свободны от этой трематодозной инвазии.
Заключение. Изучение краевой эпизоотологии фасциолеза буйволов позволит более успешно бороться с данным зоонозным биогельминтозом с учетом особенностей местности и видового состава возбудителей. Знание особенностей жизненного цикла трематод также является важной составляющей в проведении мероприятий по борьбе и профилактике паразитарных болезней.

Ключевые слова: Республика Дагестан, равнинная зона, фасциолез, *Fasciola hepatica*, буйвол, зараженность, экстенсивность и интенсивность инвазии, сезонность, возрастная динамика

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INTRODUCTION

Animal husbandry is a crucial branch of agriculture, important for a country's economy. The spread of infectious and invasive diseases in animal husbandry is a significant barrier to industry development [1, 2].

Trematodiases, particularly fascioliasis caused by the liver fluke *Fasciola hepatica*, represent a significant global health and veterinary concern. This helminth is a widespread parasite of numerous mammalian species, including both domestic livestock and wildlife within the Russian Federation and beyond. Infection with *F. hepatica* induces immunosuppression, heightening host susceptibility to secondary viral and bacterial infections, which can result in mortality [3, 4, 5, 6].

Episodic and incomplete anthelmintic treatment of livestock fails to significantly reduce the incidence of fascioliasis. The success of a deworming program is not absolute but is mediated by several critical variables. The local climate and habitat define the parasite's environmental viability, the parasite's development cycle in mollusks dictates transmission windows, and agricultural practices – notably the timing and length of grazing, which are often linked to rainfall – determine host exposure risk. Favorable environmental conditions stimulate pond snail population growth, which in turn increases the host population available for infection by larval fasciolids, facilitating parasite transmission. This, in turn, leads to a high incidence of livestock infection with trematodes. Consequently, current prophylactic and therapeutic strategies against fascioliasis do not consistently achieve the eradication of sexually mature parasites from livestock hosts [7, 8, 9, 10, 11].

Fascioliasis is a widespread parasitic disease of cattle, particularly in southern Russia and the North Caucasus region. Notably, the prevalence of infection on specific farms in the region can be as high as 54.7%. Concurrent infection with *F. hepatica* and *F. gigantica* is frequently observed in livestock populations of southern zones. Studies in Kalmykia indicate an average bovine fascioliasis prevalence of 17.8%, ranging from 15.9 to 20.8%. The highest levels are recorded during the winter, and both infection rate and the number of helminth eggs shed increase significantly with host age. The prevalence peak occurs at 6 years of age [12].

Kabardino-Balkaria also demonstrates high prevalence of fascioliasis among food-producing animals. Helminthoscopic analysis revealed a cattle infection rate of 48.2% and a mean fecal egg count of 47.7 fasciola eggs per gram. Post-mortem helminthological examination revealed an infection prevalence of 52.3% and a mean intensity of infection (II) of 99.8 fasciola individuals per animal. Infection of adult cattle is observed year-round, with the rates ranging from 52.5% in August to 76.6% in February. Studies demonstrate that the II with fascioles in farm animals in Kabardino-Balkaria varies significantly depending on the time of year. In particular, there are different infection rates with adult trematodes and young fascioles [13, 14, 15].

The predominant species of fascioles affecting livestock in the region is *F. hepatica* (78.3%). However, the prevalence of infection with *F. gigantica* is higher in irrigated areas (from 6.4 up to 30.3%). In juvenile cattle, the first fasciola eggs are detected in fecal samples in July, after which the extensity of infection (EI) increases. Overall prevalence of fasciola infection among young animals is 22.7%. According to S. B. Cherkesov and A. K. Oshkhunova, the development of *F. hepatica* and *F. gigantica* eggs occurs in Kabardino-Balkaria from mid-March to mid-November. During winter, fasciola eggs exposed to freezing air temperatures desiccate and die, while up to 38.1% of fasciola eggs submerged in water can remain viable [16].

Studies in Kabardino-Balkaria demonstrate that the summer-autumn season provides the most favorable conditions for the reproduction of *Lymnaea auricularia* and *Lymnaea truncatula* snails, the intermediate hosts of fasciola. At this time, the infection rate of mollusks with parasite larvae reaches 3.8–9.5%. The distribution of mollusks depends on the landscape: *L. truncatula* prefers mountainous and foothill areas, *L. auricularia* prefers irrigated lands. It is important to note that the metacercarial cysts of fasciola are highly resistant to adverse conditions, remaining viable on hay during winter and on pasture vegetation. However, intense sunlight (insolation) quickly desiccates and destroys metacercarial cysts on exposed vegetation. Parasites at all developmental stages can be found in animal hosts throughout the year. The parasite population exhibits clear seasonal dynamics: adult forms dominate in the liver and gallbladder during the winter-spring period, while preimaginal stages predominate during the summer-autumn period [17, 18].

A comparable pattern of fascioliasis epidemiology is observed in cattle populations across the North and Central Caucasus. The highest prevalence of fasciola infection (70.7%) was recorded in January among the adult cattle. The first cases of fascioliasis in young cattle were detected at the end of July. The high reproductive capacity of fasciola, particularly during the summer months, is a key driver of widespread invasion in the host population [19].

Studies in the Chechen Republic have revealed varying prevalence rates of fasciola infection among domestic livestock and wild animals. Prevalence in sheep was 33.2% (II – 8–59 individuals/animal), 8.1% in goats (II – 3–11 individuals/animal), 19.2% in cattle (II – 12–108 individuals/animal), 13.4% in buffaloes (II – 5–42 individuals/animal), 4.8% in horses (II – 3–7 individuals/animal), 5.7% in hares (II – 2–3 individuals/animal) and 0.9% in roe deer (II – 3 individuals/animal). Along with *F. gigantica, F. hepatica* was reported in these animals. *F. gigantica* dominates in the lowland Chechen regions, while *F. hepatica* is predominant in the mountainous regions. The researchers also established the following infection rates in farm animals: 28% for cattle (II – 14–117 individuals/animal), 34.8% for sheep (II – 9–243 individuals/animal), 26.6% for goats (II – 5–24 individuals/animal) and 23.3% for buffaloes (II – 21–84 individuals/animal) [7, 20].

Fascioliasis in buffaloes in the North Caucasus region is understudied. The liver fluke disease caused by *F. hepatica*, is widespread in ruminant populations worldwide, occurring sporadically and locally. The parasite has established persistent, localized reservoirs of infection in specific natural habitats. Inadequate herd management practices, particularly the absence of structured pasture rotation, exacerbate the environmental contamination and transmission of *F. hepatica* in ruminant populations. Analysis of epidemiological data reveals that *F. hepatica* infection is most prevalent in adult cattle and small ruminants within regions of high humidity [21].

Susceptibility to *F. hepatica* increases significantly with host age, a pattern primarily attributed to the cumulative effect of repeated exposure and reinfection over time. In adult ruminants with prolonged pasture exposure, the liver can harbor both mature adult flukes and juvenile stages simultaneously [22, 23, 24].

Epidemiological data on infection seasonality are essential for determining the most effective timing of strategic deworming to control liver fluke burdens in ruminants.

In central Russia, periods of high humidity – particularly in spring and autumn – elevate the risk of fascioliasis outbreaks in grazing ruminants. The larval stages develop most successfully during the warmer months when water temperatures remain within a permissive 12–30 °C range. Any divergence from this thermal threshold directly disrupts development within the intermediate mollusk, effectively breaking the transmission cycle. Beyond temperature, the life cycle of *F. hepatica* is critically regulated by ambient humidity and solar radiation [25, 26, 27, 28, 29].

The prevalence of fascioliasis in buffalo populations exhibits clear age dependence, with infection rates consistently higher in adult animals than in juveniles. Sexually mature *F. hepatica* can survive within the definitive ruminant host for up to 4 years. Persistent grazing on infective floodplain meadows is the primary driver for the elevated intensity of invasion most frequently recorded in adult livestock [30].

The intensity of the trematode life cycle is influenced by host population metrics – such as abundance, density, and distribution – as well as by the extensity of infection and abiotic factors like the hydrothermal regime (including precipitation, humidity, and temperature) during the vegetation season. This situation is a complex environmental problem that has a negative impact on the food security of the local population [31, 32, 33].

The freshwater biotopes of the North Caucasus include 10 species of mollusks: *Lymnaea truncatula, Lymnaea auricularia, Galba oblonga, Lymnaea peregra, Physa fontinalis, Lymnaea stagnalis, Physa acuta, Succinea putris, Planorbis planorbis* and *Lymnaea ovata*. Among these, *L. truncatula* serves as the obligate intermediate host for the trematode *F. hepatica*. The distribution of *L. truncatula* covers a wide range of natural and artificial water reservoirs. The intensity of invasion of *L. truncatula* with by *F. hepatica* larvae ranges from 3.05 to 27.14% (13.10% in average) depending on the biotope. A parallel variation in the morphological features of *L. truncatula* was observed, which correlated with the type of water body. The greatest susceptibility to invasion is observed in mollusks of the third stage of development [34, 35].

Analysis of the literature indicates a correlation between mollusk population density and their level of parasitic infection. Higher mollusk density is associated with a greater prevalence of parasitic infections. As common parasites of mollusks, trematodes act as key agents of natural population control, significantly influencing host numbers. They also alter fundamental ecosystem processes by reshaping trophic networks and modifying system-wide structure and function. The complex, multi-host life cycle of trematodes is a key driver of endemic mollusk infection, creating significant and persistent risks to regional and national food security [36].

MATERIALS AND METHODS

From March 2023 to March 2024, a cross-sectional study was conducted in the lowland zone of the Republic of Dagestan to investigate the prevalence of fascioliasis in buffalo across different age groups. Fecal samples were subsequently collected from the buffaloes and analyzed for fasciola infection using established laboratory techniques.

Fecal samples were collected from 240 buffaloes categorized into four age groups (n = 60 per group): calves (< 1 year), young stock (1–2 years), adults (2–5 years), and mature animals (> 5 years). All samples were analyzed for fasciola eggs using a standardized flotation coproscopy technique.

All study animals were maintained on pastures known to be environmentally contaminated with parasite stages and situated near populated areas.

The age-related dynamics of *F. hepatica* infection in buffalo were studied using both intravital and postmortem diagnostic techniques. Intravital detection was based on coproscopic examination, while postmortem assessment involved helminthological autopsy of the liver and gallbladder, performed according to the method of K. I. Scriabin.

The intensity of fasciola invasion was assessed in autumn via helminthological post-mortem examination. Livers and gallbladders from 20 buffaloes, with five animals sampled from each of the four age groups, were analyzed for parasite burden.

Seasonal dynamics of *F. hepatica* development was studied by coproscopic examination according to the method of N. V. Demidov.

The distribution of biotopes inhabited by intermediate snail hosts of fasciola was surveyed monthly across the study pastures (5–7 hectare area). Three biotopes (5 to 10 m^2) were examined. A total of 1,428 mollusks belonging to the family *Lymnaeidae* were collected from 34 water reservoirs. The collected specimens comprised the following species: *L. palustris*, *L. stagnalis*, *L. auricularia* and *L. truncatula*. The mollusks were identified using the N. D. Kruglov guide[1].

During the grazing season, mollusk populations were monitored monthly for infection with larval stages of *Fasciola* spp. Parasites were initially identified using the compression slide technique under a light microscope. Subsequently, the intensity of infestation was quantified.

At the onset of the grazing season, field visits were conducted to buffalo pastures to sample and examine lymnaeid snails. This preliminary work aimed to establish the species composition and population density of these mollusks. Concurrently, natural biotopes serving as habitats for the intermediate host of *F. hepatica* were identified. Pond snails were collected using tweezers and placed in plastic containers.

Following collection, the mollusks were dissected. The soft body tissue was carefully extracted from the shell using fine-pointed scissors. In cases where the shell was exceptionally robust – particularly in larger specimens – it was first cracked open with a hammer to facilitate access. During this procedure, hemolymph – which may contain helminthic larval stages such as cercariae and rediae – was released.

To prevent workplace contamination and facilitate parasite examination under the microscope, all mollusk dissections were performed within a contained vessel – either a Petri dish, a watch glass, or a dissection cuvette. Following dissection, the mollusk's body was separated into discrete anatomical sections or organs. Each of these tissues was then mounted between a microscope slide and a coverslip (compression slide technique) for detailed microscopic examination.

For very small mollusks, the entire specimen was often examined *in situ*–directly within the intact shell. In this preparation, live cercariae – if present – would actively emerge from the mollusk's tissue into the surrounding liquid medium. Their intrinsic motility significantly facilitated detection and identification under the microscope.

Statistical data were processed using variation statistics (according to N. A. Plokhinsky) and the Biometrics software.

RESULTS AND DISCUSSION

Parasitological surveys in the Babayurt Raion indicated a high prevalence of fascioliasis in adult buffalo, with many herds showing a heavy parasite burden (infection intensity).

Our data suggest that in the shallow, hydrologically stable reservoirs of the lowlands, temperature serves as the key driver of biological activity for *F. hepatica*, maintaining the continuity of its epizootic chain from egg to miracidium. Liver fluke life cycle is shown in Figure 1.

The parasite's life cycle begins with the sexual reproduction of the adult stage, known as the marita, which resides in the bile ducts of the definitive host (humans or cattle). Eggs produced by the marita are excreted into the environment via the host's feces.

For development to proceed, these eggs must reach a freshwater habitat. In water, a ciliated larva called a miracidium hatches from the egg.

The next stage involves the free-swimming miracidium locating and penetrating a suitable intermediate host – a dwarf pond snail. Inside the snail, a phase of complex asexual multiplication begins. The miracidium transforms into a primary sporocyst, which then produces numerous rediae.

Studies confirmed the widespread fascioliasis prevalence among buffaloes inhabiting the lowlands of the region.

A direct relationship was established between the age of animals and the level of their infection (extensity and intensity) with liver fluke. The results are given in Table 1.

Prevalence of infection with fascioles in different age groups was: calves (< 1 year old) – 18.3%, in young animals (< 2 years old) – 23.3%, in buffaloes (2–5 years old) – 36.7%, and in > 5 years old animals – 48.3%.

The intensity of the infection, determined by counting the number of helminth eggs per gram of faeces, also increased with age: in calves, it was (58.6 ± 9.9), in young animals – (88.5 ± 9.2), in 2 to 5 years old buffaloes – (127.6 ± 8.7), in > 5 years old

[1] Kruglov N. D. Molluscs of family *Lymnaeidae* (*Gastropoda, Pulmonata*) of Europe and Northern Asia. Smolensk: SGPU Publishing; 2005. 507 p. https://djvu.online/file/tffRyg0rOq4jg (in Russ.)

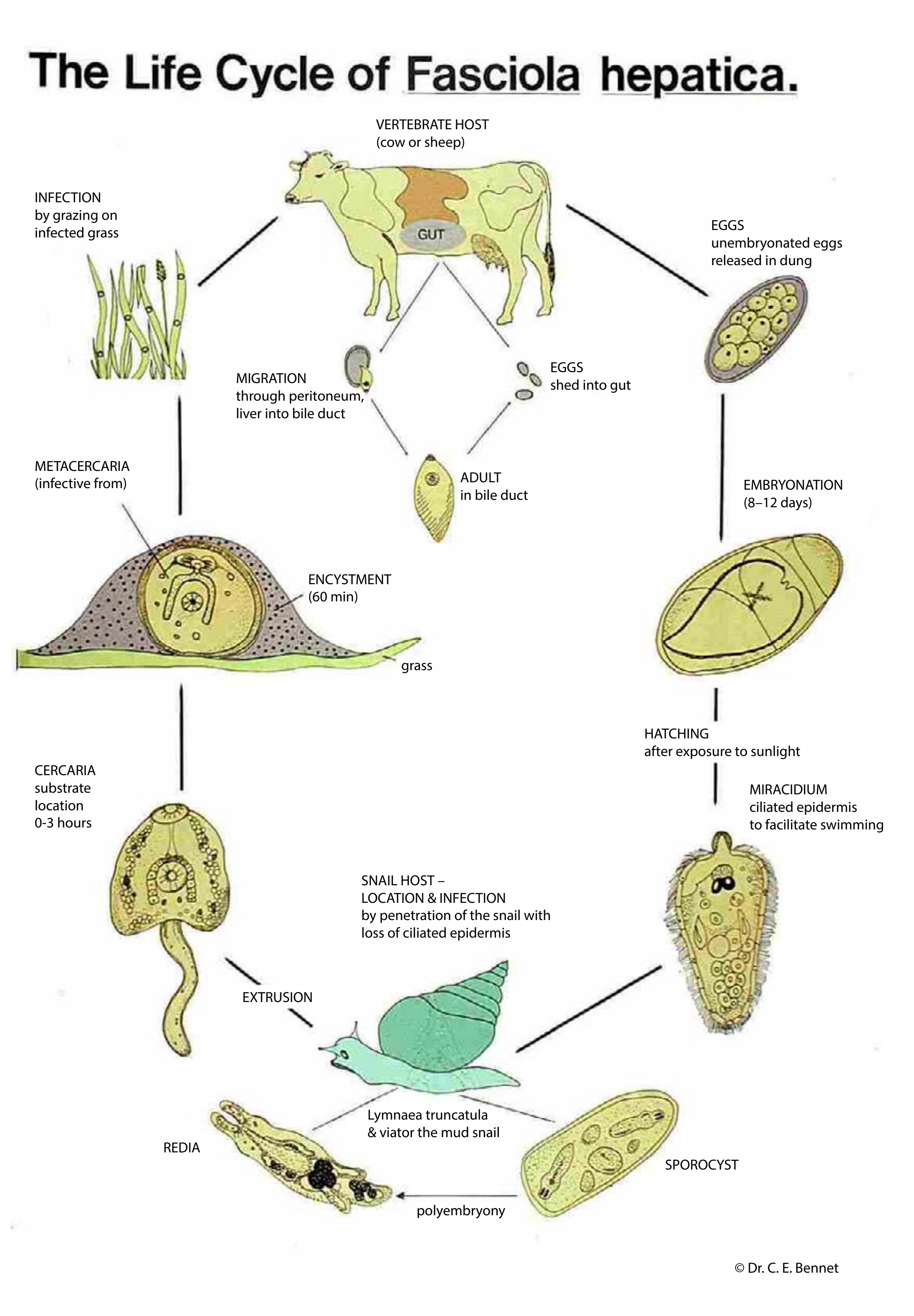

Fig. 1. Common liver fluke life cycle

animals – (166.4 ± 9.3) eggs per animal. The overall prevalence of fasciola infection in the buffalo population was 31.7%. The mean intensity of infection, measured as the number of eggs per animal, was (112.3 ± 9.2).

A direct positive correlation was confirmed between buffalo age and infection prevalence, with the highest prevalence (48.3%) observed in adult animals. The intensity of invasion in adult buffaloes is almost three times (2.8 times) higher than in calves.

Table 1
Prevalence of fasciola infection in buffaloes by age group, determined by coproscopy

Parameters	Buffalo age				
	< 1 year	< 2 years	2–5 years	> 5 years	Total
Tested, in total	60	60	60	60	240
Infected, in total	11	14	22	29	76
EI, %	18.3	23.3	36.7	48.3	31.7
Number of *F. hepatica* eggs per animal	58.6 ± 9.9	88.5 ± 9.2	127.6 ± 8.7	166.4 ± 9.3	112.3 ± 9.2

Table 2
Prevalence of fasciola infection in buffaloes by age group, determined by postmortem helminthological autopsy of the liver

Parameters	Buffalo age				
	< 1 year	< 2 years	2–5 years	> 5 years	Total
Tested, in total	5	5	5	5	20
Infected, in total	1	2	3	3	9
EI, %	20.0	40.0	60.0	60.0	45.0
Number of *F. hepatica* per animal	14.6 ± 2.3	46.7 ± 8.5	92.7 ± 9.9	132.4 ± 9.8	71.6 ± 7.6

Post-mortem examination revealed the lowest prevalence of fasciola infection in the youngest cohort (buffaloes < 1 year of age): the EI – 20.0% and the II – (14.6 ± 2.3) individuals per animal, and the highest in the oldest cohort (> 5 years old): the EI – 60.0% and the II – (132.4 ± 9.8) individuals per animal (Table 2).

Analysis of seasonal dynamics revealed an absence of *F. hepatica* infection in the examined livestock during March and April. In June and August, the average parasitic burden per animal was (19.7 ± 2.1) and (21.8 ± 2.4). In October, the number of detected fasciola increased to (49.6 ± 3.5). In December, the maximum number was recorded – (67.1 ± 6.4), Figure 2.

Field surveys in the Babayurtovsky Raion showed that pastures within the Terek River floodplain are characterized by a specific hydrology, featuring numerous small, lentic (stagnant or slow-flowing) water bodies formed by distributary streams. These water bodies are inhabited by *L. palustris* (marsh pond snail), *L. stagnalis* (great pond snail), *L. auricularia* (European ear snail) and *L. truncatula* (dwarf pond snail).

During the pasture survey, persistent wet hollows – small, groundwater-fed pools that do not dry out, were identified. These basins exhibited variable

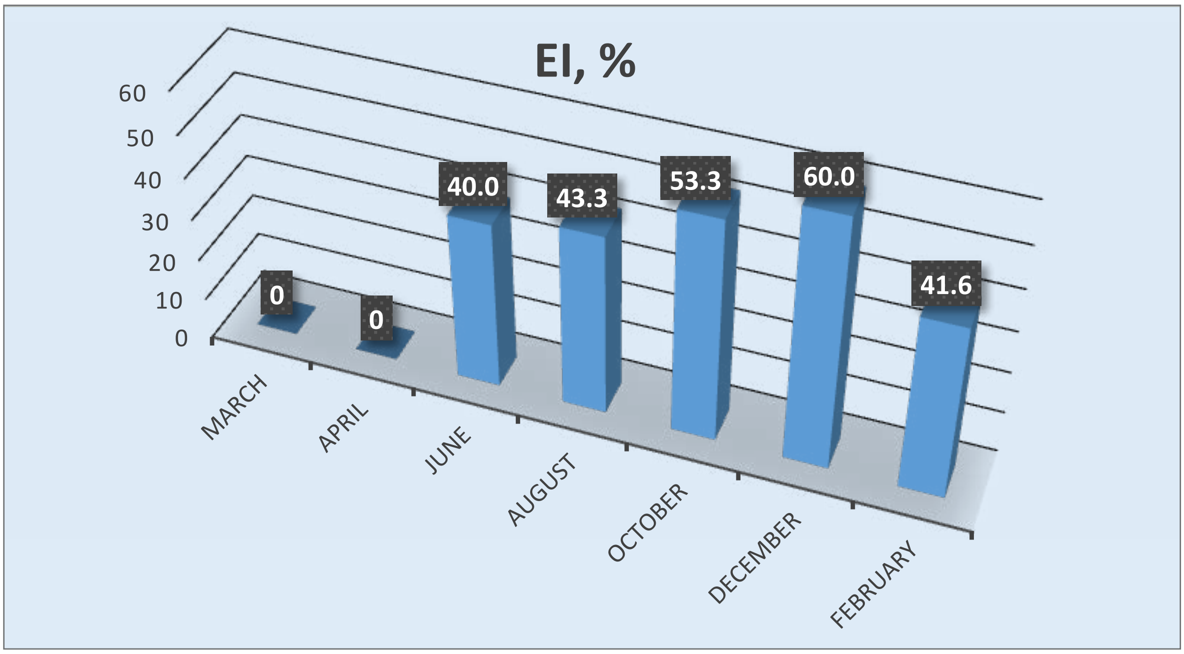

Fig. 2. Seasonal prevalence of fascioliasis in buffaloes of the Dagestan lowlands (coproscopy results)

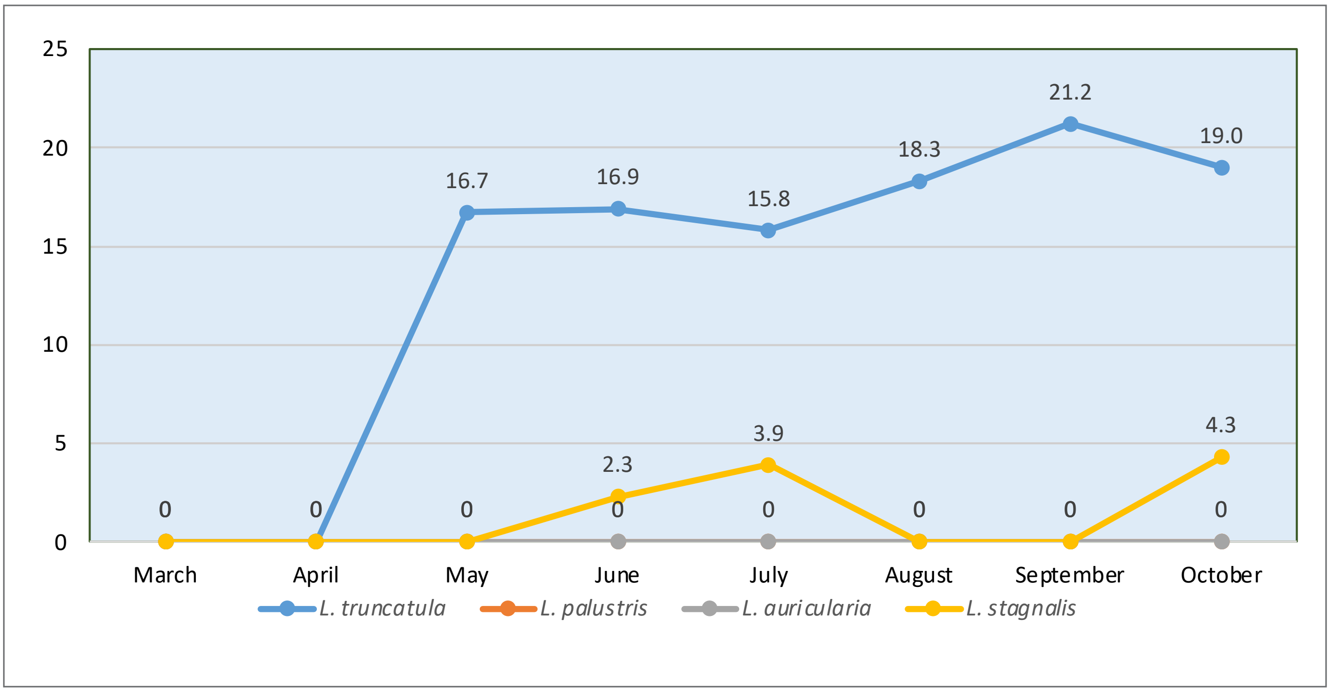

Fig. 3. Prevalence of F. hepatica infection in lymnaeid snails, March – October 2023 (%)

morphometry: surface areas started from 4 m², the substrate consisted of silty sediments, and the water column depth ranged from 3 to 25 cm. Various species of aquatic invertebrates and other freshwater inhabitants were found in these hollows. The dwarf pond snail was a predominant species among them. Its population density ranged from 50 to 70 mollusks per 1 m². The vegetation of these wet hollows was dominated by *Elytrigia repens*, *Plantago lanceolata*, and several sedge species of the genus *Carex*.

The presence of grazing livestock in the area was confirmed by direct observation of fresh hoofprints and feces. The proximity to saturated soils and streams caused animal hoofprints to fill with water, forming persistent puddles. In these microhabitats, the population density of the dwarf pond snail reached or exceeded 60 individuals per 1 m². Other mollusk species were also present within the same puddles.

In areas of peak mollusk concentration, measured parameters showed neutral to slightly alkaline conditions (pH 7.0–7.7 in water and soil, via litmus paper), with water temperature (17–22 °C) closely tracking air temperature due to the shallow nature of the water bodies.

The investigation of the malacofauna in pasture-associated aquatic habitats confirmed the presence of snail intermediate hosts of fasciola. Among all lymnaeid snails examined, only *L. truncatula* and *L. stagnalis* were found harboring larval stages of *F. hepatica*. Other lymnaeid species were not infected by these trematodes. The results are shown in Figure 3.

In rare instances, echinostomatid larvae were detected. These were distinguished from fasciola larvae by their greater motility and larger size.

From May to October, we recorded infection of dwarf pond snails with rediae and cercariae of *Fasciola* spp. This finding indicates that larval stages can partially overwinter within the mollusk host. The highest intensity of infection of the dwarf pond snail was observed in September.

Our data established that the population density of the dwarf pond snail in floodplain biotopes peaks during August and September. This period of peak snail density coincides with the highest prevalence of *F. hepatica* larval infection in the snails, creating optimal conditions for the environmental persistence and transmission of fascioliasis.

CONCLUSION

A direct positive correlation was confirmed between buffalo age and fasciola infection prevalence. In adult animals, the EI is 48.3%, and the II is 2.8 times higher than in young animals.

The studies demonstrated a clear age-dependent trend, with infection prevalence increasing significantly in older animal cohorts. In < 1 year old calves the EI was 18.3%, the II was (58.6 ± 9.9) individuals per animal; in < 2 years old animals the EI was 23.3%, the II was (88.5 ± 9.2) individuals per animal; in < 5 years old buffaloes the EI was 36.7%, the II was (127.6 ± 8.7) individuals per animal; in > 5 years showed 48.3% EI, the II was (166.4 ± 9.3) individuals per animals.

Post-mortem examination of livers from 20 buffaloes revealed fasciola infection in 9 animals, corresponding to a prevalence of 45.0%. However, in young animals < 1 year of age, the infection rate was only 20.0%, while in buffaloes over 5 years of age, this figure reached 60.0%. Moreover, the II increased with age, reaching maximum values in adult animals (on average (132.4 ± 9.8) individuals per animal). Adult buffaloes grazing in lowland areas demonstrated a consistently high intensity of infection by *F. hepatica* larvae throughout the year. This trend is primarily driven by a cumulative effect – fasciola parasites accumulate within the host over time, leading to higher prevalence and intensity in older animals.

Seasonal monitoring revealed a complete absence of fasciola parasites in buffalo during the early spring months of March and April. In June and August, the average parasitic burden per animal was (19.7 ± 2.1) and (21.8 ± 2.4). Infection intensity with the liver fluke increased significantly from August to October, peaking at (49.6 ± 3.5) parasites per animal. This seasonal peak likely corresponds to the period of highest abundance of the intermediate snail hosts in the pastures. The peak of infection intensity in buffalo was recorded in December, with a mean burden of (67.1 ± 6.4) parasites per animal.

The highest densities of lymnaeid snails were associated with specific lentic habitats: stagnant or slow-flowing reservoirs, abandoned irrigation channels, seasonal puddles, and small ponds prone to summer drying. These areas are inhabited by many species of mollusks, including the dwarf pond snail (*L. truncatula*), whose population density reached 70 individuals per 1 m^2.

Monitoring of larval trematode infections in four lymnaeid species – *L. palustris*, *L. stagnalis*, *L. auricularia* and *L. truncatula* revealed that only the dwarf and great pond snail harbored fasciola larvae. The other three species showed no evidence of infection with this parasite. In some instances, echinostomatid larvae were detected. These were distinguished from fasciola larvae by their greater motility and elongated shape.

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Recombinant antigens in serological diagnostics of transboundary and emerging bovine infections

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ABSTRACT

Introduction. Transboundary and emerging infections of cattle and small ruminants, such as peste des petits ruminants, Schmallenberg virus infection, etc., pose a serious animal health and economic threat in the context of developing globalization. Given the current geopolitical situation, the need for modern domestically produced diagnostic systems is particularly acute. Such systems can be developed using genetic engineering methods.

Objective. Analysis of domestic and foreign publications on the production of recombinant proteins of pathogens of transboundary and emerging infections of cattle and small ruminants. Creation of genetic constructs based on the processed data for further development of diagnostic tools, in particular enzyme-linked immunosorbent assay test systems.

Materials and methods. Using bioinformatics tools, codon composition of the sequences encoding the nucleocapsid proteins of peste des petits ruminants virus and Schmallenberg virus was analyzed and optimized. The optimized gene fragments were synthesized *de novo* and cloned into the pET-32b(+) expression vector. Successful insertion of the target sequence into the vector was confirmed by polymerase chain reaction and restriction analysis.

Results. Information on enzyme-linked immunosorbent assay test systems developed on the basis of recombinant antigens for the diagnosis of peste des petits ruminants and Schmallenberg virus infection is presented. The main technological aspects of obtaining recombinant antigens for their further use in a diagnostic system factored in the biological features of a particular pathogen are highlighted. Our proprietary methodology for creating protein expression vectors for the pathogens of the diseases under review is additionally described.

Conclusion. The most promising recombinant antigens for use in enzyme-linked immunosorbent assay test systems designed to detect antibodies against peste des petits ruminants virus and Schmallenberg virus are full-length and truncated virion nucleocapsid proteins. Furthermore, the biophysical properties and antigenic structure of these proteins enable their production in *Escherichia coli*. It should be noted that production of significant amounts of functional proteins in soluble form may require their expression as part of fusion proteins with tags enhancing solubility and facilitating correct folding.

Keywords: recombinant antigens, serological diagnostics, enzyme-linked immunosorbent assay (ELISA), cattle, peste des petits ruminants, Schmallenberg virus infection

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Рекомбинантные антигены в серологической диагностике трансграничных и эмерджентных инфекций рогатого скота

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РЕЗЮМЕ

Введение. Трансграничные и эмерджентные инфекции крупного и мелкого рогатого скота, такие как чума мелких жвачных животных, болезнь Шмалленберга и другие, в условиях развивающейся глобализации представляют серьезную эпизоотическую и экономическую угрозу. С учетом текущей геополитической обстановки необходимость в современных диагностических системах отечественного производства ощущается особенно остро. Подобные системы могут быть разработаны с использованием методов генной инженерии.

Цель исследования. Анализ отечественных и зарубежных публикаций, посвященных получению рекомбинантных белков возбудителей трансграничных и эмерджентных инфекций крупного и мелкого рогатого скота. Создание на основе обработанных данных генетических конструкций для дальнейшей разработки на их основе диагностических средств, в частности иммуноферментных тест-систем.

Материалы и методы. При помощи инструментов биоинформатики проведен анализ и оптимизация кодонного состава последовательностей, кодирующих нуклеокапсидные белки вирусов чумы мелких жвачных животных и болезни Шмалленберга. Оптимизированные фрагменты генов были синтезированы *de novo* и клонированы в экспрессирующий вектор pET-32b(+). Успешность вставки целевой последовательности в вектор подтверждали методом полимеразной цепной реакции и рестрикционного анализа.

Результаты. Представлена информация о разработанных на основе рекомбинантных антигенов иммуноферментных тест-системах для диагностики чумы мелких жвачных животных и болезни Шмалленберга. Освещены основные технологические аспекты получения рекомбинантных антигенов для дальнейшего их использования в диагностической системе с учетом особенностей биологии конкретного инфекционного агента, а также описана собственная методология создания векторов для экспрессии белков возбудителей обозреваемых болезней.

Заключение. Наиболее перспективными для использования в качестве рекомбинантных антигенов в иммуноферментных тест-системах, направленных на выявление антител к вирусам чумы мелких жвачных животных и болезни Шмалленберга, являются полные и усеченные нуклеокапсидные белки вирионов. При этом биофизические свойства и антигенная структура данных белков позволяют получать их в культуре клеток *Escherichia coli.* Следует отметить, что для получения значительных количеств функциональных белков в растворимой форме может понадобиться их экспрессия в составе слитых белков с повышающими растворимость и облегчающими корректный фолдинг тегами.

Ключевые слова: рекомбинантные антигены, серологическая диагностика, иммуноферментный анализ, рогатый скот, чума мелких жвачных, болезнь Шмалленберга

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INTRODUCTION

To date, the transboundary and emerging infections of cattle and small ruminants pose a serious threat. First of all, this is due to the significant impact on the economy and international trade.

Such infectious animal diseases as peste des petits ruminants (PPR) and Schmallenberg virus (SBV) infection are highly contagious and, in the context of the developing globalization and formation of tight international relations, they can easily spread beyond the borders of enzootic and infected regions. Outbreaks of these infections are accompanied by significant economic losses, which are associated with the animal disease eradication and prevention measures, decrease in animal performance and restrictions on the export of animals and animal products. At the same time, the need for reliable diagnostic tools becomes especially urgent.

The current level of molecular biology and biotechnology development, in particular, recombinant DNA technology, allows creating highly sensitive, specific and safe diagnostic tools.

The most valuable tool for the serological diagnosis of viral animal diseases is enzyme-linked immunosorbent assay (ELISA). Furthermore, the ability to produce recombinant proteins ensures that future ELISA test systems will be safe as there will be no need to handle infectious agents during their manufacture. Moreover, they will have much greater sensitivity and specificity due to a more efficient purification method used. The ability to synthesize recombinant proteins in the laboratory makes it easy to scale up the production of antigens, allowing production to be customized to the existing requirements.

It should be noted that recombinant protein-based ELISA test systems intended for the diagnosis of infectious animal diseases are being successfully developed abroad by such companies as IDvet (France), IDEXX Laboratories (USA), Ingenasa (part of Eurofins Scientific holding, Luxembourg). High sensitivity and specificity are crucial factors contributing to the use of these diagnostic tools, *inter alia* in domestic diagnostic laboratories.

Peste des petits ruminants (PPR) is an acute or subacute viral disease of sheep and goats, characterized by fever, conjunctivitis, rhinitis, necrotic stomatitis, gastroenteritis, pneumonia and mortality of the infected animals [1, 2]. The first documented reports of this disease date back to the early 20th century. From 1917 to 1929, outbreaks of a disease clinically similar to rinderpest were reported in sheep and goats in Senegal, Guinea and Nigeria. In the period from 1940 to 1942, L. Gargadennec and A. Lalanne described a disease affecting small ruminants in West Africa [3]. They detected a disease with clinical signs similar to rinderpest, but affecting only sheep and goats. Later, in 1968, peste des petits ruminants was established as an independent nosological entity [4].

The disease is caused by an enveloped RNA-containing pleomorphic virus belonging to the genus *Morbillivirus* of the *Paramyxoviridae* family. It is closely related to the viruses causing rinderpest, canine distemper, phocine distemper, and human measles [1, 2]. The virion size can vary from 150 to 700 nm. It consists of an RNA molecule, which, together with a phosphoprotein and an L-protein, is covered with a nucleocapsid envelope, all housed within a supercapsid.

The PPR virus (PPRV) genome is composed of a single-stranded, non-segmented, linear RNA molecule with negative polarity. The genome is about 16,000 nucleotides in size and encodes six structural proteins such as nucleocapsid protein (N), phosphoprotein (P), polymerase protein (L), hemagglutinin (H), fusion protein (F) and membrane protein (M). Furthermore, two nonstructural proteins, C and V, are translated from the P-encoding transcript using an alternative reading frame [1].

Today, PPR is enzootic in most parts of Africa, Middle East, South Asia, and China. Due to the high morbidity and mortality in the primary outbreaks (up to 100%), the disease is of great economic significance for the enzootic regions [2]. It should be noted that in recent years, the PPR outbreaks have been reported in countries with which the Russian Federation maintains trade and economic relations. This is indicative of the threat of PPRV introduction into Russia from the disease infected countries [5].

The main source of the PPR causative agent are the diseased and/or convalescent animals. The most common transmission routes involve contact and airborne ones. Goats, sheep, and wild small ruminants are susceptible to the PPRV. Moreover, there are reports of the PPR causative agent detection in cattle, camels, pigs and buffaloes. It should be noted that such hosts are atypical for this virus, and in case of infection, further disease and spread of the pathogen do not occur [2, 6].

As soon as the virus enters the body of a susceptible animal through the oral cavity or nasopharynx, the virions are captured by cells of the monocyte macrophage system and transported to regional lymph nodes and lymphoid tissue clusters, where primary and secondary replication of the pathogen occurs. Next, the virus is disseminated to organs and tissues remote from the primary replication foci. It should be noted that the PPRV demonstrates pronounced tropism to lymphoid tissues. Its dissemination throughout the organism causes profound immunosuppression due to the destruction of a significant part of leukocytes. In addition, necrotic lesions are observed in the spleen, thymus, and pulmonary lymph nodes [1].

The PPR incubation period ranges from 2 to 7 days. Viremia develops during 1–2 days before the clinical signs onset, and the active virus shedding begins, which contributes to the spread of the infection [2]. The PPR clinical signs include pyrexia, erosive stomatitis, ocular and nasal discharge, and diarrhea. Death usually occurs within 4–6 days after the fever onset. It should be noted that the form of the disease and its severity can vary depending on the individual animal characteristics, such as species, age, breed and production type [7].

As soon as the PPRV enters the body of a susceptible animal, a strong virus-specific immune response is reported despite significant immunosuppression. The key protective cellular and humoral immune responses are targeted at H-, F- and N-proteins of the virion. Activation of the cytotoxic T-cells is observed, which destroy the virus-infected cells. Furthermore, a large quantity of virus-neutralizing antibodies is produced, primarily targeting the glycoproteins of the virion envelope – the H- and F-proteins. In case of recovery, the animal acquires a stable lifelong immunity against re-infection.

Successful control of the disease spread, in particular, prevention of the infection in previously PPR-free regions, includes the use of a number of diagnostic tools, aimed primarily at detecting specific antibodies.

Several methods have been developed for PPR serological diagnosis, such as virus neutralization test, diffuse precipitation test, indirect immunofluorescence test, direct and indirect enzyme-linked immunosorbent assay (ELISA), as well as competitive ELISA [8]. It is important to note that the virus neutralization test, while considered the most accurate method, has significant drawbacks despite its advantages, i.e. labor-intensive and lengthy procedure. ELISA is, therefore, most often used for the routine studies [7].

The first ELISA kits for the detection of PPRV antibodies utilized inactivated and purified virus. It is worth mentioning that this approach was characterized by low specificity due to the presence of cell culture-contaminating proteins. The use of recombinant protein technology has solved this problem. Furthermore, production safety has been significantly enhanced. For this reason, to date, the majority of the PPR serological diagnostic tools are recombinant protein-based [9].

Recombinant N-protein is used as an antigen for ELISA kits to detect the PPRV antibodies, owing to the high degree of conservation of the gene encoding this protein. The protein is involved in the formation of the virion nucleocapsid, therefore it is expressed in large numbers by the affected cells [10]. In addition, N-protein has different epitopes, enabling its use for the detection of antibodies against PPR viruses of different genetic lineages [11], since all genetic lineages are serologically indistinguishable.

One of the first papers devoted to the production of the recombinant PPRV nucleocapsid protein was published in 1995 by G. Libeau et al. [12]. To produce the protein, they utilized an insect cell expression system using *Spodoptera frugiperda* (Sf9) cells. The protein-encoding sequence was derived from PPRV Nigeria 75/1 strain. The recombinant baculovirus *Autographa californica* served as the transfer vector, generated through recombination with the transfer vector pAcYM1 containing the inserted target gene under the control of a baculovirus promoter.

The competitive ELISA developed on the basis of a recombinant protein and monoclonal antibodies to it demonstrated high sensitivity and specificity. Moreover, a good correlation of the results ($r = 0.94$) was shown when comparing this method with the virus neutralization test [12].

In 2005, K.-S. Choi et al. [13] developed a competitive ELISA variant based on recombinant N-protein, which was specified by a short assay time of less than one hour. The recombinant antigen for this kit was also produced using Sf9 insect cell system. In addition, the antigen isolation included additional purification using affinity chromatography.

In 2006, employees of the Federal Centre for Animal Health N. V. Vavilova and A. V. Scherbakov developed an indirect ELISA version for PPR diagnosis. In contrast to the previously cited studies, the recombinant antigen for this diagnostic tool was produced in *Escherichia coli* culture. The N-protein-encoding sequence was amplified with the RNA of the native virus deposited in the Centre's collection of strains of microorganisms using reverse transcription polymerase chain reaction (RT-PCR) and inserted into the plasmid vector pQE under the control of the T5 promoter. Protein purification was performed using metal chelate affinity chromatography. The developed test system complied with the required sensitivity, specificity and reproducibility parameters. Over 200 sheep and goat serum samples from various regions of the Russian Federation were tested using this test system [8].

In addition to the full-length N-protein, its truncated forms were also tested for their suitability as antigens for various ELISA variants. In 2006, V. Yadav et al., having amplified the full-length and truncated N-protein-encoding sequences by RT-PCR, inserted them into the pET33b vector under the control of the T7 promoter. Using these genetic constructs, *E. coli* BL21 strain cultures were transformed and recombinant PPRV N-proteins were obtained, which showed successful results, when used for diagnostic purposes [10].

In the paper published in 2011 G.-R. Zhang et al. demonstrated the recombinant protein production method, which differed significantly from traditional ones. The PPRV N-protein-encoding nucleotide sequence was obtained from GenBank database (FJ905304), and its assembly was carried out using a set of 20 primer pairs through several overlapping PCRs. Furthermore, upon completion of the target sequence synthesis, several nitrogenous bases in the chain were replaced using site-directed mutagenesis. After verification of the assembled genetic construct, it was introduced into *E. coli* BL21 culture to produce a recombinant protein. The sensitivity and specificity of the indirect ELISA variant based on the obtained antigen was 96.7 and 96.1%, respectively, according to the results of 697 sera testing [11].

In 2019, D. Yu. Morozova et al. published a paper on the production of the recombinant PPRV N-protein. It should be noted that in addition to the encoding sequence, obtained by amplification of the native virus genome fragment, the resulting recombinant plasmid contained the thioredoxin gene. This allowed increasing the amount of protein synthesized by bacteria in the soluble form [9]. Later, an ELISA technique was developed based on the obtained protein, which demonstrated successful results [8].

Thus, the most promising antigen for routine diagnostics is the partial or full-length nucleoprotein N, produced using various genetic engineering systems, which is widely used in both international and domestic practice.

Schmallenberg virus (SBV) infection is an emerging viral vector-borne disease of ruminants, accompanied by depression, diarrhea, decreased milk production, as well as abortions, stillbirths, and congenital malformations in young animals [14, 15].

In 2011, outbreaks of an infection of unknown etiology were reported in cattle in Germany and the neighboring Netherlands. The disease was accompanied by a mild clinical course [16].

In herds where the new disease was reported, an increase in the number of calves with congenital pathologies was observed in the subsequent months. Severe neurological disorders, as well as musculoskeletal pathologies were noted. As a rule, calves with such abnormalities were inviable and died within a few days or weeks. In addition, the number of abortions and stillbirths increased [16, 17].

Using metagenomic analysis of blood samples from the diseased animals, a new virus was identified, named after the place where the first disease cases had been reported – Schmallenberg virus [18]. Later, during experimental infection of calves with the blood of cows that showed a positive result when tested for the virus presence by RT-PCR, the disease clinical signs were reproduced [17]. Thus, it was established that the cause of the outbreaks was a new virus that had not previously been reported in Europe.

Phylogenetic analysis of the viral genome sequences allowed assigning SBV to the *Bunyaviridae* family, genus *Orthobunyavirus*, Simbu serogroup, which comprises over 170 viruses, including pathogens that cause diseases in both humans (Oropouche fever and La Crosse encephalitis viruses) and ruminants (viruses causing Akabane disease, Aino disease and Cache Valley fever). It is worth mentioning that most representatives of the Simbu serogroup are spread in the Middle Eastern, African and Oceanian regions [14, 19].

The virus genome is a negative sense single-stranded RNA and it includes three segments (large, medium, and small), which encode RNA polymerase, surface glycoproteins, and nucleocapsid proteins, respectively [14, 20].

It was demonstrated that domestic and wild artiodactyls are SBV susceptible. Herewith, the susceptibility does not depend on gender or age. It is important to note that among domestic artiodactyls, sheep are the most susceptible to the infection, followed to a lesser extent by cattle and goats. Moreover, SBV-specific antibodies were detected in domestic pigs, wild boars, dogs, and elephants by various methods [14, 19, 20].

The pathogen can be transmitted both horizontally and vertically. In the first scenario, the virus enters the body of a susceptible animal through the bites of blood-sucking insects, primarily midges of the genus *Culicoides*. In the second case, the pathogen is transmitted from the infected mother to the fetus [14, 15, 19]. When infected with the SBV, the incubation period can range from one day to 4–5 days. The infection spread efficiency and rate depends on the regional natural and climatic conditions as well as on the activity of insect vectors. Up to 90% of the animal population can be infected over the relatively short period. Mortality from the disease is low and rarely exceeds 3–5%. Herewith, the number of abortions and stillbirths can reach 60% of the total number of pregnant animals [2, 15, 21].

It is worth mentioning that after the disease discovery in Germany, the virus rapidly spread across Europe. It was detected in biological samples collected from cattle, sheep, goats, bison and roe deer. Antibodies to the virus were detected in the sera of alpacas, fallow deer, mouflons, deer, and water buffaloes [18].

It was established that SBV exhibits a pronounced tropism for the nervous tissue. Upon entry of the pathogen into the body of a susceptible animal, viremia develops, lasting for several days. At the same time, a stable protective immunity is formed in parallel. However, when a pregnant animal not previously exposed to the pathogen is infected, the virus can cross the hematoplacental barrier and affect the fetus. It is important to note that the susceptibility of developing embryos and fetuses depends on the gestational age and the stage of placental development [22, 23].

The disease is characterized by a subclinical or subacute course with mild clinical signs, including fever, diarrhea, and decreased milk production in dairy animals, followed by recovery to the herd average within 2–3 weeks. The number of stillbirths increases, as does the frequency of newborns with congenital developmental pathologies such as arthrogryposis, brachygnathia, ankylosis, torticollis, and hypoplasia of the brain and spinal cord [20].

To effectively control the spread of the new viral infection and further monitor the animal disease situation, a range of diagnostic tools has been developed, including several serological ELISA systems based on the recombinant nucleocapsid protein of the virus.

The N-protein was chosen as the target due to its highest prevalence – this antigen is found both in virions and in infected cells. In addition, recombinant N-proteins of SBV-related hantaviruses (*Hantaviridae*), produced in *E. coli* culture, insect cells, or yeast, are successfully used for serological diagnosis of human hantavirus infections [17].

In 2013, E. Bréard et al. published a paper on the development of the recombinant N-protein-based ELISA systems for detecting antibodies to SBV. The basis for producing the recombinant nucleocapsid protein was an artificially synthesized nucleotide sequence obtained from GenBank database (accession number HE649914). It was cloned into an expression vector under the control of the T7 promoter. The expression of this genetic construct in *E. coli* BL21 (DE3) pLysS culture resulted in the production of a recombinant protein. In addition to the amino acid sequence of the SBV nucleocapsid protein, this recombinant protein contained six histidine residues at its N-terminus. After expression, the protein was denatured in the presence of urea and purified by immobilized metal affinity chromatography. The antigen produced in such way was then used as an antigen for testing sera of the infected and intact animals. It should be noted that the ELISA system based on the produced recombinant nucleocapsid protein demonstrated high sensitivity and specificity parameters. Moreover, the effectiveness of detecting positive samples was compared with the virus neutralization test, resulting in 98.9% agreement [16].

In the same year, 2013, a group of Chinese researchers led by Y. Zhang obtained the recombinant N-protein of the SBV. A fundamental difference in their work involved the use of the native nucleotide sequence of the produced protein. In addition, two types of genetic constructs based on pET-28a-c(+) and pMAL-c5X vectors were used to express the recombinant protein. They encoded proteins containing hexahistidine labels and maltose-binding protein, respectively. It should be noted that the expression of recombinant protein with maltose-binding protein increases its solubility. This greatly facilitates the subsequent isolation and purification of the protein. After expression, the proteins were purified under native conditions and subsequently used for monoclonal antibody generation and as antigens for ELISA [18].

Later, in 2014, J. Lazutka et al. [17] produced the SBV nucleocapsid protein in yeast cell culture. Similar to E. Bréard et al. [16] they used in their work an artificially synthesized sequence encoding the viral N-protein. The gene was inserted into the expression plasmid pFX7-SBV-6-HisN under the control of a galactose-induced promoter. The construct also contained an N-terminal hexahistidine tag for subsequent purification. The resulting protein was tested as an ELISA antigen on bovine sera. The sensitivity was 95%.

The published data allows for the conclusion that SBV infection is an emerging viral disease of ruminants posing high economic significance due to its pronounced impact on the reproductive performance of susceptible livestock.

For the SBV infection serological diagnosis, use of a recombinant antigen represented by a nucleocapsid protein is advisable. However, the accumulation of the recombinant protein during prokaryotic expression is accompanied by the formation of inclusion bodies, which necessitates the use of denaturing conditions for purification.

The work was aimed at the production of plasmid vectors based on the pET-32b(+) plasmid and codon-optimized gene sequences encoding the PPRV and SBV nucleocapsid proteins, for their expression in *E. coli* cells.

MATERIALS AND METHODS

The nucleotide sequences of the genes encoding the PPRV and SBV nucleocapsid proteins were received from GenBank database (accession numbers NC_006383.2 and NC_043582.1, respectively). Optimization of the codon composition for expression in *E. coli* was performed using the GenScript Rare Codon Analysis tool [24], taking into account the codon adaptation index (CAI). To eliminate mRNA secondary structure regions and restriction sites, the UGENE software was used [25]. The gene synthesis was made by "DNA-Synthesis" Company (Russia), followed by cloning into pUC57 vector.

E. coli XL1-Blue strain (Evrogen, Russia) of genotype recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac F′ proAB lacIq ZΔM15 Tn10 (Tetr)] was used for cloning. The cells were cultured in LB medium (Helicon Company, Russia) in an incubator shaker at 37 °C and 180 rpm. For the selection of recombinant clones, ampicillin was added to the medium to a final concentration of 100 μg/mL.

The pET-32b(+) plasmid vector (Novagen, USA) was linearized using NcoI and HindIII restricting enzymes (TransGen Biotech, China) at 37 °C for 1 hour. The synthesized genes were amplified using high-fidelity DNA polymerase and primers containing restriction sites (Table).

Table
Specifications of the primers used

Infectious disease	Primer	Sequence 5′→3′	Enzyme
Peste des petits ruminants	PPRV Hind 3620	ATAT**AAGCTT**CTGCGAGGCAATCTCGCTAAC	HindIII
	PPRV NcoI 3620	AAAA**CCATGG**CTACACTGTTAAAATCGCTC	NcoI
Schmallenberg virus infection	SBV_HindIII_R	CTCT**AAGCTT**GTGTATATTTATCCCGAACTGTTGCAGGAATG	HindIII
	SBV_NcoI	ATAT**CCATGG**ATGAGCTCGCAGTTTATCTTCGAG	NcoI

Recognition sites for restriction enzymes are in bold.

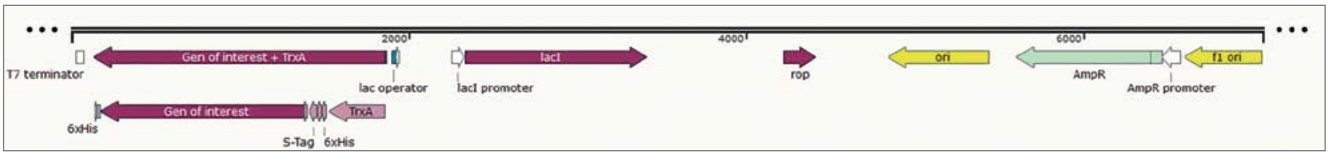

Fig. 1. Design of pET-32b(+) plasmid-based vector: T7 terminator – T7 phage transcription terminator; 6×His – histidine tag; Gen of interest – target gene encoding PPRV or SBV nucleocapsid protein; S-Tag – ribonuclease A fragment; TrxA – thioredoxin tag; Lac operator – lac promotor operator; LacI promoter – promotor of lacI protein; LacI – repressor protein; Rop – plasmid replication regulator; Ori – plasmid origin of replication; AmpR – ampicillin resistance gene; AmpR promoter – AmpR gene promoter. The scheme was constructed using SnapGene software[1]

The DNA fragments were purified using a gel extraction kit (Evrogen, Russia). Ligation was performed with T4 DNA ligase (Evrogen, Russia) with vector-to-insert molar ratio of 1:3 at 14 °C for 16 hours.

Competent cells were transformed using the heat shock method, after which 1 mL of LB medium was added and incubated for 1 hour at 37 °C. Selection was performed on plates with LB agar medium containing 100 µg/mL ampicillin.

For primary screening, bacterial colony PCR was used. For this purpose, primers complementary to the regions flanking the insertion site were used – T7 Promoter (TAA TAC GAC TCA CTA TAG GG) and T7 Terminal (GCT AGT TAT TGC TCA GCG G). The reaction was performed using Taq polymerase (Syntol, Russia). To confirm cloning, plasmid DNA was isolated using the CleanUp S-Cup kit (Evrogen, Russia) and restriction analysis was performed.

RESULTS AND DISCUSSION

Genetic constructs based on pET-32b(+) vector were developed for the expression of PPRV and SBV nucleocapsid proteins. The gene sequences obtained from GenBank database (NC_006383.2 and NC_043582.1) underwent codon optimization. The design of the developed vectors is shown in Figure 1.

After *de novo* synthesis of the target genes, they were amplified using high-fidelity polymerase and primers containing restriction enzyme recognition sites. The amplification results are shown in Figure 2.

The resulting amplicons were purified from the agarose gel and prepared for further restriction digestion alongside the vector. The restriction digestion results are shown in Figure 3.

The resulted DNA fragments, after purification and ligation via sticky ends using T4 DNA ligase, were used for the chemical transformation of competent *E. coli* XL-1 Blue cells. The presence of the insert in the transformant clones was verified by colony PCR using primers flanking the insertion region. The results are shown in Figure 4.

After the required amount of plasmid DNA containing the target gene inserts was accumulated, the restriction analysis was performed. The results are shown in Figure 5.

Fig. 2. Target PPRV and SBV gene amplification results: 1 – DNA molecular weight marker (310, 603, 872, 1,078, 1,353 bp); 2 – amplicon of the target PPRV nucleocapsid protein-encoding gene, estimated size – 1,053 bp; 3 – amplicon of the target SBV nucleocapsid protein-encoding gene, estimated size – 719 bp

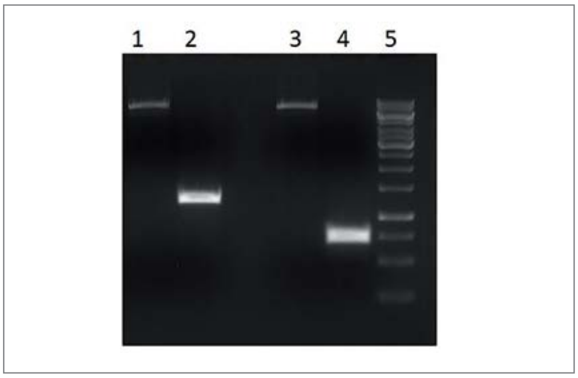

Fig. 3. Restriction digestion of target gene amplicons and pET-32b(+) vector: 1, 3 – pET-32b(+) vector, digested with NcoI and HindIII restriction enzymes, estimated size 5,861 bp; 2 – target gene amplicon encoding PPRV nucleocapsid proteins and digested with NcoI and HindIII restriction enzymes, estimated size 1,045 bp; 4 – target gene amplicon encoding SBV nucleocapsid protein and digested with NcoI and HindIII restriction enzymes, estimated size 710 bp; 5 – DNA molecular weight market (250, 500, 750, 1,000, 1,500, 2,000, 2,500, 3,000, 3,500, 4,000, 5,000, 6,000, 8,000, 10,000 bp)

[1] https://www.snapgene.com

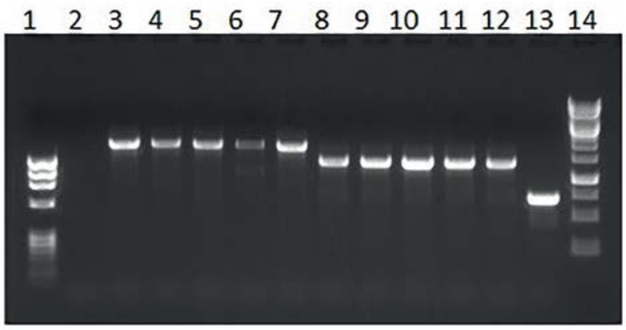

Fig. 4. Screening clones for target gene-containing plasmid inserts: 1 – DNA molecular weight marker (310, 603, 872, 1,078, 1,353 bp); 2 – negative PCR control; 3–7 – clones containing pET-32b(+) plasmid with PPRV nucleocapsid protein-encoding gene, estimated size 1,876 bp; 8–12 – clones containing pET-32b(+) plasmid with SBV nucleocapsid protein-encoding gene, estimated size 1,653 bp; 13 – positive PCR control (insertion-free pET-32b(+) vector); 14 – DNA molecular weight marker (250, 500, 750, 1,000, 1,500, 2,000, 2,500, 3,000, 3,500, 4,000, 5,000, 6,000, 8,000, 10,000 bp)

Thus, two recombinant plasmid vectors based on pET-32b(+) plasmid were obtained. They contained codon-optimized sequences encoding the PPRV and SBV nucleocapsid proteins.

It should be noted that the expression of codon-optimized genes in prokaryotic systems such as *E. coli* offers a number of key advantages due to the specifics of the bacterial translational machinery.

Firstly, the frequency of codon use in prokaryotes differs significantly from that in eukaryotes. This can lead to a shortage of corresponding tRNAs and, as a result, to translation delays, incorrect protein folding or premature termination. Codon optimization allows adaptation of a gene's nucleotide sequence to the preferred codons of the host, thereby increasing the translation speed and efficiency [26].

Secondly, it facilitates the increase in the yield of recombinant protein by reducing the likelihood of forming mRNA secondary structures that can interfere with the movement of the ribosome.

Thirdly, codon optimization minimizes the risk of amino acid misincorporation errors, which can occur when rare codons are used due to mispairing with non-canonic tRNAs. Furthermore, when expressing heterologous proteins (e.g., of human origin) in *E. coli*, codon optimization is often essential to achieve physiologically relevant production levels, as the native sequences may contain multiple codons that are rare in bacteria. Therefore, the use of codon-optimized genes in prokaryotic expression systems is an important tool for increasing the yield, stability, and functionality of recombinant proteins, which is particularly relevant for biotechnological and biopharmaceutical applications [27].

Such dangerous animal diseases as PPR and SBV infection, considering their current spread,

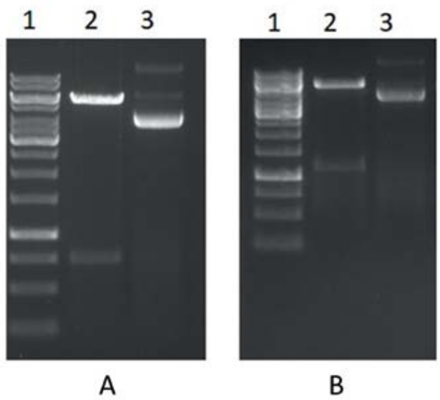

Fig. 5. Results of the restriction analysis of recombinant plasmids isolated from screening-positive clones. A: 1 – DNA molecular weight marker (250, 500, 750, 1,000, 1,500, 2,000, 2,500, 3,000, 3,500, 4,000, 5,000, 6,000, 8,000, 10,000 bp); 2 – plasmid containing SBV nucleocapsid protein gene and digested with NcoI and HindIII restriction enzymes, estimated size 5,861 and 785 bp; 3 – negative control, plasmid not digested with restrictases; B: 1 – DNA molecular weight marker (250, 500, 750, 1,000, 1,500, 2,000, 2,500, 3,000, 3,500, 4,000, 5,000, 6,000, 8,000, 10,000 bp); 2 – plasmid containing PPRV nucleocapsid protein gene and digested with NcoI and HindIII restriction enzymes, estimated size 5,861 and 1,202 bp; 3 – negative control, plasmid not digested with restriction enzymes

remain highly relevant. International cooperation and trade in small ruminants and cattle create conditions for the transmission of the causative agents of these diseases. Currently, the Russian Federation is actively pursuing an import substitution program in the fields of human and animal health, particularly in the development and production of diagnostic test systems, driven by the lack of high-quality domestic equivalents. This leads to the challenge of timely and high-quality diagnostics using our own technological arsenal. Domestic manufacturers have developed many ELISA systems based on the use of native antigens. However, such systems can frequently demonstrate non-specific results. Recombinant proteins, in turn, demonstrate significant advantages over native antigens, including higher product purity, reproducibility of results, and capacity to produce large amounts of material. Furthermore, the ELISA systems based on recombinant proteins are safer for personnel, as there is no need to produce large quantities of infectious material to obtain a viral antigen preparation.

It should be noted that there are a number of limitations to the PPR serological diagnosis. This is related to the specific characteristics of the disease course. Often, the diseased animals die earlier than the virus-specific antibodies are produced. Thus, the direct ELISA variant is the most suitable for emergency tests of the animals. At the same time, the indirect variant, including the one based on recombinant antigens, allows differentiation of vaccinated, convalescent and immune animals, for example, during monitoring studies.

CONCLUSION

It should be noted that ELISA technologies based on recombinant proteins continue to evolve, enabling improvements in the test sensitivity and specificity, thereby opening up new opportunities for laboratory diagnostics and fundamental research. Particular attention should be given to optimizing the conditions for protein expression, purification, and conjugation, which directly impacts the quality of the final diagnostic product.

A significant advantage of ELISA based on recombinant proteins is that, if the virus cannot be cultured for any reason, one can quickly respond to newly emerging viral strains of existing pathogens, provided the gene sequence is available. In addition, the obtained recombinant antigens can be used to produce poly- and monoclonal antibodies and formulate appropriate vaccines.

The continued refinement of recombinant protein production technology will contribute to the advancement of veterinary medicine and enhance the efficacy of infectious disease diagnostics.

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Effect of bacterial lysate-based bioactive supplement on immunological blood parameters in grower pigs

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ABSTRACT

Introduction. Modern pig farming in Russia is showing steady growth, which is accompanied by the introduction of new technologies aimed at increasing productive performance and reducing dependence on antibiotics. This causes increased interest in biologically active products with immunostimulatory and immunomodulatory properties. Multiple studies confirm their positive effect on the intestinal microflora, immune status and overall productive performance of animals. However, the morphofunctional and biochemical aspects of the action of these agents remain understudied, which highlights the necessity of further research in this field.

Objective. To justify the expediency of using immunomodulatory drug Immbaclys C for pigs during grower stage based on the analysis of the published resources and experimental data.

Materials and methods. Sixty biological samples (blood) collected from grower pigs on the commercial pig farm in Kolomna Municipal Okrug, Moscow Oblast in April – July 2024 were studied. The samples were tested using enzyme-linked immunosorbent assay, flow cytometry, and microscopy. The data was processed using the statistical analysis software Statistica v.13.0.

Results. Course administration of Immbaclys C to grower pigs (22–113 days old) induced statistically significant enhancements in cellular and humoral immunity markers, including elevated T- and B-lymphocyte counts, neutrophil phagocytosis, and IgG/IgM levels, demonstrating activation of immune defense pathways.

Conclusion. The dynamics of the parameters throughout the study period indicate a cumulative effect of the drug, particularly with respect to the relative count of B-lymphocytes and the level of IgM, which may suggest its prolonged action upon repeated administration. These findings position Immbaclys C as an effective immunoprophylactic agent with potential for incorporation into veterinary health programs to control and prevent immunodeficiency in intensively reared young pigs.

Keywords: replacement gilts, immunity, immunomodulators, immunostimulators, Immbaclys C, T- and B-lymphocytes, immunoglobulins, phagocytosis

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Изменение иммунологических показателей крови у поросят на доращивании под воздействием биологически активной добавки на основе лизата бактерий

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РЕЗЮМЕ

Введение. Современное свиноводство в России демонстрирует стабильный рост, который сопровождается внедрением новых технологий, направленных на увеличение продуктивности и снижение зависимости от антибиотиков. Это вызывает повышенный интерес к биологически активным препаратам, обладающим иммуностимулирующими и иммуномодулирующими свойствами. Множество исследований подтверждают их положительное влияние на кишечную микрофлору, иммунный статус и общую продуктивность животных. Однако морфофункциональные и биохимические аспекты действия этих средств остаются недостаточно изученными, что подчеркивает необходимость дальнейших исследований в этой области.

Цель исследования. На основе анализа существующей литературы и экспериментальных данных обосновать целесообразность использования препарата «Иммбаклиз С», обладающего иммуномодулирующими свойствами, для поросят в период их доращивания.

Материалы и методы. Исследовали 60 образцов биологического материала (крови), полученного от поросят на доращивании в апреле – июле 2024 г. на свиноводческом комплексе промышленного типа, расположенном на территории Коломенского городского округа Московской области. Исследования проводились методами иммуноферментного анализа, проточной цитометрии, микроскопии. Обработка данных осуществлялась с использованием пакета статистического анализа Statistica v.13.0.

Результаты. Курсовое введение препарата «Иммбаклиз С» поросятам на доращивании в возрасте 22–113 сут обусловило статистически значимое повышение показателей клеточного и гуморального звеньев иммунной системы, включая увеличение абсолютного и относительного содержания Т- и В-лимфоцитов, фагоцитарной активности нейтрофилов, а также концентрации иммуноглобулинов классов G и M, что указывает на активацию специфических и неспецифических механизмов иммунной защиты.

Заключение. Динамика показателей в течение исследуемого периода свидетельствует о накопительном эффекте препарата, особенно в отношении относительного содержания В-лимфоцитов и уровня IgM, что может указывать на его пролонгированное воздействие при многократном применении. Полученные данные позволяют рассматривать «Иммбаклиз С» как эффективное средство иммунопрофилактики, потенциально пригодное для включения в ветеринарные схемы оздоровления и профилактики иммунодефицитных состояний у молодняка свиней, выращиваемого в условиях интенсивных технологий.

Ключевые слова: ремонтные свинки, иммунитет, иммуномодуляторы, иммуностимуляторы, «Иммбаклиз С», Т- и В-лимфоциты, иммуноглобулины, фагоцитоз

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Конфликт интересов: Федотов С. В. является членом редколлегии журнала «Ветеринария сегодня», но никакого отношения к решению опубликовать эту статью не имеет. Рукопись прошла принятую в журнале процедуру рецензирования. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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INTRODUCTION

Modern commercial pig farming in Russia is showing steady growth, which is accompanied by the introduction of new technologies aimed at increasing productive performance and reducing dependence on antibiotics. This causes increased interest in biologically active products with immunostimulatory and immunomodulatory properties [1, 2]. Multiple studies confirm their positive effect on the intestinal microflora, immune status and productive performance of pigs [3, 4, 5]. However, the morphofunctional and biochemical aspects of the action of these agents remain understudied, and the differences in the formulations of the products call for a systematic method of their administration [6].

Russia holds a leading position in global pork production: by the end of 2023, the increase in live weight on the commercial farms reached 340 ths tons, which is 6.5% higher than in the previous year [1]. The industry's development is being driven by vertically integrated holdings and environmentally focused technologies implemented by the largest agricultural establishments [7]. A significant role is played by the state support program implemented since 2018 and aimed at modernizing production, reducing the environmental burden and increasing efficiency [1, 2].

The prospects of the industry are associated with the introduction of environment-friendly technologies, expansion of export markets and support for small-scale farming [2, 8]. Of particular relevance is the development and introduction of antibiotic-alternative products with immunostimulatory and immunomodulatory effects, including immunostimulating and other biologically active compounds [4, 9].

The aim of the present study is to justify the efficacy of the immunomodulator Immbaclys C for grower pigs, based on the analysis of the published data and experimental material. The objectives of the study involve experimental assessment of the immunomodulatory effect of Immbaclys C and determination of its effect on the immunological parameters of pig blood.

MATERIALS AND METHODS

The study was conducted on the commercial pig farm in the Moscow Oblast, Kolomna Municipal Okrug, Industria settlement, from April to July 2024. Sixty grower pigs were included in the experiment. The pigs were selected using the matched-pairs method according to the following parameters: Landrace and Large White cross-breeds, females, 22–113 days of age, 7–48 kg body weight. All animals were raised at agricultural cooperative "Mashkino"; they were not moved during the study and stayed in the standard housing conditions. The animals were divided into two groups of 30 animals: control and experimental groups. Pigs in both groups were housed under identical conditions, with standardized management of environmental factors, feeding, veterinary monitoring, and light cycles [10]. The diet of the experimental group was supplemented with Immbaclys C, a bioactive additive demonstrating expressed immunomodulatory and probiotic properties.

Immbaclys C (rights holder; registration certificate holder: NITA-FARM) is an immunotropic agent and immunomodulator (pharmacotherapeutic group; ATC code recommended by the World Health Organization: other immunomodulators). It is formulated as a modified-release coated granules for oral administration. Each 1 g of the product contains a protein-lipopolysaccharide complex of antigens derived from lysate of *Bordetella bronchiseptica*, *Haemophilus parasuis*, *Streptococcus suis* – 10 mg, as well as the following excipients: monosodium glutamate, D-mannitol, propyl gallate, macrogol cetostearyl ether (polyethylene glycol-25-cetostearyl ether), sugar, povidone K-30, quinoline yellow food colorant (E104), and chalk.

The substance was administered in accordance with the manufacturer's instructions (0.6 g per 1 kg of feed) in a dosage calculated for the body weight of the animal, daily throughout each course of treatment. The control group received a standard diet without any additional supplements. The experiment design included three recurring courses: course I: April – May, course II: May – June, course III: June – July 2024. Each course lasted 14 days with 21-day intervals. In each of the three courses, four blood samples were collected from the jugular vein: day 0 – before the start of the product administration, day 8 – at the stage of the immune response formation, day 15 – at the peak of the therapeutic effect, day 22 – at the end of the course. Laboratory blood tests were performed at the Department of Veterinary Medicine, Russian State Agrarian University – Moscow Timiryazev Agricultural Academy.

The volume of blood collected in one sample did not exceed 10 mL, which is within the permissible norms and has no adverse effect on the physiological state of the animals [11]. The use of the test product Immbaclys C concurrently with routine vaccinations and antibiotics was not allowed in order to avoid distortion of the study results. The following indicators were analyzed: concentration of IgG and IgM using enzyme-linked immunosorbent assay (ELISA); relative and absolute count of T- and B-lymphocytes using flow cytometry; phagocytic activity using microscopy. The data was processed using the statistical analysis software Statistica v.13.0. To assess the reliability of the differences, Fisher's exact test and the Mann – Whitney U test were used. The level of statistical significance was assumed to be $p \leq 0.05$.

The study procedure was approved by the local ethics committee. All procedures involving animals adhered to ethical standards for humane treatment [12]. During the entire experiment, no behavioural or physiological deviations were reported in any animal.

The selection of the analytical parameters was based on the sensitivity of T- and B-cell-mediated immunity to the immunomodulatory agent [13]. The studies show that when biologically active substances are used, an increase in immunoglobulin titres and activation of phagocytosis are reported [6]. Biologically active substances also contribute to the normalization of the intestinal microbiota and suppression of inflammatory processes [14]. According to the preliminary data, Immbaclys C, can be classified as a prolonged-action supplement. Its efficacy may increase when administered in courses with 30-day intervals.

The physiological characteristics of growing pigs aged 22–113 days involve active morphogenetic restructuring of the intestines [15]. At this time, the crypts form and the villi lengthen in the small intestine, while the lymphoid tissue matures [16]. Biologically active additives administered during this period improve the body resistance to opportunistic microflora. This fact is supported by the experimental data on increased production of protective mucus and activation of small intestine epithelial cells [17].

The repeatability of experimental conditions was ensured through the automated feed and water supply systems [18]. Temperature, humidity, and ammonia levels were monitored daily. The feed lots were standardized and tested for mycotoxins [19]. Such control of external factors excluded any influence on the immune response [8], which corresponded to the reproducibility principles in veterinary research [20].

The trial design incorporated a comprehensive approach integrating immunological, physiological, and hygiene control parameters. At each stage, not only laboratory parameters were monitored, but also such factors as behavior, productive performance, and feed intake. The pigs from both groups were provided with equal opportunities for physical activity. The animals were housed in individual pens. This allowed minimizing the variability within the groups.

The study protocol complied with the internationally recognized guidelines for the use of farm animals in research [13]. Herewith, both daily and seasonal changes in physiological functions were recorded [21]. All the animals were kept in the same room throughout the experiment. The animal handlers had no information about which group was the control one, which eliminated potential bias. The use of this blinded design contributed to increased objectivity of the obtained results.

RESULTS AND DISCUSSION

To analyze the immune status indicators of the replacement gilts after Immbaclys C course treatment, data obtained by calculating group means (control and experimental groups) were used, based on individual animal health assessments. Based on these data, mean values for each immunological parameter were calculated, and tables were prepared to enable comparative analysis between the groups. Dynamics of the product's action within each course, as well as summary data, were analyzed.

Following the first course of Immbaclys C administration, pigs in the experimental group demonstrated a significant increase in T-lymphocyte levels (Table 1). The absolute cell count of this population was 5.20×10^9/L compared to 4.55×10^9/L in the control group (an increase of 14.29%, $p < 0.001$). The relative count of T-lymphocytes also increased – 25.12% compared to 20.10% in control animals (a difference of 5.02 percentage points (pp), $p < 0.001$). These changes indicate activation of the cellular immune response, which is consistent with the results obtained for immunomodulators capable of enhancing T-cell activity in farm animals [11, 18]. Immbaclys C, as a drug with pronounced immunostimulatory properties, aids to the enhancement of the functional activity of the lymphocyte immunity and formation of strong cellular protection.

Comparative analysis of the humoral immunity parameters also revealed positive dynamics in pigs from the experimental group. The absolute B-lymphocyte count reached 4.01 × 10^9/L compared to 3.39 × 10^9/L in the control group (an increase of 18.30%, $p < 0.001$), and the relative count was 18.77% compared to 15.15% (a difference of 3.62 pp, $p = 0.0102$). An increase in the B-cell count indicates the activation of antibody production, which is especially important in the context of early immunoprophylaxis in pigs. According to the published data, immunomodulators activate the humoral immunity by stimulating B-lymphocyte differentiation and enhancing immunoglobulin synthesis [22, 23, 24]. Such agents enable the formation of the functionally complete humoral response already at the early stages of rearing [17, 25].

The neutrophil phagocytic activity in the experimental group was 64.88% compared to 56.98% in the control group (a difference of 7.90 pp, an increase of 13.86%, $p < 0.001$). This indicates an increase in nonspecific resistance and activation of the innate immune response. An increase in phagocytic function indicates the systemic effect of Immbaclys C on non-specific protection, including activation of the lysosomal apparatus and cytokine secretion [9, 10]. Immunostimulating substances also enhance the expression of phagocyte surface receptors thus increasing their antigen recognition capacities [9, 25].

The immunoglobulin levels confirm the overall trend towards the increase of the specific immune response in pigs from the experimental group. The IgG level was 7.58 mg/mL compared to 6.25 mg/mL in control animals (an increase of 21.28%, $p < 0.001$), and IgM level reached 2.82 mg/mL compared to 2.40 mg/mL in the control group (an increase of 17.50%, $p < 0.001$). These changes are interpreted as activation of the primary and secondary humoral responses, especially under repeated immunostimulatory exposures. Literature sources emphasize that immunomodulators enhance IgG and IgM synthesis through activation of B-cells and improved cooperation between T- and B-lymphocytes [21, 26]. Such dynamics allows for the formation of stable and balanced humoral immunity in young animals [12, 16].

During the second course of the experiment, the positive dynamics of changes in immunological parameters in pigs from the experimental group continued compared to the control group (Table 2). The absolute T-lymphocyte count was 5.42 × 10^9/L, whereas in the control group it was at the level of 4.58 × 10^9/L (an increase of 18.34%, $p < 0.001$). The relative T-cell count reached 32.63% compared to 20.23% in control animals (the difference was 12.40 pp, $p < 0.001$). These data indicate the cumulative activating effect of Immbaclys C on the cellular component of the immune system. According to the literature sources, prolonged use of immunostimulators promotes enhanced T-lymphocyte differentiation and strengthens the population immune response in young pigs [4, 14, 15].

A similar trend of changes was reported for B-lymphocytes. Their absolute count in the experimental group was 3.87 × 10^9/L compared to 3.62 × 10^9/L in the control group (an increase of 6.90%, $p = 0.0149$), and their relative count was 28.76% compared to 15.31% (a difference of 13.45 pp, $p < 0.001$). This may indicate the continued activation of the humoral immune system, which promotes the specific antibody production. As demonstrated in a number of studies, the immunostimulators activate B-cells, increase effectiveness of their antigen-presenting function, and stimulate the immunoglobulin production [22, 23, 24]. These mechanisms are important at the stage of adaptive immunity formation and increase the young animals' resistance to infectious agents [17, 25].

Phagocytic activity in the experimental group reached 65.80%, while in the control group it was 55.41% (a difference of 10.39 pp, an increase of 18.75%, $p < 0.001$). The data indicate a sustained enhancement of the innate immune response following repeated administration of Immbaclys C. Immunostimulators can increase the activity of neutrophils and macrophages, as well as enhance their capacities for pathogen recognition and destruction [10, 27]. According to literature sources, the use of immunomodulating agents is accompanied by stimulation of phagocytosis due to the activation of receptor complexes and production of innate immunity mediators [9, 18].

Table 1
Mean immunological parameters in pigs of control and experimental groups after course I (April – May 2024)

Indicator	Experimental group	Control group
T-lymphocytes, 10^9/L	5.20 ± 0.11	4.55 ± 0.05
T-lymphocytes, %	25.12 ± 0.68	20.10 ± 0.18
B-lymphocytes, 10^9/L	4.01 ± 0.08	3.39 ± 0.03
B-lymphocytes, %	18.77 ± 0.35	15.15 ± 0.13
Phagocytic activity, %	64.88 ± 0.30	56.98 ± 0.17
IgG, mg/mL	7.58 ± 0.17	6.25 ± 0.13
IgM, mg/mL	2.82 ± 0.10	2.40 ± 0.08

Fisher's exact test $p \leq 0.005$.

Table 2
Mean immunological parameters in pigs of control and experimental groups after course II (May – June 2024)

Indicator	Experimental group	Control group
T-lymphocytes, 10^9/L	5.42 ± 0.39	4.58 ± 0.26
T-lymphocytes, %	32.63 ± 6.59	20.23 ± 1.09
B-lymphocytes, 10^9/L	3.87 ± 0.51	3.62 ± 0.16
B-lymphocytes, %	28.76 ± 9.89	15.31 ± 0.80
Phagocytic activity, %	65.80 ± 1.26	55.41 ± 0.81
IgG, mg/mL	7.42 ± 1.10	6.61 ± 0.34
IgM, mg/mL	3.05 ± 0.41	2.73 ± 0.51

Fisher's exact test $p \leq 0.005$.

The immunoglobulin indicators demonstrate further strengthening of humoral immunity. The IgG level in the experimental group was 7.42 mg/mL compared to 6.61 mg/mL in the control group (an increase of 12.25%, $p = 0.0005$), and IgM was 3.05 mg/mL compared to 2.73 mg/mL (an increase of 11.72%, $p = 0.0097$). This reflects activation of both the primary and secondary immune responses and indicates a sustained immunostimulating effect of Immbaclys C. According to the published data, immunomodulators enhance expression of genes responsible for IgG and IgM synthesis, as well as promote the interaction between T- and B-lymphocytes, which contributes to the comprehensive activation of the immune system [21, 26]. These observations are supported by the experimental results of the current study and emphasize the effectiveness of the course-based administration of the agent.

During the third course of Immbaclys C biologically active supplement administration, a consistently positive dynamics of immunological parameters was reported in pigs in the experimental group as compared to the control group (Table 3). The absolute T-lymphocyte count in the experimental group was 5.56×10^9/L, which exceeds the value in the control group (4.55×10^9/L) by 22.20% ($p < 0.001$). The relative T-cell count was also higher: 33.10% compared to 19.94% in the control animals (the difference was 13.16 pp, $p < 0.001$). This confirms the continued stimulatory effect of Immbaclys C on the cellular compartment of the immune system. Similar data were obtained with prolonged use of immunomodulatory agents that promote enhanced differentiation of T-lymphocytes and maintain their functional activity [4, 14, 15].

The B-lymphocyte counts in the experimental group also demonstrated an increase. The absolute count was 3.94×10^9/L compared to 3.49×10^9/L in the control group (an increase of 12.90%, $p < 0.001$), and the relative count was 29.00% compared to 15.06% (a difference of 13.94 pp, $p < 0.001$). This indicates the continued activation of the humoral immune system. Such shifts are typical for the course-based use of immunostimulators that enhance production of the antibodies and increase the functional maturity of the B-cells [22, 23, 24]. According to the published data, the effect of immunomodulators on adaptive immunity is manifested in enhanced activation of B-lymphocytes and increased immunoglobulin levels [17, 25].

Phagocytic activity in the experimental group was 67.11% compared to 57.23% in the control

Table 3
Mean immunological parameters in pigs of control and experimental groups after course III (June – July 2024)

Indicator	Experimental group	Control group
T-lymphocytes, 10^9/L	5.56 ± 0.37	4.55 ± 0.57
T-lymphocytes, %	33.10 ± 7.15	19.94 ± 1.40
B-lymphocytes, 10^9/L	3.94 ± 0.41	3.49 ± 0.27
B-lymphocytes, %	29.00 ± 10.13	15.06 ± 0.47
Phagocytic activity, %	67.11 ± 2.39	57.23 ± 1.56
IgG, mg/mL	7.88 ± 0.83	6.48 ± 0.27
IgM, mg/mL	3.17 ± 0.54	2.21 ± 0.41

Fisher's exact test $p \leq 0.005$.

group (a difference of 9.88 pp, an increase of 17.26%, $p < 0.001$). These data indicate the maintenance of a high level of nonspecific resistance, which is one of the indicators of the immunostimulator action. Immbaclys C promotes the activation of neutrophils and macrophages, as well as an increase in the expression of molecules responsible for the pathogen destruction [3, 9, 18, 27].

The immunoglobulin level in the experimental group was also higher than in the control group. IgG was 7.88 mg/mL compared to 6.48 mg/mL (an increase of 21.60%, $p < 0.001$), and IgM was 3.17 mg/mL compared to 2.21 mg/mL (an increase of 43.44%, $p < 0.001$). These parameters indicate a powerful stimulation of both the primary and secondary humoral immune responses. Long-term administration of immunomodulators contributes to the sustainable synthesis of immunoglobulins, as reported by a number of authors who note increased expression of IgG and IgM genes in animals after repeated administration of the immunostimulating agents [21, 26].

A comparative analysis of the mean immunological parameters between the experimental and control groups based on the results of three courses of Immbaclys C administration revealed significant differences in favor of the experimental group (Table 4). The absolute T-lymphocyte count was 5.39×10^9/L compared to 4.56×10^9/L (an increase of 18.20%, $p < 0.001$), and the relative count was 30.29% compared to 20.09% (a difference of 10.20 pp, $p < 0.001$). The resulted data indicate maintenance of the agent's activating effect on the cellular immunity and support its prolonged effect when administered course-based. The immunomodulatory action of Immbaclys C contributes to the maintenance of the functional activity of T-lymphocytes and their sustained circulation in peripheral blood [4, 14, 15].

B-lymphocyte counts also demonstrated higher levels in the experimental group. The absolute count was 3.94×10^9/L compared to 3.50×10^9/L in the control group (an increase of 12.57%, $p < 0.001$), and the relative count was 25.51% compared to 15.17% (a difference of 10.34 pp, $p < 0.001$). This indicates an increased humoral immune response associated with the B-cell proliferation and activation against the background of the agent's systematic exposure. Immunostimulators, including Immbaclys C, promote activation of the B-cell immunity and stimulate the immunoglobulin synthesis at all stages of the immune response [22, 23, 24].

The phagocytic activity of neutrophils in the experimental group was 65.93% compared to 56.54% in the control group (a difference of 9.39 pp, an increase of 16.60%, $p < 0.001$). This confirms the enhancement of the innate resistance under the agent's effect. Immbaclys C demonstrates the ability to sustain the activation of non-specific immune defense mechanisms, including the stimulation of phagocytosis and the expression of functional receptors on innate immune cells [3, 9, 18, 27].

Analysis of the immunoglobulin content revealed an increase in IgG levels up to 7.62 mg/mL in the experimental group compared to 6.45 mg/mL in the control group (an increase of 18.14%, $p < 0.001$), and IgM levels up to 3.01 mg/mL compared to 2.45 mg/mL, respectively (an increase of 22.86%, $p < 0.001$). These data indicate high activity of the humoral immunity of the immune system and maintenance of the immunostimulatory effect throughout the entire period of exposure to the agent. The immunomodulators activate the antibody production by enhancing the interaction between T- and B-lymphocytes and expression of genes responsible for the immunoglobulin synthesis [21, 26].

CONCLUSION

Course-based administration of the immunomodulatory agent Immbaclys C to grower pigs aged 22–113 days resulted in a statistically significant growth of the cellular and humoral components of the immune system, including an increase in the absolute and relative T- and B-lymphocyte counts, phagocytic activity of neutrophils, as well as IgG and IgM concentration, thus indicating activation of specific and nonspecific immune defense mechanisms.

The differences between the experimental and control groups across all key immunological parameters in all three courses were of high statistical significance ($p < 0.05$–0.001), which confirms the reliability of the results obtained and enables confident assessment of the pronounced immunostimulatory effect of the agent under commercial use.

Table 4
Generalized mean immunological parameters in pigs from the control and experimental groups over the entire experimental period

Indicator	Experimental group	Control group
T-lymphocytes, 10^9/L	5.39 ± 0.33	4.56 ± 0.33
T-lymphocytes, %	30.29 ± 6.36	20.09 ± 0.94
B-lymphocytes, 10^9/L	3.94 ± 0.35	3.50 ± 0.19
B-lymphocytes, %	25.51 ± 8.92	15.17 ± 0.50
Phagocytic activity, %	65.93 ± 1.72	56.54 ± 1.25
IgG, mg/mL	7.62 ± 0.75	6.45 ± 0.28
IgM, mg/mL	3.01 ± 0.39	2.45 ± 0.41

Fisher's exact test $p \leq 0.005$.

The dynamics of the parameters over the study period demonstrates a cumulative effect of Immbaclys C, particularly in respect to the relative B-lymphocyte count and IgM level, which may suggest a prolonged action of the agent upon its repeated administration.

These findings position Immbaclys C as an effective immunoprophylactic agent, with a potential for incorporation into veterinary health programs to control and prevent immunodeficiency in intensively reared young pigs.

The limitations of the research included the use of animals of the same age category and homogeneous genotype, as well as conducting experiments within the same production facility. This necessitates a careful approach to the interpretation of the results and their extrapolation to other populations and housing conditions.

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Serological monitoring of Newcastle disease in the Russian Federation in 2023–2024

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ABSTRACT

Introduction. Newcastle disease is a highly contagious viral infection of birds that is reported in many countries around the world. Newcastle disease cases shall be notified to the World Organization for Animal Health.

Objective. The objective of this research is to ensure monitoring of Newcastle disease using serological methods and analyze the findings obtained for 2023–2024 in the Russian Federation.

Materials and methods. The Territorial Administrations of Russian Federal Service for Veterinary and Phytosanitary Supervision sampled biological material in 74 subjects of the Russian Federation (more than 66,700 samples of avian sera). Tests for antibodies to Newcastle disease virus were conducted at the Reference Laboratory for Avian Viral Diseases, housed within the Federal Centre for Animal Health (Vladimir, Russia). Enzyme-linked immunosorbent assay and hemagglutination inhibition assay were performed using diagnostic kits manufactured by the Federal Centre for Animal Health.

Results. The conducted tests revealed significant variations of seroprevalence in commercial and backyards poultry flocks and in wild birds. High Newcastle disease virus seroprevalence was observed in chickens and turkeys within closed commercial farming systems due to routine mass vaccination against the disease. At the same time, the overall seropositivity rate for all poultry species was 74% in 2023, increasing to 81% in 2024. In backyards, antibodies to Newcastle disease virus were detected in 35% of all the tested sera samples from chickens and turkeys in 2023 and in 53% of the tested samples in 2024. Specific antibodies were also detected in samples from the vaccinated guinea fowl and pheasants and from non-vaccinated geese and ducks. Antibodies to Newcastle disease virus were also detected in wild birds across several Russian regions, suggesting their role of a natural reservoir for Newcastle disease virus strains of varying pathogenicity.

Conclusion. Therefore, the monitoring data indicate that routine flock vaccination helps to control successfully Newcastle disease in commercial poultry flocks, creating a stable epizootological situation. However, a significant risk of Newcastle disease virus introduction and spread from infected backyard poultry and wild bird reservoirs still persists.

Keywords: Newcastle disease, epizootology, monitoring, poultry, wild birds, synanthropic birds

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Серологический мониторинг ньюкаслской болезни в Российской Федерации в 2023–2024 гг.

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РЕЗЮМЕ

Введение. Ньюкаслская болезнь – высококонтагиозная вирусная инфекция птиц, которая регистрируется во многих странах мира. О случаях инфицирования вирусом ньюкаслской болезни необходимо уведомлять Всемирную организацию здравоохранения животных.

Цель исследования. Проведение в течение 2023–2024 гг. на территории Российской Федерации мониторинговых исследований по ньюкаслской болезни с использованием серологических методов и анализ полученных результатов.

Материалы и методы. Биологический материал (более 66 700 проб сыворотки крови птиц) был отобран территориальными управлениями Россельхознадзора в 74 субъектах Российской Федерации. Исследования выполнены на базе референтной лаборатории вирусных болезней птиц ФГБУ «ВНИИЗЖ» (г. Владимир) с использованием диагностических наборов для выявления антител к вирусу ньюкаслской болезни иммуноферментным методом и в реакции торможения гемагглютинации производства ФГБУ «ВНИИЗЖ».

Результаты. Проведенные исследования показали разную степень серопревалентности у сельскохозяйственной птицы промышленных птицеводческих хозяйств, индивидуального сектора и дикой птицы. Для кур и индеек в промышленных хозяйствах закрытого типа была установлена высокая серопревалентность по ньюкаслской болезни, что связано с массовой вакцинацией птиц против данного заболевания. При этом доля выявленной серопозитивной птицы (в целом по всем видам сельскохозяйственной птицы) была равна 74% в 2023 г. и 81% в 2024 г. В индивидуальном секторе антитела к вирусу ньюкаслской болезни были обнаружены в 35% случаев от числа всех исследованных проб сывороток крови кур и индеек в 2023 г. и в 53% случаев – в 2024 г. Специфические антитела были выявлены также в пробах от вакцинированных цесарок и фазанов и от непривитых гусей и уток. В нескольких регионах Российской Федерации антитела к вирусу ньюкаслской болезни обнаружены у птиц дикой фауны, которые, вероятнее всего, являются естественным резервуаром возбудителя ньюкаслской болезни различной степени патогенности.

Заключение. Таким образом, результаты мониторинговых исследований свидетельствуют о благополучной ситуации по ньюкаслской болезни в промышленных птицеводческих хозяйствах, обусловленной плановой вакцинацией поголовья. В то же время сохраняется угроза заноса и распространения ньюкаслской болезни птиц из неблагополучных индивидуальных хозяйств и дикой фауны.

Ключевые слова: ньюкаслская болезнь, эпизоотология, мониторинг, домашняя птица, дикая птица, синантропная птица

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INTRODUCTION

Newcastle disease (ND) is a highly contagious viral disease of birds that poses a general threat to global poultry industry resulting from significant economic losses [1].

The ND pathogen is the RNA-containing virus of *Avian orthoavulavirus javaense* species, which belongs to the *Paramyxoviridae* family, *Avulavirinae* subfamily, *Orthoavulavirus* genus, previously classified as *Avian paramyxovirus* 1, or Newcastle disease virus (NDV) [2]. Dimitrov K. M. et al. in 2019, offered a classification based on the genetic properties of NDV [3]. Out of the two distinguished NDV classes, class I included genotype 1 only. Class II consisted of at least 20 different genotypes, which were divided into subgenotypes. In recent decades, genotype V, circulating in Americas, and genotype VII, circulating in other countries of the world, have become the most crucial for poultry industry. In the Russian Federation, NDV isolates are represented by different genetic and biological groups, including virulent and avirulent viruses, as well as vaccine strains [4].

Newcastle disease virus is capable of infecting over 200 avian species. Domestic *Galliformes* demonstrate the highest susceptibility to NDV. Infected birds have pathological lesions in the respiratory and digestive tracts, as well as the central nervous system. Mortality rates in non-vaccinated poultry flocks can be up to 100% [5]. Disease cases are also registered in geese, pheasants and guinea fowls [6]. Quails are susceptible to NDV infection: experimental infection with NDV virulent strains resulted in clinical signs that occur on days 3 to 14 post infection and mortality rates lower than in chickens [7, 8]. At the same time, specific antibodies are recorded on day 14 post infection. In quails vaccinated against ND (La Sota strain), the antibody peak is observed on day 40 post vaccination, with further decrease after day 46.

Synanthropic (magpies, pigeons, sparrows, etc.) and wild birds are natural NDV carriers [9, 10]. Wild waterfowl and migratory birds serve as the primary natural reservoirs for NDV. The disease exhibits seasonal patterns, largely driven by the annual migrations of wild birds. Waterfowl (domestic ducks and geese) are considered NDV reservoirs, since these birds are resistant to strains that are highly virulent for chickens.

However, since the 1990s there have been reports of ND outbreaks in domestic waterfowl in the Asian countries, including Korea, Japan and China. In the infected duck flocks, a drop in egg production was observed, with a morbidity rate of approximately 80% and a mortality rate ranging from 30 to 50%. Affected birds exhibited both diarrheal and neurological symptoms. Similar ND outbreaks were reported in geese flocks in China [6, 11]. Virulent NDV strains detected in geese across Asia during the 2000s were classified as genotype VIId. Xu Q. et al. [11] demonstrated that experimental infection of geese with virulent genotype VIId NDV strain induced a robust early cellular immune response, a finding associated with the unique characteristics of ND pathogenesis in geese [11]. Wan H. et al. described virus transmission from infected geese to chickens under contact housing conditions [6].

Newcastle disease is reported in many countries worldwide and there is a mandatory requirement to send corresponding notifications on disease cases triggered by highly virulent isolates of the *Avian orthoavulavirus javaense* to the World Organization for Animal Health (WOAH).

Newcastle disease outbreaks, notified to the WOAH over the past four years, were registered in more than 50 countries around the world (in Asia, Europe, America and Africa), including Russia. According to the WOAH data, ND outbreaks in poultry were reported over a four-year period in backyards of 16 subjects included into to the Central, Volga, Ural, Siberian, Far Eastern and Southern Federal Districts of the Russian Federation. Recent ND epizootics in numerous Asian and European countries have been caused by viruses from various subtypes of genotype VII [12, 13]. In the Russian Federation, this genotype was first isolated from chickens in 2006, during an ND outbreak on a poultry farm in the Amur Oblast. Subsequently, NDV genotype VII caused sporadic outbreaks among backyard poultry in different regions of the country [14, 15, 16, 17]. ND cases in pigeons are recorded annually in Russia [9, 15, 16].

In order to prevent ND, some countries, including the Russian Federation, vaccinate poultry with various vaccines [18, 19, 20]. Vaccination efficacy is assessed by measuring specific antibody titers against NDV before immunization and at various time points thereafter, using hemagglutination inhibition (HI) or enzyme-linked immunosorbent assay (ELISA) methods [21, 22]. Serological tests are of limited value for ND surveillance and diagnosis owing to the widespread implementation of poultry vaccination programs [23, 24].

This paper provides results of serological monitoring program for ND in poultry, implemented in 2023–2024. The surveillance was carried out under the state mandate of the Russian Federal Service for Veterinary and Phytosanitary Supervision (Rosselkhoznadzor) for the oversight of highly dangerous infectious diseases.

MATERIALS AND METHODS

Biological material tested. The Rosselkhoznadzor Territorial Administrations sampled biological material (avian sera) in 2023–2024.

Test methods. The collected sera samples were tested using commercial diagnostic kits produced by the Federal Centre for Animal Health (Russia), i.e. HI test-kit for detection of antibodies against Newcastle disease virus and single-dilution ELISA test-kit for detection of antibodies against Newcastle disease virus in compliance with the instructions for use.

Treatment of the tested samples. Before the test procedure, all sera samples received for the tests were inactivated at 56 °C for 30 minutes in a serum inactivator (or in a water bath).

RESULTS AND DISCUSSION

This work was conducted under the state assignment for epizootiological monitoring, as stipulated by Decrees No. 1915 (dated 20 December 2022) and No. 1630 (dated 22 December 2023) of the Russian Federal Service for Veterinary and Phytosanitary Surveillance (Rosselkhoznadzor). More than 66,700 samples were tested for antibodies to NDV. Avian sera were delivered from 69 and 74 regions of the Russian Federation in 2023 (35,005 samples) and in 2024 (31,766 samples), respectively.

Currently, poultry farming in the Russian Federation is represented by large commercial poultry farms using intensive farming systems, small family-operated farms and backyards.

26,983 and 26,004 sera samples were collected from commercial poultry in 2023 and 2024, respectively. Table 1 gives information on antibodies against NDV detected in poultry sera (from chickens, turkeys, ducks, geese, and quails) taken on commercial poultry farms in the Russian Federation.

In 2023, monitoring tests included samples from 237 commercial farms (establishments) of 60 RF Subjects, in 2024 – from 280 farms of 74 RF Subjects. In 2024, more positive samples were detected in the Russian Federation as a whole than in the previous year (81 and 74%).

Results of detecting antibodies to NDV in chicken sera (collected on commercial poultry farms in 2023–2024 and tested in HI and ELISA) are illustrated in Figures 1 and 2.

Table 1
Antibodies to NDV detected in poultry sera collected on commercial poultry farms of the Russian Federation in 2023–2024

Federal District	Number of samples tested in 2023		Number of farms / RF Subjects in 2023	Number of samples tested in 2024		Number of farms/ RF Subjects in 2024
	total	positive		total	positive	
Northwestern	2,646	1,640	23/7	2,540	1,936	20/8
Central	5,915	4,964	55/12	6,916	5,480	61/13
Volga	9,413	6,481	64/14	5,993	5,073	75/14
Ural	2,221	1,390	23/4	2,650	2,002	28/6
Siberian	1,907	1,497	32/9	2,601	2,234	38/11
Far Eastern	1,076	973	10/7	1,571	1,336	17/8
Southern	3,080	2,618	27/5	2,601	2,054	31/9
North Caucasian	725	419	3/2	1,132	941	10/5
Total	26,983	19,982 (74%)*	237/60	26,004	21,056 (81%)	280/74

* percentage of positive samples from the total number of the tested ones.

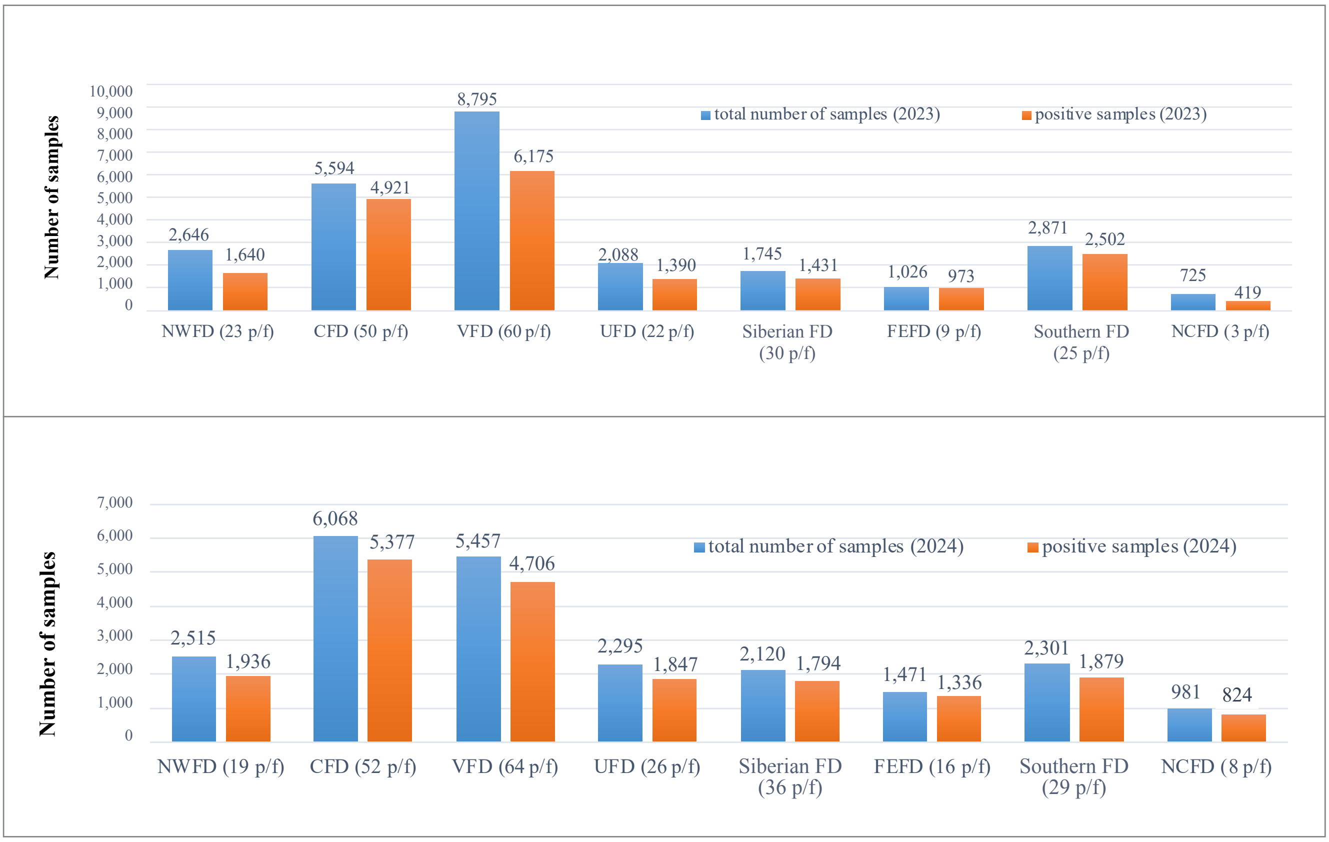

Fig. 1. Detection of antibodies to NDV in chicken sera collected on commercial poultry farms (p/f) in 2023–2024

In 2023, 25,490 samples from chicken collected on 222 commercial poultry farms in 8 RF Federal Districts were tested, antibodies to NDV were detected in 19,451 samples (76%). In 2024, antibodies to NDV were detected in 19,699 (85%) out of 23,208 samples from 250 poultry farms (Fig. 1).

ND serological tests conducted in 2023 revealed the minimum percentage of positive samples (58%) in the North Caucasian Federal District (NCFD). Slightly more positive samples, between 62 and 70%, were detected in chickens from the Northwestern (NWFD), Ural (UFD) and Volga (VFD) Federal Districts. In the Siberian (Siberian FD), Southern (Southern FD) and Central (CFD) Federal Districts, number of positive samples ranged between 82 and 88%, and the maximum number (95%) was detected in the Far Eastern (FEFD) Federal District. In 2024, in 7 out of 8 Federal Districts, with the exception for the NWFD (77%), the proportion of seropositive chickens ranged from 80 to 91% (Fig. 2).

Results of detecting antibodies to NDV in sera from other poultry species collected on commercial poultry farms are illustrated in Figures 3 and 4. Turkey sera were tested using two methods: ELISA and HI test; only HI was used for geese, ducks and quails.

989 turkey sera samples collected in 2023 on 8 poultry farms in 4 RF Federal Districts (CFD, VFD, Siberian FD and Southern FD) were tested and antibodies were detected in 529 (54%). In 2024, antibodies to NDV were detected

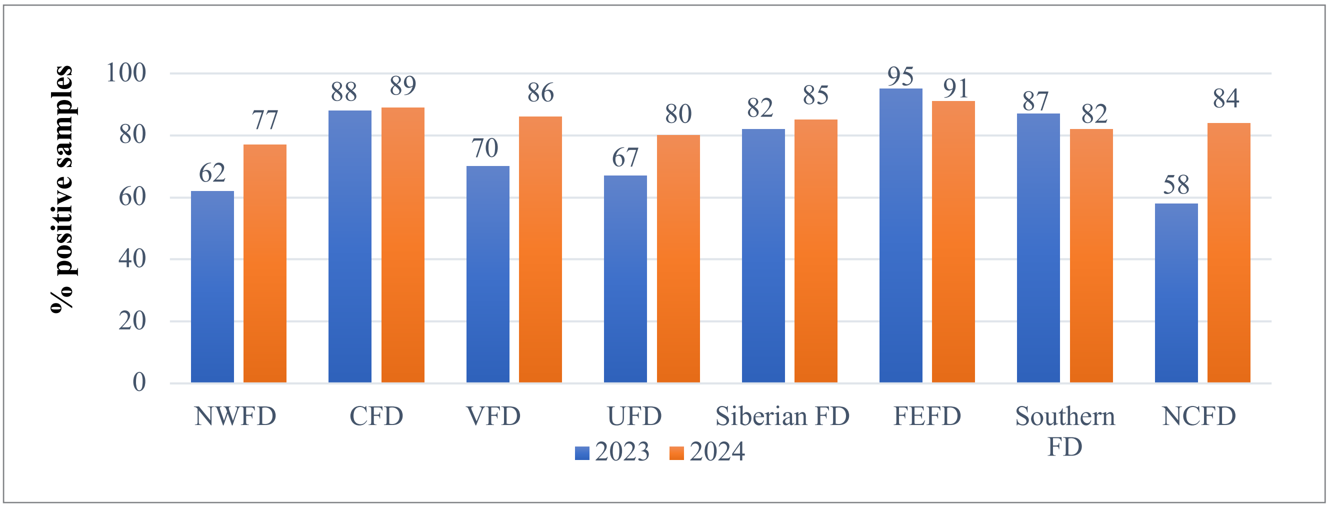

Fig. 2. Percentage of positive samples (chicken sera) detected in 2023–2024 on commercial poultry farms in various Federal Districts of the Russian Federation

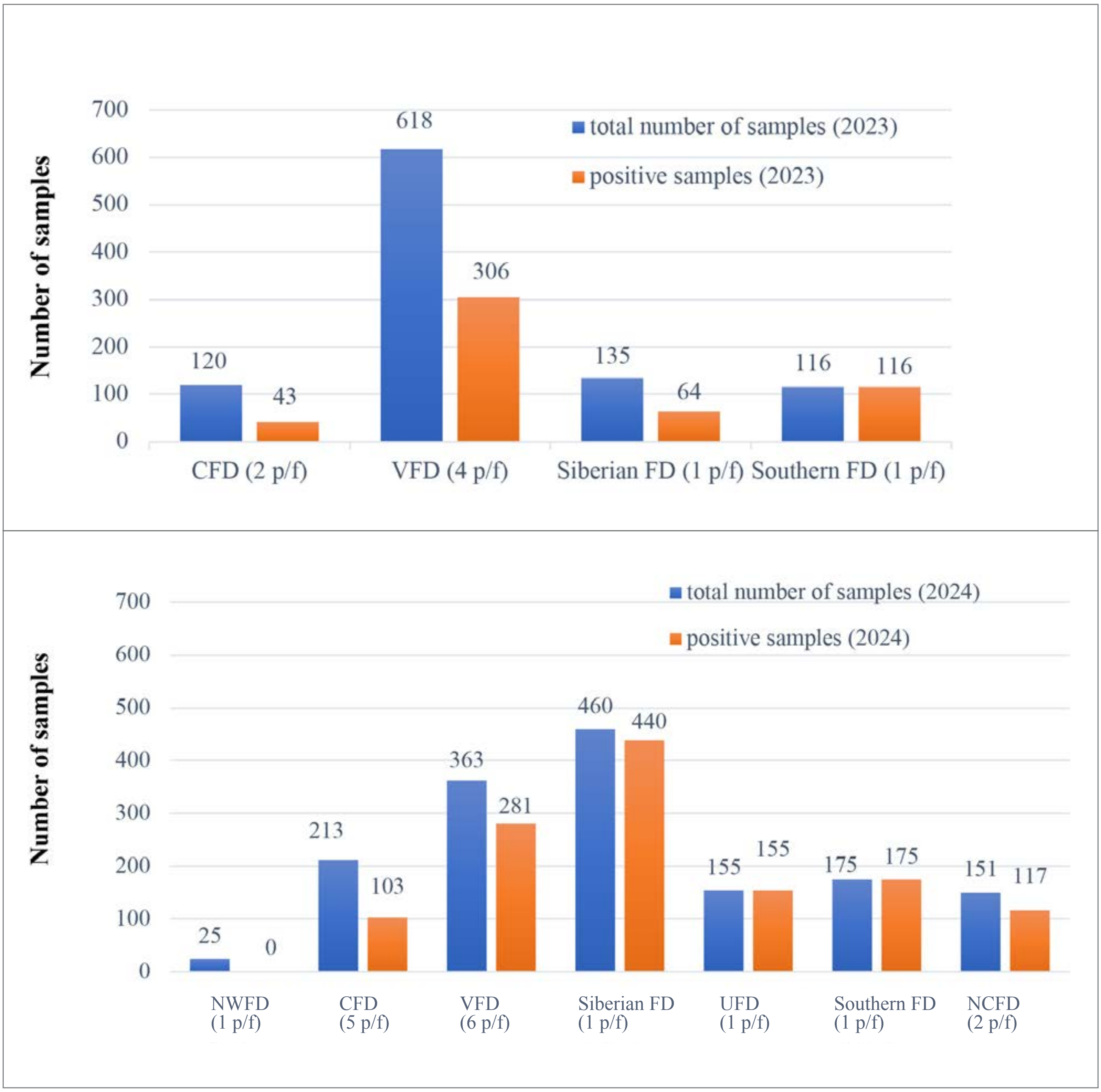

Fig. 3. Detection of antibodies to NDV in turkey sera collected on commercial poultry farms (p/f) in 2023–2024

in 1,271 (82%) out of 1,542 sera samples from 17 poultry farms in 7 Federal Districts (excluding the FEFD). Samples from turkeys that are not vaccinated against ND from the NWFD showed negative results (Fig. 3).

As illustrated by the accompanying documents from the farms, the detected specific antibodies were produced in response to live or inactivated ND vaccines. Number of seropositive poultry on farms depended on a number of factors, including the vaccine type and the vaccination schedule. Use of new, more effective vaccines, including those inactivated and produced from virulent NDV strains, increased the proportion of immunized birds in the flock and enhanced the level of the immune response [25, 26, 27].

Sera from ducks were delivered from the CFD, the Siberian FD and the Southern FD: 290 samples from 4 poultry farms in 2023 and 591 samples from 4 poultry farms in 2024. Antibodies specific to NDV were detected in only 2 samples (0.7%) on one poultry farm in the Siberian FD in 2023 (Fig. 4).

In 2023, 133 samples from geese were collected on the UFD poultry farms and tested; no antibodies to NDV were detected. In 2024, 373 samples taken from 6 poultry farms in the VFD and UFD were tested. Antibodies to NDV were detected in 86 samples (23%) from geese not vaccinated against ND (from the Republics of Bashkortostan and Tatarstan). Domestic waterfowl (ducks and geese) are rarely vaccinated against ND on commercial farms, since these avian species are less susceptible to ND than chickens, but at the same time they can be the disease reservoir on poultry farms [11, 28].

No antibodies to NDV were detected in quail sera collected in two Federal Districts (CFD and FEFD) and tested within two years, including those from the vaccinated birds (Fig. 4). The absence of specific antibodies in the vaccinated quails observed in our monitoring tests

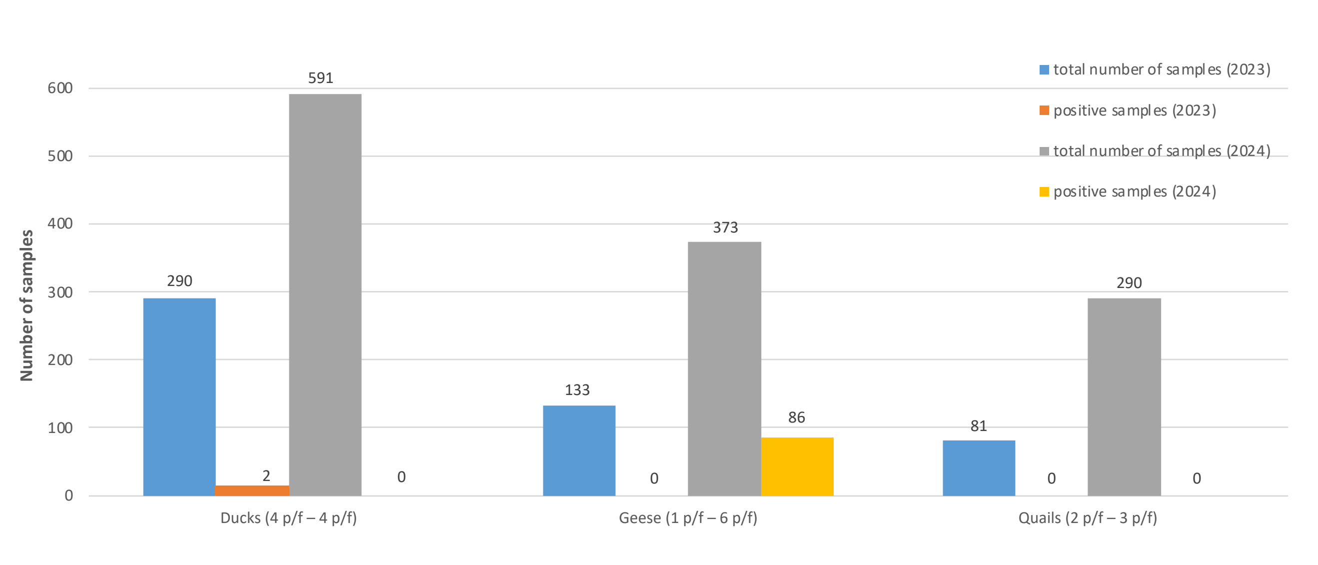

Fig. 4. Detection of antibodies to NDV in sera collected on commercial poultry farms (p/f) in 2023–2024

may result from incorrect sampling time after vaccination or an incorrect vaccination schedule.

In 2023 and 2024, 7,796 and 5,484 poultry sera samples were collected in backyards and on family-operated farms, respectively, in 39 RF Subjects of 8 Federal Districts and tested for antibodies to NDV (Tables 2 and 3).

Tests of poultry sera collected in backyards and on family-operated farms detected antibodies specific to NDV in chickens, turkeys, ducks, geese and guinea fowls in 2023 and in chickens, turkeys, geese and pheasants in 2024.

As for chickens and turkeys, antibodies to NDV were detected in 35 and 53% of samples tested in 2023 and 2024, respectively.

As indicated in the accompanying documents, ND vaccination was not universally implemented across

Table 2

Antibodies to NDV detected in chicken and turkey sera collected in backyards and on family-operated farms of the Russian Federation. Samples tested in ELISA and HI assay (data for 2023–2024)

Federal District	Poultry species	Number of samples (2023)		Number of samples (2024)	
		total/positive	% pos. samples	total/positive	% pos. samples
Northwestern	chickens	96/15	16	54/0	0
Central	chickens	928/253	27	751/298	40
Volga	chickens	2,023/689	34	1,227/669	55
	turkeys	45/0	0	n/t	n/t
Ural	chickens	200/41	21	120/15	13
Siberian	chickens	607/250	41	278/165	59
	turkeys	10/0	0	10/0	0
Far Eastern	chickens	1,222/223	18	643/286	45
	turkeys	167/146	87	117/54	46
Southern	chickens	500/411	82	759/538	71
	turkeys	n/t	n/t	25/25	100
North Caucasian	chickens	981/334	34	689/437	63
	turkeys	10/10	100	n/t	n/t
	Total	6,789/2,372	35	4,673/2,487	53

n/t – not tested; pos. – positive.

Table 3
Antibodies to NDV detected in sera from various poultry species collected in backyards and on family-operated farms of the Russian Federation. Samples tested in HI assay (data for 2023–2024)

Federal District	Poultry species	Number of samples (2023)		Number of samples (2024)	
		total/positive	% pos. samples	total/positive	% pos. samples
Northwestern	ducks	75/0	0	106/0	0
	guinea fowls	5/0	0	10/0	0
	pheasants	5/0	0	10/0	0
	ostriches	4/0	0	n/t	n/t
	peacocks	5/0	0	n/t	n/t
Central	ducks	14/0	0	61/0	0
	geese	96/6	6	30/0	0
	quails	50/0	0	73/0	0
Volga	ducks	171/10	6	28/0	0
	geese	378/61	16	269/24	9
	quails	20/0	0	75/0	0
	guinea fowls	30/30	100*	n/t	n/t
Ural	quails	5/0	0	10/0	0
Siberian	geese	8/0	0	15/0	0
	quails	10/0	0	n/t	n/t
Far Eastern	ducks	10/0	0	24/0	0
	quails	75/0	0	75/0	0
	geese	46/0	0	n/t	n/t
Southern	pheasants	n/t	n/t	25/25	100*
	Total	1,007/107	11	811/49	6

n/t – not tested; * samples from poultry vaccinated against ND; pos. – positive.

all backyards and family-operated poultry farms. Poultry for backyards were mainly purchased from poultry farms, where ND vaccination was implemented. Vaccine was typically administered at a single dose. Live vaccines, which were more frequently utilized, often induced a short-term immunity. The increase in vaccination coverage against ND in backyard flocks is evidenced by the rise in positive test results ranging from 35% in 2023 to 53% in 2024.

528 and 314 samples from domestic geese were delivered for tests in 2023 and 2024, respectively. Antibodies to NDV were detected in 67 (2023) and 24 (2024) samples from non-vaccinated geese brought from the CFD and the VFD. Antibodies were detected in sera from domestic ducks in 10 samples out of the 270 tested ones (VFD). In 2024, no specific antibodies to NDV were detected in duck sera. Presence of antibodies in domestic waterfowl not vaccinated against ND may be associated with circulation of avirulent NDV strains, since samples for the test were taken from clinically healthy birds [11, 28]. Antibodies to NDV were detected in all samples from the vaccinated guinea fowl and pheasants.

Many species of wild birds are natural reservoirs and carriers of infectious disease pathogens [4, 17], and therefore monitoring in wild fauna makes it possible to control occurrence and spread of dangerous avian infections, including ND.

Table 4 shows HI results for sera from wild birds collected during 2023–2024.

Testing sera samples from wild birds collected in seven RF Subjects revealed antibodies to NDV in 74 out

Table 4
Antibodies to NDV detected in wild birds using HI assay

Federal District (RF Subject)	Poultry species	Number of samples (2023)		Number of samples (2024)	
		total	positive	total	positive
Northwestern (Vologda Oblast)	wild ducks	85	6	41	2
	wild geese	54	0	62	6
	seagulls	1	0	15	1
Central (Lipetsk and Smolensk Oblasts)	synanthropic birds	17	0	35	0
	wild birds	17	0	35	0
	wild ducks	7	0	n/t	n/t
Volga (Republic of Tatarstan)	wild birds	20	0	n/t	n/t
	pigeons	14	3	n/t	n/t
Far Eastern (Primorsky Krai)	zoo birds	11	0	n/t	n/t
Siberian (Krasnoyarsk Krai, Omsk Oblast)	pigeons	n/t	n/t	90	56
Total		226	9 (4%)*	278	65 (23%)

n/t – not tested; * percentage of positive samples from the total number of the tested ones.

of 504 submitted samples. Samples from birds from the NWFD, the VFD and the Siberian FD were positive: 59 samples from synanthropic birds (pigeons) and 15 samples from wild birds (ducks, geese and seagulls). Two-year monitoring in the Vologda Oblast revealed specific antibodies to NDV in samples from wild birds. Positive samples from pigeons were obtained from the Republic of Tatarstan, the Krasnoyarsk Krai and the Omsk Oblast. In most cases, they were collected next to large commercial poultry farms, thus suggesting a threat of infection spread to poultry.

CONCLUSION

In 2023–2024, ND situation in the Russian Federation was assessed using field sera samples from various poultry species and wild birds. Due to routine vaccination with live and inactivated vaccines throughout the poultry rearing cycle, commercial poultry demonstrated a high level of seropositivity to NDV. Due to insufficient protection against ND, backyard poultry pose a persistent threat as a potential source of a primary disease outbreak driven by virulent strains of the virus. Antibodies to NDV were detected in wild and synanthropic birds in 4 regions of the Russian Federation.

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Investigating the infectious process in chickens infected with Newcastle disease virus genotype VII via different routes

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ABSTRACT

Introduction. Newcastle disease is a notifiable disease and is a major threat for commercial poultry. There are many known genotypes of the Newcastle disease virus (NDV), which differ in virulence. In recent years, there is an increasing interest in NDV genotype VII that stems from its prevalence and high pathogenicity in chickens and other species of commercial poultry, causing severe disease with up to 100% mortality.

Objective. Investigation of the infectious process and other clinical and post-mortem signs in chickens infected with Newcastle disease virus via different routes.

Materials and methods. Thirty-day-old chicks were experimentally infected with NDV genotype VII via three different routes: intranasal, oral and intramuscular. Forty eight hours post infection, six intact chickens were introduced in each group. Over the next 10 days, the clinical condition of the infected and contact poultry was assessed. Oropharyngeal and cloacal swabs were collected and tested by polymerase chain reaction. Dead chicks were subjected to post-mortem examination.

Results. The experiment demonstrated that NDV/chicken/rus/Saratov/2403-3/22 isolate causes poultry mortality within 5–7 days. Intramuscular infection led to faster disease progression and death in poultry compared to oral or intranasal routes. The NDV genome was identified in samples of oropharyngeal and cloacal swabs tested by polymerase chain reaction. While nonspecific signs of the disease were recorded in all individuals, the predominant clinical presentation varied with the infection route. Pronounced neurological symptoms were observed in birds infected via the intramuscular and oral routes. In contrast, respiratory signs were characteristic of infections via the oral and intranasal routes. The autopsy results indicate that specific pathological signs characteristic of Newcastle disease developed within 24 hours of the disease onset. A number of post-mortem lesions were found in the internal organs of individuals that died early. However, these lesions were not informative for a diagnosis of Newcastle disease.

Conclusion. The Newcastle disease virus NDV/chicken/rus/Saratov/2403-3/22 strain (genotype VII) was pathogenic to chickens during experimental infection. The disease was easily reproduced by intramuscular, intranasal, and oral routes of infection and was characterized by a peracute course with respiratory and neurological symptoms.

Keywords: Newcastle disease, infectious process, *Orthoavulavirus javaense, Paramyxoviridae,* genotype VII

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Изучение инфекционного процесса у кур при различных способах заражения вирусом ньюкаслской болезни генотипа VII

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РЕЗЮМЕ

Введение. Ньюкаслская болезнь птиц входит в перечень нотифицируемых болезней и является актуальной проблемой современного птицеводства. К настоящему времени известно о существовании различных генотипов возбудителя, отличающихся друг от друга по вирулентности. Все больший интерес в последние годы вызывает вирус ньюкаслской болезни генотипа VII, который инициирует тяжелую форму болезни среди кур и других видов коммерческой птицы вплоть до 100%-й летальности поголовья.

Цель исследования. Изучение инфекционного процесса, а также клинических и патолого-анатомических особенностей ньюкаслской болезни птиц при экспериментальном заражении кур разными способами.

Материалы и методы. Провели экспериментальное заражение вирусом ньюкаслской болезни генотипа VII 30-суточных цыплят тремя разными способами: интраназально, перорально и внутримышечно. Через 48 ч после инфицирования в каждую группу поместили по 6 интактных цыплят. В течение последующих 10 сут оценивали клиническое состояние зараженной и контактной птицы, собирали и исследовали методом полимеразной цепной реакции ротоглоточные и клоакальные смывы и проводили патолого-анатомическое вскрытие павшей птицы.

Результаты. В ходе поставленного эксперимента было установлено, что изолят NDV/chicken/rus/Saratov/2403-3/22 вызывает гибель птицы в течение 5–7 сут. При внутримышечном заражении болезнь и гибель птицы наступали быстрее, чем при пероральном и интраназальном инфицировании. В исследованных методом полимеразной цепной реакции образцах ротоглоточных и клоакальных мазков был выявлен геном вируса ньюкаслской болезни. Неспецифические признаки болезни были зафиксированы у всех особей, однако преобладание определенного симптомокомплекса зависело от способа заражения: у птиц, инфицированных внутримышечно и перорально, отмечались ярко выраженные неврологические симптомы; респираторные признаки были характерны при пероральном и интраназальном заражениях. Результаты вскрытия свидетельствуют о том, что специфические патолого-анатомические признаки, характерные для ньюкаслской болезни, развивались после 24 ч с момента начала болезни. У особей, павших ранее, был обнаружен ряд патологических изменений внутренних органов, которые тем не менее не являлись информативными для диагностики ньюкаслской болезни при вскрытии.

Заключение. Штамм вируса ньюкаслской болезни генотипа VII NDV/chicken/rus/Saratov/2403-3/22 является патогенным для кур при экспериментальном инфицировании. Болезнь легко воспроизводится при внутримышечном, интраназальном и пероральном способах заражения и характеризуется молниеносным течением с развитием респираторных и неврологических симптомов.

Ключевые слова: ньюкаслская болезнь птиц, инфекционный процесс, *Orthoavulavirus javaense, Paramyxoviridae,* генотип VII

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INTRODUCTION

Newcastle disease has threatened global poultry farming for over a century, continuing to cause significant economic damage worldwide. Despite ongoing vaccination efforts, recurrent Newcastle disease outbreaks in recent decades have underscored the virus's continued economic impact on the poultry industry [1]. Newcastle disease is caused by the virulent avian orthoavulavirus (*Orthoavulavirus javaense*, OAVJ). This virus is a member of the *Paramyxoviridae* family [2] and was previously referred to as avian paramyxovirus serotype 1 (APMV-1) [3]. Newcastle disease virus (NDV) genome is a negative-sense, single-stranded RNA virus in the order *Mononegavirales*, with a spiral (helical) capsid symmetry, and its replication occurs in the host cell's cytoplasm [4, 5]. Despite all NDV strains belong to OAVJ species, there is significant genetic and antigenic diversity between different genotypes [6]. NDV isolates are categorized into two classes, I and II, based on the genome length and unique genomic features [7, 8]. Class I strains are genotypically uniform, whereas the more diverse class II strains are currently divided into 21 genotypes based on the phylogenetic analysis of the F gene coding sequence [7, 9, 10, 11].

Many bird species are susceptible to NDV, but the disease's severity and outcome vary significantly by species [12]. Numerous bird species are susceptible to NDV, including domestic poultry like broilers, laying hens, ducks and turkeys, as well as game birds such as pigeons, peacocks, and pheasants, and even some non-poultry birds like ostriches and parrots (*Psittacidae*). First emerging in the 1990s, genotype VII has since become prevalent on multiple continents, including Asia, the Middle East, Europe, and parts of Africa and South America, garnering significant scientific attention in recent years [13]. Due to its high virulence, it causes a severe form of the disease in chickens and other commercial poultry, with mortality rates that can reach 100%. Initial classification divided genotype VII into two subgenotypes: VIIa, which emerged in the Far East in the 1990s and spread to Europe and other parts of Asia, and VIIb, which also originated in the Far East before spreading to South Africa [14]. The classification has since evolved, dividing genotype VII into eight subgenotypes. This includes the recently identified VII-L, which has been associated with Newcastle disease outbreaks in Iran [15] and other countries. Currently, based on new nomenclature criteria for NDV, genotype VII is now subdivided into three subgenotypes: VII.1.1, VII.1.2, and VII.2 [16]. It is known, that subgenotype VII.1.1 caused the third panzootic in pigeons in 1980s, and caused the fourth (starting in 1985) and the last, fifth panzootic [17].

Newcastle disease virus is traditionally divided into four pathotypes based on virulence: velogenic, mesogenic, lentogenic, and asymptomatic intestinal. However, the clinical signs associated with these pathotypes may not always be distinct [18]. Velogenic strains are further divided into two main categories: viscerotropic, which cause widespread hemorrhaging in the internal organs, and neurotropic, which are characterized by neurological and respiratory signs [19]. The disease is primarily transmitted via inhalation or ingestion of the virus shed in feces and respiratory secretions by infected birds for variable lengths of time [20, 21]. The virus can also be transmitted through the conjunctiva. Efficient bird-to-bird virus transmission requires the presence of infectious virus [22]. The infection may occur through inhalation of fine aerosols or large droplets containing the virus; however, the alimentary route of infection is probably the main one [23]. The disease develops rapidly, with symptoms appearing in a flock within two days of aerosol transmission, but the incubation period can be longer, up to 15 days, with fecal-oral infection, especially in caged birds [24, 25]. Some sources state that the incubation period for Newcastle disease in experimental infection ranges from 2 to 6 days [23]. According to the World Organization for Animal Health (WOAH), the maximum incubation period for Newcastle disease is 21 days.

The disease manifests with signs affecting the respiratory, digestive, and nervous systems, with symptoms varying based on viral virulence, bird age, and immune status [26]. Classic symptoms of Newcastle disease in birds include depression, decreased appetite, ruffled feathers, conjunctivitis, and green or white diarrhea [23]. The green color is a result of the disease affecting the digestive system, likely due to factors like a viral-induced disruption of digestive enzymes and bile production [27]. Respiratory symptoms include coughing and wheezing, while neurological classic symptoms are tremors, wing and leg paralysis, and torticollis (twisted neck). The neurological signs typically appear later in the disease, often after respiratory and digestive signs have started or have become severe [23].

Newcastle disease causes a range of pathological changes in birds, with their severity dependent on the virus's virulence and the host's susceptibility. Significant pathological lesions are typically induced exclusively by velogenic strains of the NDV. Gross post-mortem lesions consist of petechiae on serous membranes and hemorrhages affecting the mucosal surface of the pancreas and the intestinal serosa. These are accompanied by multifocal, necrotic-hemorrhagic lesions, particularly within lymphoid tissues such as the intestinal (caecal) tonsils [25]. The spleen may be enlarged, blotchy, and necrotic [28]. The lung tissue may exhibit hyperemia, along with multifocal hemorrhages and necrotic areas ranging from punctate to ecchymotic. Clinical findings can include cyanosis and petechiation of the comb [29]. Congestion and hemorrhages in the trachea are common signs of velogenic viscerotropic Newcastle disease in chickens. It is also reported that unlike geese the virus replicates in the brain of chickens, causing neurological signs and lesions [30]. Other sources confirm that hyperemia and multi-focal point hemorrhages can occur in the brain's membranes of chickens infected with certain strains of the NDV [25]. Infection with lentogenic NDV strains typically causes airsacculitis, characterized by thickening of the air sac membranes, and can lead to pneumonia from the virus or secondary bacteria [29].

The Newcastle disease situation deteriorated sharply across the Russian Federation in 2019, following the rapid nationwide spread of the subgenotype VII-L (VII 1.1) virus, which spread from Primorsky Krai in the east to the Kursk Oblast in the west. As a result, 17 outbreaks were reported, all of them in backyards, where non-vaccinated poultry were kept [31]. According to the WOAH, a total of 289 outbreaks of Newcastle disease were reported globally in 2023, with thirteen of these occurring in the Russian Federation[1]. In 2024, Newcastle disease was reported in 15 countries across four continents, with a total of 518 recorded outbreaks, 399 of which were concentrated in Nigeria (212) and Iraq (187)[2]. Despite vaccination, sporadic outbreaks of Newcastle disease caused by genotype VII virus occur even in vaccinated poultry in South America [32] and Asia [33]. Moreover, genotype VII is expanding its distribution, leading to diseases in waterfowl [34]. Repeated outbreaks of Newcastle disease in vaccinated poultry flocks can be due to a mismatch between vaccine strains and circulating field strains, which can have different antigens or incorrect vaccination protocols used to control the disease [13].

Currently, the most widely used Newcastle disease vaccines rely on early genotypes (I and II) isolated about 70 years ago. However, the predominant field strains now belong to later genotypes – such as V in the Americas, VII in Asia and Africa, and the globally prevalent genotype VI in pigeons – which are genetically and antigenically distinct from the vaccine strains [35].

Analysis of the global situation indicates a high risk of the virus being introduced into the Russian Federation, underscoring the critical importance of robust preventive measures. Numerous scientific studies have been conducted worldwide by various authors on the infectious process of velogenic NDV in chickens. However, there are only few Russian publications addressing this problem. Therefore, a comparative assessment of the infectious process in chickens, using different routes of infection with a virulent NDV strain, is critically needed.

To advance specific prevention strategies against Newcastle disease, it is crucial to reproduce the genotype VII infectious process experimentally. This allows for a detailed study of viral properties and establishes standardized disease signs in susceptible animals, providing a foundation for vaccine and therapy development.

MATERIALS AND METHODS

Virus. For infection, a virulent NDV/chicken/rus/Saratov/2403-3/22 strain (subgenotype VII.1.1 (VII-L), genotype VII) was used. The infectious dose was 6.0 $lgEID_{50}$ according to the WOAH recommendations[3]. The NDV isolate is classified as velogenic, based on the presence of a polybasic cleavage site in the F protein sequence and an intracerebral pathogenicity index of 1.62 [36].

Poultry. The experiment was conducted in chicks hatched from SPF eggs (VALO BioMedia GmbH, Germany). At the time of inoculation of the virus-containing material, the chicks were 30 days old. The hemagglutination inhibition assay confirmed the absence of NDV-specific antibodies in serum samples collected from birds prior to infection.

Infection routes. To simulate the infectious process, three different routes of infection were applied. The virus was administered in a suspension at different infecting doses according to the route of inoculation: intranasal (0.1 mL instilled into the nose), oral (1.0 mL provided in drinking water), and intramuscular (0.5 mL injected into the thigh muscle).

The control birds were not infected.

Study design. The experimental birds were divided into three equal groups, 8 birds per group. The groups were inoculated applying the above routes. Forty eight hours post infection, 6 intact chickens were introduced in each group. The experimental groups were formed in accordance with

[1] Global Newcastle disease situation (WOAH, 2023). https://fsvps.gov.ru/wp-content/uploads/2023/10/БН-мир-2023.pdf (in Russ.)

[2] Global Newcastle disease situation (WOAH, 2024). https://fsvps.gov.ru/wp-content/uploads/2024/06/БН-мир-2024-2.pdf (in Russ.)

[3] Newcastle disease (infection with Newcastle disease virus). *In: WOAH. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Chapter 3.3.10.* https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/3.03.10_NEWCASTLE_DIS.pdf

Table 1
Infectious process in Newcastle disease following different routes of exposure

Parameters	Experimentally infected birds			Contact birds	Control
	Inoculation route				
	Intramuscular	Oral	Intranasal		
Incubation period, days	2	2	3	3	–
Death following inoculation, days	5	6	7	6	–
Lethality, %	100	100	100	100	–
Virus genome in cloacal swabs	+	+	+	+	–
Virus genome in oropharyngeal swabs	+	+	+	+	–

the "Rules for Regulating the Veterinary Medicinal Products Circulation in the Customs Territory of the Eurasian Economic Union" (Council Decision No. 1 of the Eurasian Economic Commission dated January 21, 2022)[4]. Over the next 10 days, the clinical condition of infected and contact birds was evaluated in accordance with GOST R 58090-2018 "Clinical examination of unproductive animals. General requirements"[5]. Oropharyngeal and cloacal swabs were collected at the peak of clinical signs in accordance with the "Recommended practice of biological sample collection, storage and transportation for AIV and NDV diagnostic tests"[6]. Dead birds were necropsied in accordance with GOST R 57547-2017 "Services for non-productive animals. Pathological-anatomical study of corpses of non-productive animals. General requirements"[7]. The necropsy procedure consisted of an external examination of the carcass and an internal inspection of the viscera for pathological lesions. The reproductive organs of the experimental birds could not be adequately assessed due to age-related underdevelopment.

Birds were monitored daily for clinical signs. The progression of the disease, including the duration of each phase, the spectrum of clinical manifestations, and the incidence of mortality, was recorded. The dead birds were necropsied and all pathological lesions were recorded. The death specificity was confirmed by polymerase chain reaction (PCR).

All animal experiments were conducted in strict accordance with the interstate standard for laboratory animal keeping and handling GOST 33215-2014, adopted by the Interstate Council for Standardization, Metrology and Certification, and in accordance with the requirements of Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. The studies were approved by the Federal Centre for Animal Health Bioethics Commission (report of 25.07.2025).

Virus identification. The NDV genome in oropharyngeal and cloacal swabs was detected by real-time PCR in accordance with the "Recommended practice of RNA identification and differentiation of NDV virulent isolates by qRT-PCR"[8].

RESULTS AND DISCUSSION

Infectious process. The NDV used in the study was identified as genotype VII, subgenotype VII.1.1, and proved to be contagious for chickens across all experimental groups. The virus demonstrated efficient transmission from infected to contact birds, resulting in 100% lethality in both directly inoculated and contact-exposed poultry. Table 1 summarizes the key parameters of the infectious process for each experimental group.

The shortest incubation period (2 days) was observed with intramuscular and oral inoculation, while the longest (3 days) occurred with the intranasal route. This finding aligns with data from the WOAH and other sources [23, 25].

The time to death was shortest with intramuscular infection, intermediate with oral infection, and longest with intranasal infection (5, 6, and 7 days, respectively). Birds in all experimental groups, both infected and contact, shed the virus in their droppings and oropharyngeal secretions. The course of infection in contact chickens generally mirrored that of the experimentally infected birds.

Experimental inoculation routes that mimic natural infection – with the exception of the parenteral (intramuscular) route – resulted in an acute infectious process characteristic of velogenic NDV strains. Clinically, disease manifestation in contact chickens occurred almost simultaneously with that in directly inoculated birds, indicating high virus transmissibility and pathogenicity.

Clinical signs. The analysis included data from the infectious process as well as the clinical signs observed in both experimentally infected and contact birds. Table 2 demonstrates the variability of Newcastle disease clinical signs in chickens from different experimental groups.

Nonspecific signs, including hyperthermia (Fig. 1), diarrhea, inappetence, and depression (Fig. 2) were observed in all experimental groups. During episodes of diarrhea, the feces were watery and green in color.

Following the incubation period, intramuscularly infected birds developed a more severe and rapid disease course, leading to early mortality. However, the clinical presentation was often ambiguous and limited to non-specific signs. In addition, birds infected via the intramuscular and oral routes exhibited pronounced neurological signs. Respiratory signs – including coughing, sneezing, and

[4] https://www.alta.ru/tamdoc/22sr0001/?ysclid=mghl9evtve540241408 (in Russ.)
[5] https://files.stroyinf.ru/Data2/1/4293738/4293738274.pdf?ysclid=mghllls82495439970 (in Russ.)
[6] Andreychuk D. B., Andriyasov A. V., Volkova M. A., Chvala Ir. A., Volkov M. S., Chvala Il. A. Recommended practice of biological sample collection, storage and transportation for AIV and NDV diagnostic tests: approved by the Federal Centre for Animal Health on 24.06.2019. Vladimir; 2019. 17 p. (in Russ.)
[7] https://files.stroyinf.ru/Data2/1/4293744/4293744536.pdf?ysclid=mghmowmxuy341824070 (in Russ.)
[8] MU 47-16. Recommended practice of RNA identification and differentiation of NDV virulent isolates by qRT-PCR: approved by Rosselkhoznadzor on 06.06.2016. Vladimir; 2016. 11 p. (in Russ.)

wheezing – were observed in the orally and intranasally infected groups. Notably, nasal discharge was specific only to the intranasal infection route. Neither the directly inoculated chickens nor the contact-exposed birds exhibited ocular discharge or conjunctivitis. This finding contrasts with reports from numerous authors, who identify these clinical signs as characteristic of NDV infection [25, 37]. The clinical presentation of Newcastle disease in contact-exposed birds across all three groups mirrored that of the intramuscularly inoculated birds.

Post-mortem examination. Post-mortem examination revealed no gross pathological lesions characteristic of Newcastle disease in the viscera of birds that died within 24 hours of clinical onset. The birds exhibited below-average body condition and ruffled plumage. The beak and eyes were closed, no discharge observed. Minor petechiae were observed on the serosal surface of the sternum. Visceral changes included mild mucosal hyperemia of the intestine, without hemorrhages or necrotic foci, and slight splenomegaly. Cerebral edema with marked vascular hyperemia and scattered petechial hemorrhages were observed.

Birds that succumbed > 24 hours post-onset exhibited distinct pathological changes that varied with the route of exposure and clinical presentation. The birds exhibited below-average body condition and ruffled plumage. The cloaca was occluded, with the surrounding feathers soiled by greenish fecal material. Serous edema was present within the subcutaneous and interstitial tissues of the head, neck, and particularly the thoracic region. Pallor, sometimes progressing to cyanosis, was noted in the comb and wattles. Birds that succumbed following intranasal infection had exudate accumulated on the beak surface and around the nares. The oral mucosa in all experimental groups was cyanotic and exhibited catarrhal inflammation. The mucosa of the pharynx and esophagus was erythematous with multifocal hemorrhages. The oral cavity and pharynx contained abundant mucous exudate (Fig. 3). Hemorrhages were noted on the posterior pharyngeal wall and trachea (Fig. 4) in orally and intranasally infected birds. The blood within the cardiac chambers and major vessels was clotted. In some cases, the myocardium was flaccid. The lungs were hyperemic and edematous. The spleen was cyanotic and the capsule was tense. Diffuse hyperemia was present throughout the intestinal mucosa, accompanied by multifocal hemorrhages and areas of necrosis. The lymph nodes were enlarged. In some cases, the liver was flaccid and mottled, while the kidneys were enlarged, extending beyond the renal fossae. Cerebral edema with marked vascular hyperemia and scattered petechial hemorrhages were observed.

Table 2
Influence of exposure route on the clinical manifestation of Newcastle disease in chickens

Clinical signs	Experimentally infected birds			Contact birds
	Inoculation route			
	Intramuscular	Oral	Intranasal	
Depression	+	+	+	+
Loss of appetite	+	+	+	+
Diarrhea	+	+	+	+
Hyperthermia	+	+	+	+
Coughing, sneezing	–	+	+	–
Wheezing	–	+	+	–
Nasal discharge	–	–	+	–
Ocular discharge, conjunctivitis	–	–	–	–
Neurological symptoms (unsteady gait, torticollis, tremors of the head and limbs)	+	+	–	+

Birds that succumbed 72–96 hours post-onset exhibited distinct gross pathological lesions upon both external and internal examination. The birds were emaciated, with ruffled plumage soiled by liquid, greenish fecal material. Scalp, comb and wattles were cyanotic, without hemorrhage. Petechiae and small ecchymoses were present on the pancreatic serosal surface. The mucosal lining of the gizzard was loose and easily detached. In some birds, the mucosa at the junction of the pancreas and the proventriculus was hyperemic and exhibited band-like hemorrhages (Fig. 5). The spleen was dark in color, with nectrotic foci. The intestine exhibited catarrhal inflammation, with hemorrhages and necrotic foci involving the intestinal tonsils and associated lymphoid tissue. Cerebral edema

Fig. 1. Hyperthermia in infected chicks

Fig. 2. Ruffled feathers and general depression (right chick) and a clinically healthy contact chick (left)

Fig. 3. Accumulation of mucosal exudate in the mouth and pharynx

Fig. 4. Submucosal hemorrhages in the posterior oropharynx and trachea

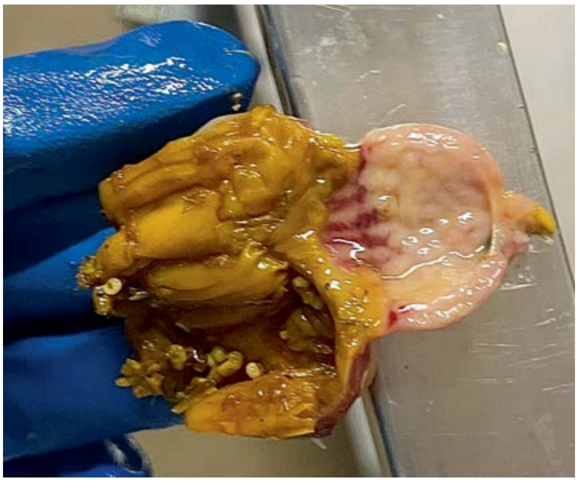

Fig. 5. Hemorrhagic banding at the proventricular-ventricular junction

Fig. 6. Cerebral edema with associated hemorrhages

with marked vascular hyperemia and scattered petechial hemorrhages were observed (Fig. 6).

The overall pathological presentation observed in this study aligns with the characteristic lesions of Newcastle disease documented in prior literature [23, 37]. Neuropathological changes were present in all experimental birds, irrespective of clinical presentation, infection route, or time of death. Although some sources report no significant splenic changes in Newcastle disease, splenomegaly and discoloration were frequently observed pathological signs in the present study [38]. The development of specific lesions was contingent on survival beyond the incubation period; in birds surviving more than 24 hours post-incubation, pathological changes appeared and intensified as the clinical disease advanced. In cases of acute disease resulting in early mortality, pathological changes were mild and nonspecific; consequently, gross necropsy was not a reliable method for postmortem diagnosis of Newcastle disease.

CONCLUSION

Investigating the biological properties of NDV genotype VII strains and the resulting infectious process is essential for refining prevention strategies and preventing new outbreaks.

This study experimentally confirmed the contagiousness of the NDV isolate NDV/chicken/rus/Saratov/2403-3/22 (genotype VII, subgenotype VII-L) in chickens via intramuscular, oral, and intranasal infection routes. It also demonstrated the virus's ability to be shed into the environment through the respiratory and digestive tracts. An infecting dose of 6.0 lg EID_{50} was uniformly lethal for 30-day-old SPF chicks, resulting in 100% mortality under the experimental conditions.

The specific clinical presentation of Newcastle disease varied with the infection route. Neurological signs predominated in intramuscularly infected and contact-exposed birds, while respiratory signs were primary following intranasal inoculation. Orally infected birds exhibited a mixed clinical presentation, featuring both neurological and respiratory symptoms. Necropsy revealed that the pathological presentation of Newcastle disease is highly variable. The spectrum of lesions was influenced more by the time from disease onset to death than by the route of infection.

The velogenic isolate NDV/chicken/rus/Saratov/2403-3/22 (genotype VII-L) induced the most severe disease and highest mortality in birds following intramuscular administration. Although intramuscular infection is a non-natural route, it is a standard method for challenge tests and is recommended by the WOAH for evaluating

Newcastle disease vaccine efficacy. Therefore, intramuscular route for inoculation of a virus-containing suspension of NDV/chicken/rus/Saratov/2403-3/22 isolate is optimal for the experimental modeling of Newcastle disease

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Antibiotic resistance of bacterial pathogens circulating on a dairy farm in Sverdlovsk Oblast

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ABSTRACT

Introduction. Currently, there is a need to develop a unified strategy for rational antibiotic therapy, including monitoring the sensitivity of microorganisms, medicinal product rotation, and the use of alternative treatment methods to reduce the spread of antibiotic-resistant bacterial isolates.

Objective. Identification of bacterial pathogens that cause mastitis in cows, with an assessment of their resistance to antimicrobial medicinal products used at a livestock farm located in Sverdlovsk Oblast, for subsequent rotation of antimicrobial agents and the development of individual recommendations.

Materials and methods. The research was conducted in 2022–2024 on the basis of an agricultural farm located in Sverdlovsk Oblast. The identification of grown colonies was performed using MALDI-ToF mass spectrometry, susceptibility to antimicrobials medicinal products was determined by the disk diffusion method, and antibiotic resistance genes were detected by qPCR.

Results. In 2022, test results showed the presence of *Streptococcus* spp. (70.6%), *Escherichia coli* (52.9%), *Staphylococcus aureus* (35.3%), and *Streptococcus agalactiae* (23.5%) in mammary gland secretions. Isolates of *Escherichia coli* and *Staphylococcus aureus* were resistant to several groups of antimicrobials: aminoglycosides, penicillins, tetracyclines and fluoroquinolones (ciprofloxacin), and vancomycin. Resistance genes were identified: *bla*DHA, *bla*CTX-M, and *bla*OXA-10 in *Escherichia coli* (5%); *Erm*B in the group of bacteria *Staphylococcus* and *Streptococcus* (4%); *Mec*A in *Staphylococcus aureus* (isolated cases). Upon repeated testing in 2023, it was observed that all isolated bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Enterobacter* spp., *Streptococcus* spp., *Enterococcus faecalis/faecium*) were sensitive to all antimicrobials medicinal products. The *bla*VIM and *bla*NDM genes were detected in one *Pseudomonas aeruginosa* isolate. The test results obtained in 2024 showed the predominance of *Escherichia coli* and *Staphylococcus* spp. (100%), *Klebsiella pneumonia* (30%), *Enterobacter* spp. (20%), *Enterococcus faecalis/faecium* (10%) in mammary gland secretion samples. Eight different antimicrobial resistance genes were identified, along with the detection of carbapenem-resistant bacteria and vancomycin-resistant *Enterococcus* spp. (*Van*B gene). Based on laboratory tests conducted in 2022–2024 at a livestock farm in Sverdlovsk Oblast, measures to control antimicrobial resistance in bovine mastitis pathogens have been developed and tested.

Conclusion. Replacement of outdated treatment regimens (tetracyclines, aminoglycosides, cephalosporins of the II generation) with cephalosporins of the I/III/IV generations and fluoroquinolones temporarily reduced resistance. However, returning to the previous protocols in 2024 caused a sharp increase in multidrug resistance. Therefore, recommendations have been provided. These include continuous monitoring of pathogen resistance, strict adherence to antibiotic rotation schedules, long-term application of the revised treatment protocols, and the implementation of additional molecular genetic methods to detect bacterial resistance genes. These measures are aimed at controlling the situation at the livestock farm.

Keywords: monitoring, antibiotic resistance, antimicrobials, antimicrobial rotation, laboratory testing, cattle, disinfectants

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Антибиотикорезистентность бактериальных патогенов, циркулирующих на молочнотоварном предприятии Свердловской области

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РЕЗЮМЕ

Введение. В настоящее время возникла необходимость разработки единой стратегии рациональной антибиотикотерапии, включающей мониторинг чувствительности микроорганизмов, ротацию препаратов и использование альтернативных методов лечения, позволяющих сократить распространение антибиотикорезистентных изолятов бактерий.

Цель исследования. Определение бактериальных патогенов, вызывающих мастит у коров, с оценкой их устойчивости к антимикробным препаратам, применяемым на животноводческом предприятии, расположенном на территории Свердловской области, для последующей ротации антимикробных средств и разработки индивидуальных рекомендаций.

Материалы и методы. Исследования проведены в 2022–2024 гг. на базе сельскохозяйственного предприятия Свердловской области. Идентификацию выросших колоний производили методом MALDI-ToF масс-спектрометрии, чувствительность к антимикробным препаратам определяли диско-диффузионным методом, гены резистентности к антибиотикам выявляли с помощью полимеразной цепной реакции в режиме реального времени.

Результаты. В 2022 г. результаты исследований показали наличие в секрете молочной железы *Streptococcus* spp. (70,6%), *Escherichia coli* (52,9%), *Staphylococcus aureus* (35,3%), *Streptococcus agalactiae* (23,5%). Изоляты *Escherichia coli* и *Staphylococcus aureus* обладали резистентностью к нескольким группам антимикробных препаратов: аминогликозидам, пенициллинам, тетрациклинам и фторхинолонам (ципрофлоксацину), ванкомицину. Установили гены устойчивости: *bla*DHA, *bla*CTX-M и *bla*OXA-10 – у *Escherichia coli* (5%); *Erm*B – у группы бактерий *Streptococcus* (4%); *Mec*A – у *Staphylococcus aureus* (единично). При повторном исследовании в 2023 г. наблюдали, что все изолированные бактерии (*Staphylococcus aureus*, *Escherichia coli*, *Enterobacter* spp., *Streptococcus* spp., *Enterococcus faecalis/faecium*) были чувствительны ко всем антимикробным препаратам. У одного изолята *Pseudomonas aeruginosa* выявлены гены *bla*VIM, *bla*NDM. Результаты, полученные в 2024 г., показали преобладание в пробах секрета молочной железы *Escherichia coli* и *Staphylococcus* spp. (100%), *Klebsiella pneumonia* (30%), *Enterobacter* spp. (20%), *Enterococcus faecalis/faecium* (10%). Были выявлены 8 различных генов резистентности к антимикробным препаратам, также обнаружены карбапенем-устойчивые бактерии и ванкомицин-устойчивый *Enterococcus* spp. (ген *Van*B). На основе лабораторных исследований, проведенных в 2022–2024 гг. на животноводческом предприятии Свердловской области, разработаны и апробированы меры контроля антимикробной резистентности возбудителей мастита у коров.

Заключение. Замена устаревших схем лечения (тетрациклины, аминогликозиды, цефалоспорины II поколения) на цефалоспорины I/III/IV поколений и фторхинолоны временно снизила резистентность. Возврат к прежним схемам в 2024 г. вызвал резкий рост полирезистентности. В связи с чем даны рекомендации, включающие непрерывный мониторинг резистентности возбудителей, строгое соблюдение ротации антибиотиков, долгосрочное применение схем лечебных мероприятий, внедрение дополнительных молекулярно-генетических методов для детекции генов устойчивости бактерий в целях контроля ситуации на животноводческом предприятии.

Ключевые слова: мониторинг, антибиотикорезистентность, антимикробные препараты, ротация препаратов, лабораторная диагностика, крупный рогатый скот, дезинфицирующие средства

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INTRODUCTION

The irrational use of antimicrobials in animal husbandry has led to livestock becoming a reservoir of antibiotic-resistant bacteria. Resistant strains of microorganisms pose a threat not only to animal health, but also to human health as they can also enter the human body with products of animal origin (meat, eggs, and dairy products). There is now a pressing need to develop a unified strategy for the rational use of antimicrobials, which includes monitoring of microbial susceptibility, rotation of veterinary medicinal products and use of alternative methods that allow reducing their use. Important measures also include a transition to extensive farming systems, reducing animal stress, and maintaining high hygiene standards. Scientists worldwide emphasize the global nature of the antimicrobial resistance (AMR) problem and the importance of international cooperation in solving it [1, 2, 3, 4]. Foreign authors stress the need for coordinated global, regional, and national strategies, based on the "One World, One Health" approach, to reduce the use of antimicrobials and find alternatives [5, 6, 7]. The World Health Organization and the World Organization for Animal Health have developed lists of critically important antibiotics for human medicine and veterinary medicine in order to limit their irrational use [3].

Russian scientists have experimentally established that the repeated use of the same antibiotics in treatment and prevention protocols both in cattle and in poultry leads to the AMR development in pathogenic microflora. This reduces efficacy of veterinary medicinal products, negatively impacts productivity, and increases risks to animal health [8, 9, 10].

Experience from leading international medical researchers indicates that the periodic rotation of antibiotics can help reduce the risk of AMR development. Rotation of veterinary medicinal products can significantly increase the susceptibility of antibiotic-resistant bacterial strains. Modified treatment protocols, routinely applied in practice, can yield positive results even after several years. The authors have also conducted multi-center studies to confirm these findings and to optimize both the frequency and rotation options of antibiotics [11, 12].

Experts agree that an effective countermeasure against AMR necessitates an integrated approach, combining optimized antibiotic therapy, stringent infection control, innovative methods (such as rapid resistance diagnostics), and AMR monitoring to achieve maximum effect [13, 14].

Modern Russian publications also take into account the ecological status of the territories of the Russian Federation when developing measures to control AMR. Authors discuss enhanced monitoring of radionuclides and heavy metals in feed, as well as antibiotic resistance on farms in industrial zones, alongside the development of adaptive livestock farming technologies to reduce animal stress in polluted areas [15]. Researchers emphasize the need for widespread application of alternative methods, such as vaccination, probiotics, phytobiotics, bacteriophages, bacteriocins, rotation of antibiotics, and controlled application of these alternative methods in industrial livestock and poultry farming [1, 16, 17]. However, despite promising results from using these methods, most of them require additional research, particularly within the context of specific agricultural farms [17, 18, 19, 20].

Research aimed at identifying antibiotic resistance in bacterial pathogens is highly relevant due to the complex AMR situation in animal husbandry, which poses a serious threat to both animal and human health through the food chain. The irrational use of antimicrobials has led to the emergence and spread of resistant microbial strains, significantly reducing treatment efficacy and necessitating new approaches to managing infectious diseases in livestock. In Sverdlovsk Oblast, a region with developed livestock sectors, the AMR problem is particularly significant, underscoring the need for localized monitoring and development of tailored recommendations for specific farms.

The novelty of this study is twofold. First, it provides a comprehensive analysis of the dynamics of the microbial landscape and the resistance profiles of mastitis pathogens on the operational farm in Sverdlovsk Oblast. Second, it develops and validates a practical algorithm for rotating antimicrobials, based on regular molecular genetic monitoring, which has proven effective in a commercial herd.

This study aimed to identify the primary bacterial pathogens responsible for mastitis in cows on the farm in Sverdlovsk Oblast and assess their resistance to antimicrobials. The findings provide a basis for implementing an antimicrobial rotation strategy and delivering tailored farm-specific recommendations.

MATERIALS AND METHODS

This research was conducted as part of the Russian Ministry of Science and Higher Education's state assignment "Development of Methodological Approaches for Monitoring, Controlling, and Containing Antibiotic Resistance of Opportunistic Microorganisms in Animal Husbandry" (No. 0532-2021-0004). The work was carried out across several departments of the Ural Federal Agrarian Scientific Research Centre, Ural Branch of the Russian Academy of Sciences: the Department of Genomic Research and Animal Selection, the Laboratory of Microbiological and Molecular Genetic Research Methods, and the Laboratory of Biological Technologies within the Department of Veterinary Laboratory Diagnostics and its testing facility.

The study involved monitoring circulation of pathogenic and opportunistic microorganisms, determining their susceptibility to standard antibiotics and the antimicrobials/disinfectants in use, identifying resistance genes, and developing recommendations for rotating antimicrobials used in treatment of bovine mastitis. This was implemented and evaluated over a three-year period (2022, 2023, 2024) on the dairy farm located in Sverdlovsk Oblast.

Sampling was conducted as follows: in 2022, 10 samples of mammary-gland secretion were collected from cows with clinical mastitis; in 2023, 3 composite samples were collected from 15 cows with subclinical mastitis on the same farm; and in 2024, 16 samples were collected.

Microbiological tests were performed in accordance with the "Methodological Guidelines for the Bacteriological Examination of Milk and Udder Secretions from Cows" (No. 115-69, approved by the Main Veterinary Directorate of the USSR Ministry of Agriculture on December 30, 1983)[1].

The following nutrient media were used in this study: "Columbia Blood Agar Base" (Bio-Rad Laboratories, Inc., France), defibrinated sheep blood (EKOlab, Russia), dry nutrient medium for accumulation of *Salmonella* (magnesium medium), bismuth sulfite agar, Ploskirev's agar, GRM nutrient agar for microorganism cultivation (State Research Center for Applied Microbiology and Biotechnology, Russia), Sabouraud Dextrose agar with 2% glucose and chloramphenicol, Mueller – Hinton agar (SIFIN diagnostics GmbH, Germany), and trypticase soy broth with 20% glycerol (Condalab, Spain).

Grown colonies were identified using MALDI-ToF mass spectrometry (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight) on a Vitek® MS device (bioMérieux, France). For this purpose, bacterial biomass was applied to a target slide spot, then covered by 1 µL of matrix (*α*-cyano-3-hydroxycinnamic acid), and air-dried at room temperature, and its ribosomal mass spectra were read with a special device and were compared with ones from the database using the MYLA® software (bioMérieux, France).

Antibiotic susceptibility was determined by a disk-diffusion test on Mueller – Hinton agar (Bio-Rad Laboratories, Inc., France) following European Committee on Antimicrobial Susceptibility Testing (EUCAST) standard guidelines and disks impregnated with preparations of a specific concentration (Bio-Rad Laboratories, Inc., France). Antibiotic susceptibility patterns were read by an ADAGIO automatic analyzer (Bio-Rad Laboratories, Inc., France). Interpretation of susceptibility categories was performed following EUCAST criteria: Clinical breakpoints-bacteria (v 10.0).

The antibiotic disks used in the study included: amoxicillin / clavulanic acid, gentamicin, oxytetracycline, tigecycline, levofloxacin, norfloxacin, cefepime, cefixime, cefoperazone, cefotaxime, cefpodoxime, ceftazidime, ceftriaxone, ciprofloxacin, and ceftiofur (Bio-Rad Laboratories, Inc., France). Microbiological tests also included determining susceptibility to combined antibacterials used on the farm for treating bovine mastitis (2023–2024), which contained antibiotics from the following classes: cephalosporins, aminoglycosides, tetracyclines, and polypeptide antibiotics.

[1] https://base.garant.ru/72125912/?ysclid=mguhhtg7xh175440448 (in Russ.)

Table 1
Antibiotic resistance and the presence of AMR genes in bacterial pathogens isolated from cow mammary gland secretions, 2022 (*n* = 10)

Bacterium species	Resistance of the isolated bacteria to the following antimicrobials	AMR genes
E. coli	Aminoglycosides, penicillins, tetracyclines	*bla*DHA, *bla*CTX-M, *bla*OXA-10 (in 5% of cases); resistance to *β*-lactams (cephalosporins and protected penicillins)
S. aureus	Fluoroquinolones (ciprofloxacin), vancomycin, tetracyclines	*Mec*A (in a single case); resistance to cephalosporins of the II generation
Streptococcus spp.	Susceptible to antimicrobials	*Erm*B (in 4% of cases); resistance to macrolides, lincosamides, streptogramines

The isolated microbial cultures were frozen at −20 °C in tubes containing trypticase soy broth with 20% glycerol as a cryoprotectant.

Real-time polymerase chain reaction was performed using the Diatom™ DNA Prep 200 kit (Laboratory Isogene, Russia) for DNA extraction from biological materials and the "COMPLEX RESISTOM ESKAPE-V" reagent kit (Lytech, Russia) – for detecting pathogen DNA and antibiotic resistance genes. Amplification was performed in real time using a QuantStudio 5 system (Thermo Fisher Scientific Inc., USA).

Based on the laboratory findings, tailored recommendations for antibacterial therapy of bovine mammary gland diseases were developed. Antibiotic selection followed established methodological guidelines [21], ensuring a scientifically grounded approach to rotation of antibiotics, and complied with Order No. 771 of the Ministry of Agriculture of the Russian Federation (November 18, 2021)[2] on restrictions governing use of antimicrobials in veterinary medicine.

The obtained data were processed using Microsoft Excel software (Microsoft Office Pro 19).

RESULTS AND DISCUSSION

In 2022, microbiological tests using MALDI-ToF mass spectrometry of the collected biological materials (10 samples of mammary gland secretion from cows) revealed the following bacterial isolates: *Streptococcus* spp. (present in 70.6% of samples), *Streptococcus agalactiae* (23.5%), *Staphylococcus aureus* (35.3%), and *Escherichia coli* (52.9%).

Data on the antibiotic resistance and the presence of AMR genes in these bacterial pathogens are presented in Table 1.

When determining antibiotic resistance by a disco diffusion test, it was found that all identified *E. coli* isolates were resistant to several groups of antimicrobials (aminoglycosides, penicillins, tetracyclines) and sensitive to cefoxitin (cephalosporin of the II generation), ciprofloxacin (fluoroquinolone of the II generation). *S. aureus*, isolated from all 10 samples, was resistant to ciprofloxacin (fluoroquinolone of the II generation), vancomycin (a glycopeptide antibiotic), tetracyclines and was sensitive to chloramphenicol, cefoxitin, and in single cases it was sensitive to tobramycin (aminoglycoside) and linezolid (oxazolidinone). Streptococci isolates were susceptible to all tested antimicrobials. Notably, isolates of *S. aureus* and *E. coli* also exhibited resistance to chlorhexidine- and iodine-based disinfectants used for pre- and post-milking udder hygiene.

Key resistance genes were detected by qPCR: *bla*DHA, *bla*CTX-M, *bla*OXA-10 genes (conferring resistance to *β*-lactams – cephalosporins and protected penicillins) were detected in 5% of *E. coli* isolate; *Erm*B gene (responsible for resistance to macrolides, lincosamides, and streptogramins) was found in 4% of *Streptococcus* spp. isolates; *Mec*A gene (regulating resistance to cephalosporins of the II generation) was identified in one *S. aureus* isolate.

Based on these findings, the following evidence-based recommendations were developed to enhance therapeutic efficacy and curb the further spread of antibiotic resistance. Priority antimicrobials for mastitis treatment were recommended: cefazolin, ceftiofur, cefquinome (representing cephalosporins of the I, III, and IV generation, respectively), and ciprofloxacin (fluoroquinolone of the II generation). Previously used multi-component medicinal products containing tetracyclines, aminoglycosides, macrolides, and cephalosporins of the II generation were recommended for removal from treatment protocols. It was recommended to use antimicrobials of penicillin group with caution. With regard to hygiene and monitoring it was recommended to implement regular disinfection control of milking equipment and conduct a semi-annual (every 6 months) AMR monitoring program of detected pathogens.

In 2023, microbiological tests performed by the disk diffusion test on 3 pooled samples of mammary gland secretion obtained from 15 cows with subclinical mastitis revealed that single *E. coli* and *S. aureus* isolated from the biological material by MALDI-ToF mass spectrometry, possessed resistance to ciprofloxacin. Other bacterial isolates (*S. aureus, Escherichia, Enterobacter, Streptococcus* spp., *Enterococcus faecalis/faecium*) were susceptible to all tested antimicrobials. It should be noted that the *E. coli* and *S. aureus* isolates exhibited susceptibility to chlorhexidine- and iodine-based agents used for udder disinfection before and after milking. Using the qPCR method, the *bla*VIM and *bla*NDM genes, responsible for resistance to carbapenems, were detected in a single *Pseudomonas aeruginosa* isolate. The other

[2] https://fsvps.gov.ru/files/prikaz-minselhoza-rossii-ot-18-nojabrja-2021-2/?ysclid=mgqesh36jf335708795 (in Russ.)

Table 2
Antibiotic resistance and the presence of AMR genes in bacterial pathogens isolated from cow mammary gland secretions, 2024 (*n* = 16)

Bacterium species	Resistance of the isolated bacteria to the following antimicrobials	AMR genes
E. coli	Cephalosporins, carbapenems (100%)	*bla*OXA-10 (in 30% of cases), *bla*CTX-M (sporadic); resistance to cephalosporins
S. aureus	Cephalosporins, carbapenems (100%)	Not detected
Staphylococcus spp.	Cephalosporins, carbapenems (100%)	*Mec*A (in 50% of cases); resistance to *β*-lactams
K. pneumoniae	Susceptible to antimicrobials	*bla*KPC, *bla*OXA-48-like (in 50% of cases); resistance to carbapenems
Enterobacter spp.	Susceptible to antimicrobials	*bla*Ges, *bla*DHA (in 30% of cases); resistance to carbapenems, protected penicillins and cephalosporins
E. faecalis/faecium	Susceptible to antimicrobials	*Van*B (in a single case); resistance to glycopeptides (vancomycin)

bacterial isolates exhibited no genetic mutations, indicating rational use of antibacterials on the farm during the study period and the future possibility of using a broader spectrum of antimicrobials in the treatment of inflammatory diseases of the mammary gland in cows, taking into account their identification of the phenotypic antibiotic susceptibility.

Throughout 2022–2023, it was established that the detected isolates were resistant to the agents used for treatment after milking. So, the use of combinations of disinfectants with different mechanisms of action was recommended to optimize hygienic measures during milking. A product based on a polyvinylpyrrolidone-iodine complex was proposed as the disinfectant of choice for post-milking teat treatment.

Microbiological tests conducted on the same farm in 2024 showed the predominance of *E. coli* and *Staphylococcus* spp. (100% of samples) in 16 samples of mammary gland secretions collected from cows with mastitis; in contrast, *K. pneumoniae* (30%), *Enterobacter* spp. (20%) and *E. faecalis/faecium* (10%) were less frequently detected.

Data on antibiotic resistance and the presence of AMR genes in bacterial pathogens isolated from cow mammary gland secretions in 2024 are presented in Table 2.

The disk diffusion test revealed that all *E. coli*, *S. aureus*, and *Staphylococcus* spp. isolates exhibited resistance to cephalosporins and carbapenems. The *bla*OXA-10 genes, conferring resistance to cephalosporins, were detected in 30% of *E. coli* isolates by qPCR, and in single cases the *bla*CTX-M genes were detected. The *bla*KPC and *bla*OXA-48-like genes responsible for carbapenem resistance were identified in 50% of *K. pneumoniae* isolates. The *Mec*A gene, conferring *β*-lactam resistance, was confirmed in 50% of *Staphylococcus* spp. isolates. 30% of the *Enterobacter* spp. isolates harbored resistance genes (*bla*Ges, *bla*DHA) that confer resistance to carbapenems protected by penicillins and cephalosporins. *E. faecalis/faecium* carrying the *Van*B gene, associated with glycopeptide (vancomycin) resistance, were detected in single cases. Thus, microbial cultures isolated in 2024 from bovine mammary gland secretions exhibited 8 distinct AMR genes. The findings demonstrate a high prevalence of multi-drug resistance in the bacterial flora of mammary secretions, including resistance to reserve antibiotics.

All isolates detected in 2024 demonstrated susceptibility to the post-milking teat disinfectant containing polyvinylpyrrolidone-iodine complex that was recommended in 2023.

Based on the research findings, the following recommendations were provided to the farm: revision of mastitis treatment protocols with mandatory susceptibility testing of identified pathogens, enhanced biosafety measures (equipment disinfection, animal quarantine), implementation of regular antibiotic resistance monitoring. The recommendations emphasized that critically important antibiotics (cephalosporins and fluoroquinolones of the III and IV generation) should be strictly restricted to use as a last-line therapy in exceptional cases only, to preserve their efficacy.

CONCLUSION

Microbiological tests and MALDI-ToF mass spectrometry identified the following dominant bacterial pathogens in bovine mammary gland secretions: in 2022 – *Streptococcus* spp. (70.6%), *S. agalactiae* (23.5%), *S. aureus* (35.3%), and *E. coli* (52.9%) isolates; in 2023 – antimicrobial-susceptible *S. aureus*, *Escherichia coli*, *Enterobacter* spp., *Streptococcus* spp., *E. faecalis/faecium*, and *P. aeruginosa* isolates, and in single cases – ciprofloxacin-resistant *E. coli* and ciprofloxacin-resistant *S. aureus*; in 2024 – *E. coli* and *Staphylococcus* spp. were detected in 100% of samples, alongside newly emerging pathogens: *K. pneumoniae* (30%), *Enterobacter* spp. (20%), and *E. faecalis/faecium* (10%).

In 2022, *E. coli* exhibited resistance to aminoglycosides, penicillins, tetracyclines, with 5% of isolates carrying several resistance genes *bla*DHA, *bla*CTX-M and *bla*OXA-10 conferring resistance to cephalosporins and protected penicillins; *S. aureus* demonstrated resistance to fluoroquinolones, vancomycin, tetracyclines, and the *Mec*A gene resistant to cephalosporins of the II generation was identified in a single isolate; 4% of *Streptococcus* spp. group bacteria had the resistance gene to macrolides, lincosamides, streptogramins. In 2023, no AMR genes were detected in the tested isolates, except for one *P. aeruginosa* isolate, which carried the carbapenem resistance genes *bla*VIM and *bla*NDM. In 2024, *bla*OXA-10 genes were identified in 30% of *E. coli* isolates, while *bla*CTX-M genes, conferring resistance to cephalosporins, were identified in a single isolate. In 50% of *K. pneumoniae* isolates *bla*KPC/OXA-48-like carbapenem resistance genes were identified, while the *Mec*A gene conferring *β*-lactam resistance was detected in *Staphylococcus* spp.; the *bla*Ges/DHA resistance genes to carbapenems, protected penicillins, and cephalosporins were detected in 30% of *Enterobacter* spp. A few single isolates of *E. faecalis/faecium* that harbored the *Van*B gene, which confers resistance to glycopeptides, were reported.

In 2022 it has been established that multicomponent veterinary medicinal products based on tetracyclines, aminoglycosides, macrolides and cephalosporins of the II generation should be excluded from the treatment protocols used in the bovine mastitis treatment. As an alternative, the use of cefazolin, ceftiofur, cefquinome (cephalosporins of the I, III, and IV generation) and ciprofloxacin (fluoroquinolone of the II generation) was recommended. The implementation of an antibiotic rotation system based on monitoring made it possible to temporarily reduce resistance levels in 2023. However, the subsequent return to previous treatment protocols in 2024 provoked a sharp increase in multi-drug resistance among bacterial mastitis pathogens. The obtained results confirm the need for continuous monitoring of antibiotic resistance, strict adherence to recommendations for the rotation of antimicrobials, and the integration of molecular genetic methods into the veterinary control system as a tool for tracking the occurrence of AMR genes in bacteria.

In 2022–2023, an increase in resistance of bacterial isolates to the disinfectants used on the dairy farm was identified. A veterinary medicinal product based on a polyvinylpyrrolidone-iodine complex was proposed as the disinfectant of choice for post-milking teat treatment. Control studies in 2024 confirmed the effectiveness of this measure: no resistance to the disinfectant was detected, justifying its continued use at the farm.

The results of the work are of practical importance for the veterinary service of the farm and can be used in the development of regional programs for AMR control in animal husbandry.

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Detection of *Listeria monocytogenes* while testing food raw materials and products of animal origin for microbiological contamination

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ABSTRACT

Introduction. *Listeria*-contaminated food remains an ongoing concern. Consumption of raw or undercooked animal-derived products contaminated with pathogenic *Listeria* results in human infection. The Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing (Rospotrebnadzor) documented 100 listeriosis cases in 2023, with 18 cases resulting in death. In recent years, there has been an increase in *Listeria* contamination of both domestically produced and imported food products. Thus, detection of pathogenic *Listeria* in the products of animal origin, food raw materials, and ready-to-eat products remains a critical task.

Objective. Detecting *Listeria monocytogenes* contamination in products of animal origin (meat, fish, dairy) manufactured and marketed in the Nizhny Novgorod Oblast from 2023 to 2024.

Materials and methods. The samples were analysed and pure microbial cultures were identified in accordance with GOST 32031-2022 "Food products. Methods for detection of *Listeria monocytogenes* and other *Listeria* (*Listeria* spp.)".

Results. Analysis and synthesis of the obtained data revealed that out of 3,650 tested samples, 57 (1.6%) were contaminated with *L. monocytogenes* bacteria. The highest number of contaminated samples was found among such product categories as combined semi-finished meat products, beef products, and poultry meat products. The incidence of *L. monocytogenes* in samples of fishery products was 1.1%. The highest levels of contamination were detected in the following products: minced beef (10.7%), poultry meat products wrapped in dough (9.3%), mechanically deboned poultry meat (7.1%), large-cut semi-finished products (4.6%), beef offal (4.3%), and chopped semi-finished poultry meat products (4.2%).

Conclusion. The test results show that the number of contaminated samples among combined semi-finished meat products was 4.3%, non-compliance with the safety requirements of beef products was detected in 3.7%; 2.8% of poultry product samples were contaminated with *L. monocytogenes* bacteria. The number and percentage of contaminated samples among frozen and refrigerated products did not significantly differ and amounted to 0.7 and 0.8%, respectively. *L. monocytogenes* were not detected in samples of dairy and ready-to-eat meat products that do not require heat treatment.

Keywords: listeriosis, *Listeria monocytogenes*, contamination, meat, milk, food products

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Индикация бактерий *Listeria monocytogenes* при оценке микробиологической контаминации сырья и продуктов животного происхождения

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РЕЗЮМЕ

Введение. Проблема контаминации пищевых продуктов листериями не теряет своей актуальности. Употребление в пищу обсемененных патогенными бактериями рода *Listeria* продуктов животного происхождения в сыром или недостаточно термически обработанном виде приводит к заражению человека. По данным Государственного доклада Роспотребнадзора, в 2023 г. в нашей стране было зарегистрировано 100 случаев листериоза, из которых

18 – с летальным исходом. В последние годы наблюдается рост контаминации листериями пищевых продуктов как отечественного, так и зарубежного производства. Таким образом, выявление патогенных видов *Listeria* в продуктах животного происхождения, пищевом сырье и готовых пищевых продуктах является актуальной задачей.

Цель исследования. Определение контаминации бактериями *Listeria monocytogenes* продуктов животного происхождения (мясных, рыбных, молочных), произведенных и реализуемых в Нижегородской области в период с 2023 по 2024 г.

Материалы и методы. Исследование проб, а также идентификацию чистой культуры микроорганизмов проводили в соответствии с ГОСТ 32031-2022 «Продукты пищевые. Методы выявления бактерий *Listeria monocytogenes* и других видов *Listeria* (*Listeria* spp.)».

Результаты. При анализе и обобщении полученных данных было показано, что из 3650 исследованных проб бактериями *L. monocytogenes* были контаминированы 57 образцов (1,6%). В таких категориях продуктов, как полуфабрикаты мясные смешанного состава, продукты из говядины и мяса птицы, было определено наибольшее количество контаминированных проб. При исследовании проб рыбных продуктов инцидентность *L. monocytogenes* составила 1,1%. Наибольший уровень контаминации отмечен в таких видах продуктов, как фарш говяжий (10,7%), полуфабрикаты из мяса птицы в тестовой оболочке (9,3%), мясо птицы механической обвалки (7,1%), полуфабрикаты крупнокусковые (4,6%) и субпродукты (4,3%) из говядины, полуфабрикаты из мяса птицы рубленые (4,2%).

Заключение. В результате испытаний было установлено, что количество контаминированных проб полуфабрикатов мясных смешанного состава достигло 4,3%, несоответствия требованиям безопасности продуктов из говядины выявлены в 3,7% случаев, 2,8% проб продуктов птицеводства были обсеменены бактериями *L. monocytogenes.* Количество и процентное соотношение контаминированных проб замороженных и охлажденных продуктов достоверно не различались и составили 0,7 и 0,8% соответственно. Бактерии *L. monocytogenes* не были выявлены в пробах молочных и готовых мясных продуктов, не требующих термической обработки.

Ключевые слова: листериоз, *Listeria monocytogenes*, контаминация, мясо, молоко, пищевые продукты

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Конфликт интересов: Прунтова О. В. является членом редколлегии журнала «Ветеринария сегодня» с 2012 г., но не имеет никакого отношения к решению опубликовать эту статью. Рукопись прошла принятую в журнале процедуру рецензирования. Об иных конфликтах интересов авторы не заявляли.

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INTRODUCTION

Listeriosis is an infectious disease affecting most farm and domestic species (pigs, horses, cattle, sheep, goats, rabbits, chickens, and ducks) as well as humans. The disease-related economic damage includes reduced animal productivity, expenses for medical treatment and prevention, and quarantine control measures. In Russia and other countries worldwide, the incidence of foodborne diseases, including listeriosis, shows no signs of decline [1, 2, 3]. Listeriosis ranks fifth among the most frequently reported zoonoses in humans in the European Union and is one of the most significant foodborne diseases [4]. Infection caused by *Listeria monocytogenes* is highly dangerous for pregnant women (causing miscarriages), infants (resulting in newborn fatalities) and immunocompromised individuals [5]. Listeriosis in pregnant women and their newborns have been reported in the Republic of Dagestan [6]. In 2021, a *Listeria* meningoencephalitis case was diagnosed in a COVID-19 patient in the Voronezh Oblast [7]. In 2022, a case of neonatal listeriosis was registered in the Tula Oblast [8].

In the Russian Federation, animal listeriosis has been documented since 1956, while human listeriosis was officially recognized as a distinct nosological phenomenon and included in health records by the Russian Ministry of Health in 1992 [9, 10]. Pursuant to the State Report of the Rospotrebnadzor, 100 cases of listeriosis (18 deaths) were registered in the Russian Federation in 2023. Most cases were reported: in Moscow (32 cases) and St. Petersburg (19 cases) [11].

A key feature of *Listeria* is its wide growth temperature range, i.e. from 4 to 45 °C (optimum 36–38 °C) and pH range of 5–11 [12]. It has been established that *L. monocytogenes* is killed when heated to 100 °C for 3–5 minutes or to 75–90 °C for 20 minutes [13]. *Listeria* demonstrates environmental persistence [14, 15], grows in high concentrations of sodium chloride and carbon dioxide, and can survive freezing and drying. They can survive in an oxygen-free environment and are capable of intracellular parasitism [16].

Listeria infection usually occurs when eating contaminated products of animal origin, including fish and seafood that have not been properly heat-treated, as well as vegetables and fruit [17]. A large number of listeriosis cases (15–20%) are associated with consumption of contaminated meat from domestic animals and poultry (15–80%) [18]. In Ethiopia, according to X. Wei et al., *L. monocytogenes* was detected in raw and pasteurized milk [19]. As the US Centers for Disease Control and Prevention (CDC) reports, there was a listeriosis human case in 2022 caused by cheese produced by Old Europe Cheese, Inc. In 2022, Big Olaf ice cream was the reason behind listeriosis outbreaks in the States of Florida and Ohio. In 2023, CDC reported listeriosis outbreaks associated with leafy greens, as well as peaches, nectarines, and plums [20]. In 2024, in multiple U. S. States, deli meats and liver sausage products under the Boar's Head brand were contaminated with *Listeria*, resulting in human disease and death cases [21, 22]. According to several studies, the infection may develop even at relatively low bacterial concentrations in food products (10^2 CFU/g) [23].

According to Food Safety News, in 2022 Federal Office of Public Health and Federal Office for Food Safety and Veterinary Affairs of Switzerland reported a listeriosis outbreak associated with smoked trout [24].

Thus, detection of pathogenic *Listeria* in the products of animal origin, food raw materials, and ready-to-eat products remains a critical task.

The objective of this research is to detect *L. monocytogenes* contamination in products of animal origin (meat, fish, dairy) manufactured and marketed in the Nizhny Novgorod Oblast from 2023 to 2024.

MATERIALS AND METHODS

Samples of animal products, including food raw materials and ready-to-eat food products were submitted to the testing laboratory of the Nizhny Novgorod Branch of Federal Centre for Animal Health for the required tests.

Test materials. Totally, 3,650 samples from livestock and aquaculture were analyzed. The samples were collected between 2023 and 2024 from commercial poultry farms and retail chains in the Nizhny Novgorod Oblast of the Russian Federation.

Sampling. Product samples were collected at different storage time points (within the shelf-life period) in accordance with established sampling requirements for microbiological testing. They were delivered to the laboratory in a cooler bag. The delivery time did not exceed an hour.

Culture media: Fraser broth (State Research Center for Applied Biotechnology and Microbiology, Russia); ALOA – Agar *Listeria* according to Ottaviani and Agosti (Merck, Germany); *Listeria* Identification Agar Base, PALCAM agar (HiMedia Laboratories Pvt Ltd., India); blood agar (Sredoff, Russia).

Methods. The samples were analyzed and the pure microbial culture was identified based on a set of morphological and biochemical characteristics confirming they belong to *L. monocytogenes*, in accordance with GOST 32031-2022 "Food products. Methods for detection of *Listeria monocytogenes* and other *Listeria (Listeria* spp.)"[1].

Preparations from pure microbial cultures were fixed, Gram-stained, examined microscopically, and identified based on their ability to grow at 25 °C, *β*-hemolysis, catalase production, Voges – Proskauer test, fermentation of xylose and rhamnose, and lecithinase activity. API *Listeria* identification system (bioMérieux, France), which includes 10 biochemical tests, was used for identification.

Automatic miniVidas analyzer and a Vidas *Listeria* test kit (bioMérieux, France) were used to detect *L. monocytogenes* in food samples.

[1] https://docs.cntd.ru/document/1200193714

For statistical data processing and graph construction, Microsoft Excel and standard statistical data analysis methods were used.

RESULTS AND DISCUSSION

Between 2023 and 2024, the testing laboratory of the Nizhny Novgorod Branch of Federal Centre for Animal Health tested 3,650 samples of animal-derived products for *L. monocytogenes,* i.e. 680 samples of dairy products, 615 samples of fishery products, and samples of 2,355 meat products. Non-heat treated meat products included 323 beef samples, 834 poultry meat samples, 326 combined semi-finished meat products, and 288 pork samples. Moreover 584 samples of finished meat products were also tested, including 187 samples of poultry meat and 397 samples of ready-to-eat meat products, except for poultry meat products. Ready-to-eat meat products included jellied products, sausages, pâtés, as well as heat-treated meat and meat-based convenience products, excluding sausages.

The conducted tests revealed that the bacterial isolates exhibited cell morphology characteristic of *Listeria*, stained Gram-positive, were catalase-positive, were Voges – Proskauer positive, motile at (25 ± 1) °C, fermented rhamnose but did not ferment xylose, showed lecithinase activity on charcoal agar, and formed a zone of *β*-hemolysis on blood agar. Thus, identification of pure bacterial cultures isolated from the contaminated samples of the tested products demonstrated that all cultures belonged to *L. monocytogenes* species.

The test results for animal products produced and marketed in the Nizhny Novgorod Oblast from 2023 to 2024 are given in Table 1.

In 2023, tests revealed 41 *L. monocytogenes*-contaminated samples, which accounted for 2.1% of all the tested samples (*N* = 1,970). The proportion of *Listeria* detected in frozen and chilled products did not differ statistically and amounted to 1.1 and 1.0%, respectively.

Table 1
Detection of *L. monocytogenes* in products of animal origin in the Nizhny Novgorod Oblast (from 2023 to 2024)

Food product	% of positive samples	
	2023	2024
Meat products requiring heat treatment, including:	4.0 (*N* = 910)	1.6 (*N* = 861)
beef products	5.1 (*N* = 175)	2.0 (*N* = 148)
poultry meat products	3.8 (*N* = 424)	1.7 (*N* = 410)
pork products	0 (*N* = 135)	0.6 (*N* = 153)
combined semi-finished meat products	6.3 (*N* = 176)	2.0 (*N* = 150)
Dairy products	0 (*N* = 316)	0 (*N* = 364)
Fish and fishery products	1.0 (*N* = 479)	1.5 (*N* = 136)
Ready-to-eat meat products	0 (*N* = 265)	0 (*N* = 319)
Total	2.1 (*N* = 1,970)	1.0 (*N* = 1,680)

N – number of samples tested.

Testing of food products produced in 2024 revealed 16 *L. monocytogenes*-contaminated samples, which accounted for 1.0% of all the samples tested (*N* = 1,680). In frozen and chilled products, this type of *Listeria* was detected in 0.4 and 0.6% of cases, respectively.

Figure 1 shows results for food samples tested in 2023. The maximum detection rate of *Listeria* was recorded in combined semi-finished meat products – 6.3%, beef products – 5.1% and poultry meat – 3.8%. The bacteria rate detected in fishery products was 1.0% of the total number of the tested samples in this category.

Figure 2 shows results for products of animal origin tested in 2024. It was established, that 2.0% of positive samples accounted for combined semi-finished meat products and beef products. *Listeria* detections in fishery products was 1.5% of the total number of such products tested; in poultry products *Listeria* was detected in 1.7%; the lowest percentage of detections was noted in pork products – 0.6%.

No *L. monocytogenes* were detected in dairy products and ready-to-eat meat products that we tested in 2023 and 2024. In 2023, no pathogenic microorganisms were detected in pork products either.

Table 2 gives data on *Listeria* detected in poultry meat products produced from 2023 to 2024. The maximum *L. monocytogenes* detection rate was reported in poultry products (*N* = 834), i.e. in chopped semi-finished poultry meat products (including minced meat) – 1.2%, and the minimum detection rate was reported in mechanically deboned poultry meat – 0.4%.

When testing poultry products, namely poultry meat (carcasses, half-carcasses, wing, leg, thigh), *L. monocytogenes* were detected in 1.6% of the tested samples of this product category (total number *N* = 385), and in frozen and chilled products the detection number was equal – 0.8% for each category.

Listeria contamination in the tested chopped semi-finished poultry meat products, such as cutlets, kupati (i.e. spicy Georgian sausages), minced meat, etc., amounted to 4.2% of the tested samples belonging to this product group (*N* = 236), while 0.4% of detections were reported in frozen products and 3.8% of detections were reported in chilled products.

Listeria was recorded in 9.3% of samples of dough-wrapped semi-finished poultry meat products (frozen dumplings, *N* = 43), and in 7.1% of tested samples of mechanically deboned poultry meat (only in frozen products, *N* = 42).

No *L. monocytogenes* were detected in samples of poultry offal (heart, stomach, liver, raw fat), skin, uncooked convenience semi-finished poultry meat products, and cut-style poultry meat semi-finished products (including the ones soaked in marinade).

Percentage of contaminated samples from all the beef products tested in 2023–2024 (*N* = 323) (by product type): large-cut semi-finished products – 1.9% (0.6% – frozen; 1.2% – chilled products), minced beef – 0.9% (chilled products), offal, small-cut semi-finished products and semi-finished products wrapped in dough – 0.3% each. No *L. monocytogenes* were detected in chopped semi-finished meat products (cutlets, meatballs and etc.). The relevant tests results are given in Table 3.

From 2023 to 2024 *L. monocytogenes* was found in the following types of combined semi-finished meat products (*N* = 326): in chopped semi-finished products – 2.76% (1.23% – in frozen and 1.53% – in chilled products), in minced meat (chilled only) –

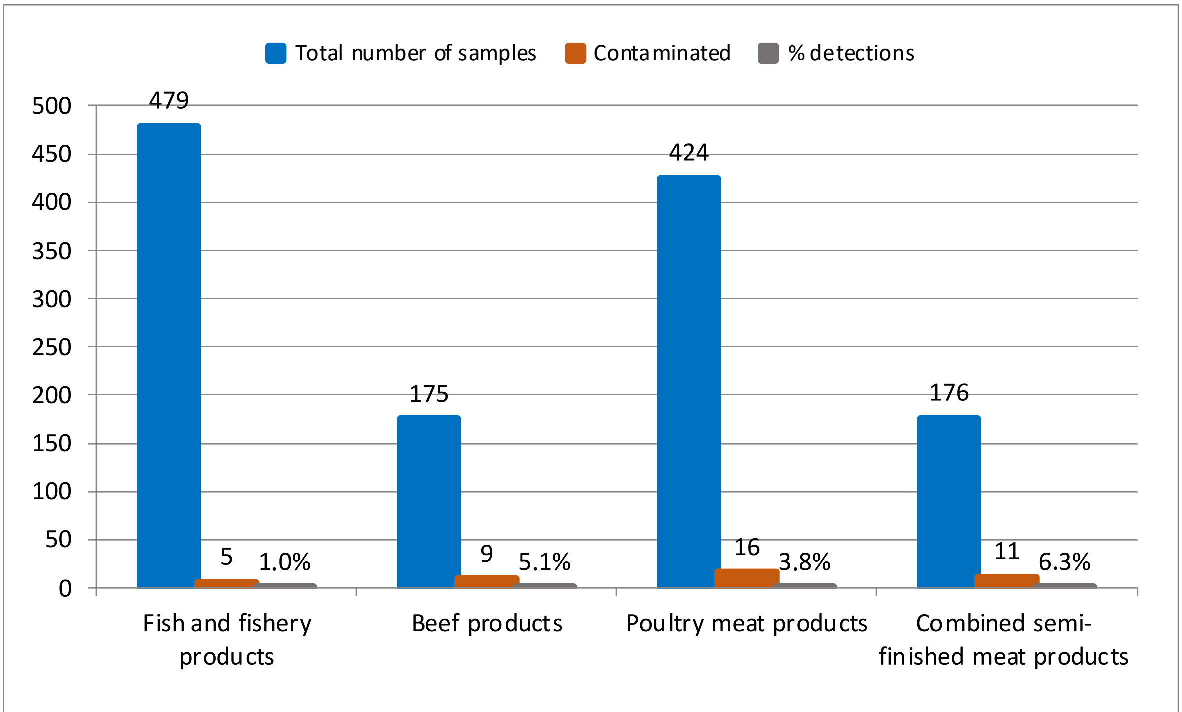

Fig. 1. Detection of L. monocytogenes in products of animal origin in the Nizhny Novgorod Oblast in 2023

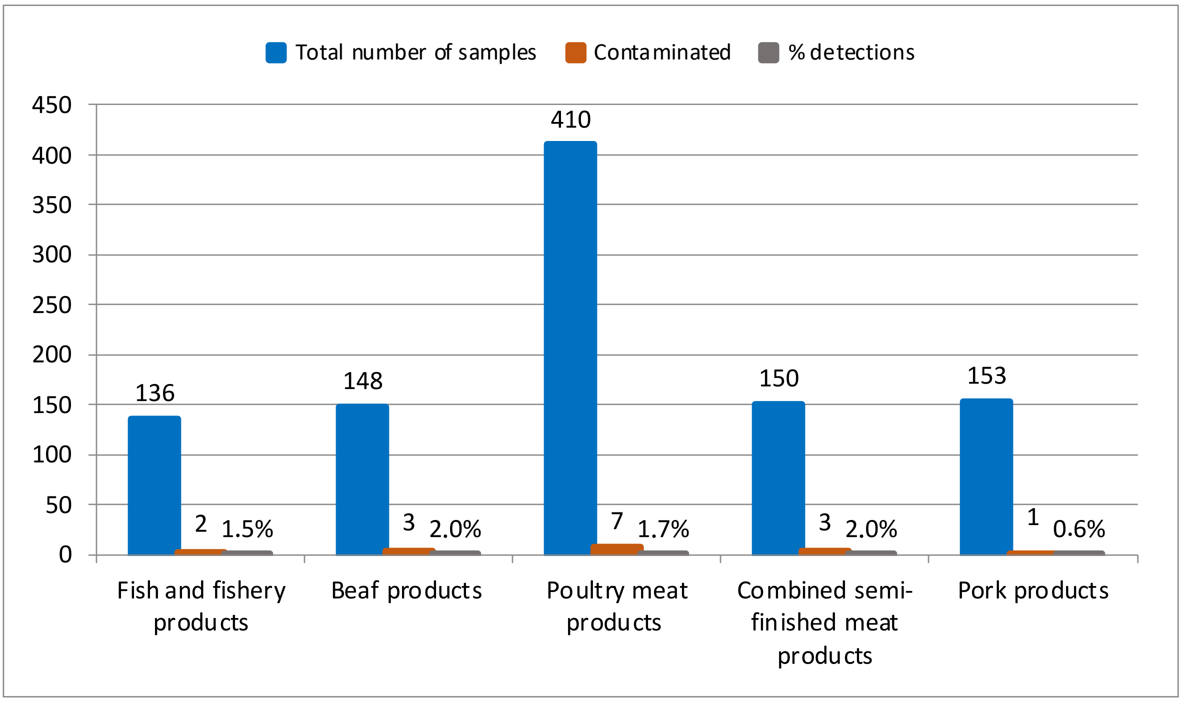

Fig. 2. Detection of L. monocytogenes in products of animal origin in the Nizhny Novgorod Oblast in 2024

in 0.6% of samples, in dough-wrapped semi-finished products – 0.9% (in frozen).

Listeria contamination was also detected in fishery products, namely in freshly frozen shrimps and semi-finished shrimp products containing a flour component (breaded cutlets).

Contamination with *L. monocytogenes* was found in one of the samples of frozen minced pork, which accounted for 0.3% of the total number of pork products samples (*N* = 288). The remaining pork processed products (large and small-cut semi-finished products, chopped products, products wrapped in dough, offal) met safety requirements and did not contain *Listeria*.

The work conducted between 2023 and 2024 resulted in detection of 57 *L. monocytogenes* contaminated samples, which accounted for 1.6% of the tested animal products. The percentage of *Listeria* detected in frozen and chilled meat products did not differ statistically – 0.7 and 0.8% of the total number of the samples tested, respectively. *Listeria* contamination in various food products ranged from 0.6% (samples of pork products) to 6.3% (samples of combined semi-finished meat products). *L. monocytogenes* detection in fishery products accounted for 1.1% of the total number of tested samples of these products (*N* = 615), in pork products – 0.3%.

Table 2
Contamination of poultry products with *L. monocytogenes*

Types of products	Number of samples	Contaminated	*Listeria* detected (%) in this type of products	*Listeria* detected (%) in the total number of poultry products
Poultry meat (carcasses, parts of carcasses)	385	6	1.6	0.7
Chopped semi-finished poultry meat products (including minced meat)	236	10	4.2	1.2
Semi-finished poultry meat products wrapped in dough	43	4	9.3	0.5
Mechanically deboned poultry meat	42	3	7.1	0.4
Offal	74	0	0	0
Skin	11	0	0	0
Convenience semi-finished poultry meat products, partially cooked	19	0	0	0
Cut-style semi-finished poultry meat products (soaked in marinade)	24	0	0	0

Table 3
L. monocytogenes contamination of beef products

Types of products	Number of samples	Contaminated	*Listeria* detected (%) in this type of products	*Listeria* detected (%) in the total number of beef products
Ground beef	28	3	10.7	0.9
Large-cut semi-finished products	130	6	4.6	1.9
Chopped semi-finished meat products	26	0	0	0
Offal	23	1	4.3	0.3
Small-cut semi-finished meat products	74	1	1.4	0.3
Dough-wrapped semi-finished products	42	1	2.4	0.3

The results we obtained align with the published scientific data from other researchers. Thus, according to the results of food monitoring in 14 EU countries, the total number of *L. monocytogenes* detections in beef products intended for human consumption in 2019 was 4.2%, in 2020 the number increased to 7.4%, and in 2021, decreased to 3.9% [4]. According to the foreign data, in 2023, number of samples contaminated with *L. monocytogenes* in category "fish" was 1.1% [3], which correlates with the results of our tests.

As foreign sources report, *L. monocytogenes* has been detected in milk, different types of cheese, butter, cream, and ice cream [4, 20, 25, 26], as well as in ready-to-eat meat products [21, 22, 27]. Absence of *L. monocytogenes* in ready-to-eat meat and dairy products, as confirmed in tests conducted in the Nizhny Novgorod Branch of Federal Centre for Animal Health, may suggest that the establishments where these food products have been produced comply with technological and sanitary standards, the products are properly heat-treated, production hygiene is maintained, quality control of raw materials is well-organized, an effective quality control system is in place, product storage and transportation of conditions meet relevant regulatory requirements. However, regardless of the good current results, continuous monitoring of products shall be in place.

The data obtained indicate that contamination of meat products with *L. monocytogenes* does not depend on their storage temperature (frozen or chilled). This confirms the cold stress adaptation mechanisms of this pathogen described by foreign researchers [28, 29, 30]

CONCLUSION

Tests of products of animal origin manufactured and sold in the Nizhny Novgorod Oblast conduceted in 2023–2024 show that 1.6% of samples were *L. monocytogenes*-contaminated. The highest level of contamination was found in combined semi-finished meat products (6.3% in 2023 and 2.0% in 2024), as well as in beef products (5.1 and 2.0%, respectively) and poultry meat (3.8 and 1.7%). At the same time, no *L. monocytogenes* was detected in dairy products and ready-to-eat meat products, which may indicate compliance of production with technological and sanitary standards.

As for poultry meat products, the highest level of contamination was detected in semi-finished poultry meat products wrapped in dough (9.3%), in mechanically deboned poultry meat samples (7.1%) and chopped products (4.2%), while in whole poultry carcasses the level was lower (1.6%). Among beef products were minced beef (10.7%) and large-cut semi-finished beef products (4.6%). As for fishery products, *L. monocytogenes* was found in 1.1% (in shrimps and fish cutlets).

Storage temperature (refrigeration/freezing) did not have a significant impact on the level of *L. monocytogenes* contamination, indicating the cold tolerance of this pathogenic microorganism.

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Study of microbial species composition in the production environment of livestock facilities

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ABSTRACT

Introduction. Livestock facilities serve as a reservoir for microorganisms of various families and genera, including both opportunistic and pathogenic microorganisms. Continuous microbiological monitoring of the production environment in livestock facilities, along with the detection and identification of microorganisms, allow for the microflora control in these facilities, thereby preventing the risks of infectious diseases and ensuring timely implementation of appropriate veterinary, sanitary, and zoohygienic measures.

Objective. Study of microbial species composition in the production environment of livestock facilities including contamination level and classification of the isolated mircoorganisms by families and disinfectant-resistant groups.

Materials and methods. Swabs from the surfaces in the production facilities for cattle (namely, dairy cow facility, calf facility, calving area, and milking hall) on the cattle farm located in the Omsk Oblast were taken for study of microbial species composition. The microorganisms were classified using MMT E24 и MMT S multi-biochemical microtests and selective nutrient medium.

Results. Tests showed that the microflora circulating in cattle facilities included both pathogenic and opportunistic microorganisms of the following species: *Escherichia coli, Proteus mirabilis, Proteus vulgaris, Klebsiella aerogenes, Citrobacter freundii, Morganella morganii, Hafnia alvei, Klebsiella ozaenae, Enterococcus faecalis, Bacillus cereus, Staphylococcus sciuri, Staphylococcus capitis, Staphylococcus simulans, Staphylococcus intermedius* and *Staphylococcus lentus.*

Conclusion. The recovered microorganisms belonged to the families *Enterobacteriaceae, Bacillaceae* and *Staphylococcaceae* and to the following disinfectant-resistant groups: low-resistant, moderately-resistant and highly-resistant. The highest microbial load was detected on floor, walls and stall dividers in the facility for dairy cows and in milking hall, the detected microorganisms demonstrated high species diversity. The lowest microbial load was detected in calving area and calf facility.

Keywords: microorganisms, microbiological load, production environment

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Изучение видового состава микроорганизмов производственной среды животноводческих помещений

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РЕЗЮМЕ

Введение. Производственные объекты животноводческих комплексов являются резервуаром микроорганизмов различных семейств и родов, среди которых есть как условно-патогенные, так и патогенные представители. Постоянный микробиологический мониторинг производственной среды животноводческих помещений, индикация и идентификация микроорганизмов дает возможность контролировать микрофлору данных помещений, тем самым предотвращать риски возникновения инфекционных заболеваний и своевременно проводить качественные ветеринарно-санитарные и зоогигиенические мероприятия.

Цель исследования. Изучение видового состава микроорганизмов производственной среды животноводческих помещений, уровня контаминации и классификация выделенной микрофлоры по семействам и группам устойчивости к дезинфицирующим препаратам.

Материалы и методы. Для изучения видового состава микрофлоры были взяты смывы с поверхностей в производственных помещениях для содержания крупного рогатого скота (коровник – дойное стадо, телятник, родильное отделение и доильный зал), расположенных в животноводческом хозяйстве Омской области. Идентификацию микроорганизмов проводили с использованием биохимических мультимикротестов ММТ Е24 и ММТ С и селективной питательной среды.

Результаты. В результате проведенных исследований установлено, что микрофлору, циркулирующую в помещениях для содержания крупного рогатого скота, составляют как патогенные, так и условно-патогенные микроорганизмы, которые представлены следующими видами: *Escherichia coli, Proteus mirabilis, Proteus vulgaris, Klebsiella aerogenes, Citrobacter freundii, Morganella morganii, Hafnia alvei, Klebsiella ozaenae, Enterococcus faecalis, Bacillus cereus, Staphylococcus sciuri, Staphylococcus capitis, Staphylococcus simulans, Staphylococcus intermedius* и *Staphylococcus lentus.*

Заключение. Выделенные микроорганизмы представлены семействами *Enterobacteriaceae, Bacillaceae* и *Staphylococcaceae* и принадлежат к следующим группам устойчивости к дезинфектантам: малоустойчивые, устойчивые и особо устойчивые. Наиболее высокая микробиологическая нагрузка наблюдалась на таких объектах, как пол, стены и ограждения в стойлах, расположенных в коровнике (дойное стадо) и доильном зале, микрофлора характеризовалась большим видовым разнообразием микроорганизмов, низкий уровень микробной диссеминации установлен в помещениях родильного отделения и телятника.

Ключевые слова: микроорганизмы, микробиологическая нагрузка, производственная среда

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INTRODUCTION

Modern large-scale animal farming is characterized by a high concentration of cattle in specialized livestock establishments. The animal farming industrialization and its transfer to a large-scale mass production imply a profound qualitative restructuring of all technological processes. Under such intensive farming conditions, biological agents accumulate at various production facilities of livestock establishments. This leads to the emergence of mass dysbiosis in animals and, as a result, to increased number of infectious diseases [1, 2, 3].

The production facilities of livestock establishments serve a reservoir of microorganisms of various families and genera, including both opportunistic and pathogenic microorganisms. Under prolonged exposure to high humidity, various microflora contaminates the building structures of livestock establishments, thereby increasing the risk of infectious diseases [4, 5, 6, 7].

Infectious diseases of farmed animals are responsible for significant losses to the livestock industry. Poor veterinary and sanitary practices on farms of various levels is the one of the main causes for infectious disease occurrence. All these often provoke the infectious gastrointestinal, respiratory and other pathologies caused by both pathogenic and opportunistic microflora (cocci, proteus, klebsiella, etc.), which virulence increases when the animal resistance weakens due to adverse factors related to feeding, care and housing condition violations [8, 9, 10].

Veterinarians have to take into account the whole range of animal habitat factors that have changed significantly due to technological progress in order to create an optimal environment. However, under modern conditions, veterinarian's attention is focused on the animal, its health and performance, as well as on protection of the environment from various contaminants associated with the large-scale livestock establishment activities. The strict observance of veterinary containment and security measures plays a crucial role in the livestock establishments. The high density of facilities and animals concentrated in a limited area requires strict measures to protect establishments from the introduction of infectious diseases [11, 12, 13].

A poorly maintained production environment is a major obstacle to effective infectious disease control. This risk extends beyond highly dangerous pathogens to include opportunistic microbes, which can turn pathogenic under suitable conditions and cause significant damage. A significant number of microorganisms are shed by animals during the physiological acts: coughing, sneezing, defecation, urination. The production environment of livestock facilities, where pathogenic and opportunistic microorganisms are shed, is typically not their natural habitat. There are often no favourable living conditions here: nutrients, optimal temperature and pH of the environment. However, in facilities containing large quantities of organic matter, such microorganisms can maintain their viability but also pathogenicity for long periods. They are detected on the surfaces of livestock buildings, vehicles, in manure, animal-origin raw materials, and many other objects. The level of production facility contamination depends mainly on the presence of infectious diseases in animals. Diseased animals constantly shed pathogens into the production environment. Pathogens become to further spread from inadequately decontaminated surfaces within the facility. One of the persistent causes of microbial contamination in the production environment is carrier animals. These animals pose even greater risk of pathogenic microflora spreading and the disease maintenance within the establishment than apparently diseased animals, since the latter can be isolated until their recovery [14, 15, 16, 17].

Animals shedding pathogenic and opportunistic microorganisms with faeces and airborne droplets are the main source of livestock production facility contamination, and the more intensely the environment is contaminated with secretions, the higher the probability of contamination of objects with the relevant pathogens. For many microorganism species, the intestine is a biotope, that is, their only habitat. Consequently, the detection of intestinal microflora in the tested material (water, feed, samples from livestock facility surfaces, etc.) serves as a direct indicator of faecal contamination of the object and possible presence of pathogens of intestinal infections (salmonellosis, yersiniosis, etc.) [18, 19, 20].

Many microorganisms circulating in livestock facilities naturally possess resistance mechanisms rooted in their cellular structure and metabolism. These include a multilayer cell wall, biofilm formation, enzymatic breakdown or active xenobiotic efflux pumps. Bacterial spores possess a unique cell membrane that enables them to withstand biocide concentrations thousands of times higher than those effective against vegetative cells. Spore dense coating membrane prevents the penetration of biocide into the cell and neutralizes the effect of those that do breach its barrier. The coating membrane accounts for up to 50% of the spore dry mass. All these features provide spores with the resistance to environmental factors, including biocides. Mycobacteria are also highly resistant to many biocides, resistant to acids, alkalis, chlorhexidine, quaternary ammonium compounds, heavy metals and dyes. Mycobacteria are able to form biofilms (for example, in water supply systems), which are more difficult to remove than enterobacterium biofilms [21, 22, 23].

Biofilm formation is one of the manifestations of bacterial survival strategy, conferring resistance to adverse factors, including biocides. Biofilm is a microbial community, often multispecies, embedded within a self-produced extracellular polymeric matrix (glycocalyx) that acts as a protective barrier against external factors. Increased resistance to biocides has been found in the following biofilm-growing species of microorganisms: *Pseudomonas*, *Burkholderia cepacia*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Legionella pneumophila*, *Salmonella typhimurium*, and *Yersinia enterocolitica* [24, 25, 26].

Numerous highly effective broad-spectrum antibiotics are widely applied in veterinary practice. They are highly effective when used for respiratory and gastrointestinal infection prevention and treatment. However, prolonged and uncontrolled use of antibiotics leads to the emergence of a significant number of resistant microorganism strains [27, 28].

The significance and innovation of this work lie in comprehensive analysis of the microbial species composition and levels of production environment contamination in livestock facilities as well as classification of the isolated microorganisms by families and disinfectant-resistant groups that enables implementation of proper and prompt veterinary-sanitary measures, such as cleaning and disinfection, for prevention of infectious disease risks.

The study was aimed at examination of microorganism species composition in the production environment of livestock facilities, and contamination level and classification of the isolated microflora by families and disinfectant-resistant groups.

MATERIALS AND METHODS

Tested materials: 184 samples taken before cleaning and disinfection from various surfaces in 4 facilities of the livestock establishment located in the Omsk Oblast.

General zoo-technical characteristics of the establishment. The establishment is surrounded by 500-meter sanitary-protective zone and located apart of other livestock/agricultural establishments and facilities including backyards. The main economic activity types are legumes cultivation and dairy cattle farming, breeding, raw milk production. The establishment has the status of a breeding farm for breeding Red Steppe dairy cattle. The dairy cow facility is a reconstructed standard tie-stall cattle facility for 200 animals. There is a "Parallel" automated milking parlour (commissioned in 2020) for 24 animals, S = 420 m^2 in the establishment. The parlour is divided into three parts: a milking hall and cow facilities on milking hall both sides.

The establishment is free from acute infectious diseases. Diagnostic tests for infectious diseases such as tuberculosis, brucellosis, leucosis, hypodermatosis, chlamydia, leptospirosis are carried out according to the plan of anti-epizootic preventive measures. Animals are vaccinated against anthrax, blackleg, brucellosis, pasteurellosis, enterococcal infection, colibacillosis, salmonellosis, klebsiellosis and proteus infection, ringworm and treated against hypodermatosis.

Annual plan comprising organizational, zootechnical, and veterinary measures for leucosis prevention is developed. Key strategies include the isolated rearing of replacement heifers and selection of heifers based on their family history.

Regular disinfection of all livestock facilities is carried out. The establishment is fully fenced, there is a sanitation checkpoint at its entrance, and there are disinfection barriers at the entrances to the cow facilities and calf facilities.

Tested facilities. Dairy cow facility (48 samples from 6 sites): stall floor (rubber covering), stall walls, the walls at the entrance, wooden window frames, stall fences, wooden door to the cow facility. Calf facility (48 samples from 6 sites): the floor inside the cages (straw), stall walls, walls at the entrance, plastic window frames, fences of cages for calves, door to the calf facility. Calving facility (48 samples from 6 sites): stall floor (rubber covering), stall walls, the walls at the entrance, wooden window frames, partitions in the stalls, door to the calving facility. "Parallel" milking parlour (40 samples from 5 sites): milking hall floor (rubber covering), tiled walls, plastic window frames, milking machines, fences of the milking plant.

The samples were taken at relative humidity of 81% in the cow facility, 72% in the calf facility, and 74% in calving facility; indoor temperature was (24 ± 2) °C; the calving facility was equipped with automated ventilation system.

Samples were taken by swabbing the surfaces of various objects according to the methodological guidelines MG 4.2.0220-20[1]. Sterile swab was moistened by dipping it into the Amies transport medium immediately before swabbing. A document including data required for unambiguous identification of the object, sampling place, reason and conditions, date and time, conditions and time periods of sample delivery to the diagnostic laboratory was drawn up.

Biochemical multimicrotests: MMT E24 and MMT C (NPO Immunotex, Russia), were used for *identification of microorganisms* belonging to the *Enterobacteriaceae* and *Staphylococcaceae* family, respectively. These

[1] MG 4.2.0220-20 Methods of sanitary and bacteriological testing of environmental objects for microbial contamination: methodological guidelines (approved by the Federal Service for Supervision of Consumer Rights Protection and Human Welfare on 4 December 2020). https://docs.cntd.ru/document/573595605?ysclid=mguk1xg4sw975021985 (in Russ.)

Table 1
Results of study of species composition of the microorganisms circulating in the cattle facility (dairy herd), *n* = 93

Microorganisms	Tested surfaces					
	Floor (rubber)	Stall walls	Walls at the entrance	Windows (wooden)	Stall fences	Door to the cow facility
Positive samples, %						
Enterobacteriaceae family microorganisms						
E. coli	100.0	87.5	0.0	0.0	62.5	0.0
P. mirabilis	75.0	87.5	0.0	0.0	0.0	0.0
K. aerogenes	75.0	0.0	0.0	62.5	62.5	0.0
C. freundii	62.5	75.0	62.5	0.0	0.0	0.0
M. morganii	62.5	75.0	0.0	0.0	0.0	0.0
E. faecalis	100.0	62.5	62.5	0.0	62.5	0.0
Bacillaceae family microorganisms						
B. cereus	0.0	75.0	0.0	0.0	0.0	0.0
Staphylococcaceae family microorganisms						
S. capitis	0.0	0.0	0.0	0.0	62.5	0.0
S. sciuri	0.0	75.0	0.0	37.5	0.0	62.5
S. simulans	0.0	0.0	0.0	0.0	62.5	0.0

multimicrotests are designed to determine the biochemical activity of enterobacteria and staphylococci during bacteriological analysis and their species identification and are based on the determination of these microorganisms' enzyme systems reacting to the corresponding substrates. *Bacillaceae* family microorganisms were identified using Donovan's selective nutrient medium containing lithium chloride (selective agent). The tests were carried out at the Diagnostic Research and Biotechnology Laboratory of the Omsk Agrarian Scientific Center.

The test results were statistically processed using the Microsoft Excel software.

RESULTS AND DISCUSSION

Test results showed that the microflora in dairy cow facility rooms includes various microorganisms. Thus, stall floors, walls and stall fences are particularly prone to significant microbial contamination. For 48 swab samples tested, the following types of microorganisms were detected in 122 cases, *n* = 93 (Table 1): swabs from the floor – *E. coli* and *E. faecalis* (100.0% of swabs), *Proteus mirabilis* and *Klebsiella aerogenes* (75.0% of swabs), *Citrobacter freundii* and *Morganella morganii* (62.5% of swabs); swabs from the walls – *E. coli* and *P. mirabilis* (87.5% of swabs), *C. freundii*, *M. morganii*, *Bacillus cereus*, and *Staphylococcus sciuri* (75.0% of swabs), *E. faecalis* (62.5% of swabs); swabs from stall fences – *E. coli*, *K. aerogenes*, *E. faecalis*, *Staphylococcus capitis* and *Staphylococcus simulans* (62.5% of swabs).

Tests revealed low microbial contamination of window frame surfaces: *K. aerogenes* (62.5% of swabs) and *S. sciuri* (37.5% of swabs); door to the cow facility: *S. sciuri* (62.5% of swabs); as well as in samples taken from the walls at the entrance: *C. freundii* and *E. faecalis* (62.5% of swabs).

For 48 swab samples collected in calf facility the following heavily contaminating microorganisms were isolated in 54 cases (*n* = 49): in swabs from floor (*Hafnia alvei* – 100.0%, *C. freundii* – 75.0% and *E. faecalis* – 75.0% of swabs), fences of cages for calves (*E. faecalis* – 87.5% and *C. freundii* – 37.5% of swabs), stall walls (*C. freundii* – 62.5% of swabs). *Staphylococcus lentus* was detected in 75.0% of swab samples from window frames, walls at the entrance and door to the calf facility (Table 2).

For 48 swab samples collected in calving facility, microorganisms were detected in 52 cases (*n* = 46). A high microbial load was detected in the samples collected from floor surface (*Klebsiella ozaenae* – 87.5%, *H. alvei* – 87.5%, *P. mirabilis* – 75.0% of swabs) and from stall walls (*Staphylococcus intermedius* – 87.5%, *H. alvei* – 62.5% and *P. mirabilis* – 62.5% of swabs).

Table 2
Results of study of species composition of the microorganisms circulating in the calf facility (up to 6 months), *n* = 49

Microorganisms	Tested surfaces					
	Floor (straw)	Stall walls	Walls at the entrance	Windows (plastic)	Cage partitions	The door to the calf facility
Positive samples, %						
Enterobacteriaceae family microorganisms						
H. alvei	100.0	0.0	0.0	0.0	0.0	0.0
C. freundii	75.0	62.5	0.0	0.0	37.5	0.0
E. faecalis	75.0	0.0	0.0	0.0	87.5	0.0
Staphylococcaceae family microorganisms						
S. lentus	0.0	0.0	75.0	75.0	0.0	75.0

Table 3
Results of study of species composition of the microorganisms circulating in the calving facility, *n* = 46

Microorganisms	Tested surfaces					
	Floor (rubber)	Stall walls	Walls at the entrance	Windows (wooden)	Partitions	Door to the calving facility
Positive samples, %						
Enterobacteriaceae family microorganisms						
K. ozaenae	87.5	0.0	0.0	0.0	25.0	0.0
H. alvei	87.5	62.5	0.0	0.0	0.0	0.0
P. mirabilis	75.0	62.5	37.5	0.0	0.0	0.0
Staphylococcaceae family microorganisms						
S. intermedius	0.0	87.5	0.0	87.5	0.0	37.5

A low microbial load was detected in swab samples taken from window frames and doors in the calving facility – *S. intermedius* (87.5% and 37.5% of swabs, respectively). *K. ozaenae* was detected in 25.0% of swabs collected from fences, *P. mirabilis* was detected in 37.5% of swabs taken from the walls at the entrance (Table 3).

For 40 samples collected in the milking hall, microflora characterized by a wide variety of microorganisms was detected, in 84 cases (*n* = 69). *E. coli*, *Proteus vulgaris* and *S. simulans* were detected in 87.5% of swab samples from floor, *H. alvei*, *C. freundii*, *M. morganii*, and *E. faecalis* were detected in 75.0% of swab samples; *E. coli* (62.5%), *H. alvei*, *M. morganii*, *E. faecalis*, and *S. intermedius* were detected in 37.5% of swab samples from the milking plant fences (Table 4).

The microbial contamination of walls, windows and milking machines was low. *S. sciuri* was detected in 75.0% and *C. freundii* was detected in 62.5% of swab samples taken from wall surfaces; *S. simulans* and *M. morganii* were detected in 62.5% and 37.5% of swab samples taken from window surfaces, respectively, and *S. sciuri* was detected in 37.5% of swab samples collected from milking machines.

The isolated microorganisms belong to the following disinfectant-resistant groups: low resistant group: *E. coli*, *P. mirabilis*, *P. vulgaris*, *K. aerogenes*, *C. freundii*, *M. morganii*, *H. alvei*, *K. ozaenae* and *E. faecalis*; moderately resistant group: *S. capitis*, *S. simulans*, *S. intermedius*, *S. sciuri* and *S. lentus*; highly resistant group: *B. cereus*.

CONCLUSION

The study results allow us to conclude that the microflora in the cattle facilities included both pathogenic and opportunistic microorganisms belonging to the *Enterobacteriaceae*, *Bacillaceae* and *Staphylococcaceae* families. The members of the first of them were: *E. coli* (causative agent of colibacillosis in young livestock animals), *P. mirabilis* (causes purulent-inflammatory processes in wounds), *P. vulgaris* (causes feed-borne toxic infections, purulent-

Table 4
Results of study of species composition of the microorganisms circulating in the milking hall, *n* = 69

Microorganisms	Tested surfaces				
	Floor (rubber)	Walls (glossy tiles)	Windows (plastic)	Milking machines (inner surface)	Fences of the milking plant (duralumin)
Positive samples, %					
Enterobacteriaceae family microorganisms					
E. coli	87.5	0.0	0.0	0.0	62.5
P. vulgaris	87.5	0.0	0.0	0.0	0.0
H. alvei	75.0	0.0	0.0	0.0	37.5
C. freundii	75.0	62.5	0.0	0.0	0.0
M. morganii	75.0	0.0	37.5	0.0	37.5
E. faecalis	75.0	0.0	0.0	0.0	37.5
Staphylococcaceae family microorganisms					
S. intermedius	0.0	0.0	0.0	0.0	37.5
S. sciuri	0.0	75.0	0.0	37.5	0.0
S. simulans	87.5	0.0	62.5	0.0	0.0

inflammatory processes in wounds, enteritis, peritonitis and sepsis), *K. aerogenes* (causative agent of opportunistic infections), *C. freundii* (causative agent of infectious urinary, respiratory and circulatory diseases), *M. morganii* (urinary tract infections), *H. alvei* (urinary tract infections, pneumonia, sepsis), *K. ozaenae* (respiratory tract infections), *E. faecalis* (urinary tract infections, endocarditis, and gastrointestinal infection). *B. cereus* belonging to *Bacillaceae* family and causing gastrointestinal infections was detected in samples collected in production facilities. The following pathogenic microorganisms belonging to *Staphylococcaceae* family were detected: *S. sciuri* (responsible for urinary and circulatory infections, endocarditis), *S. capitis* (causative agent of infectious meningitis, osteomyelitis, endocarditis), *S. simulans* (bacteraemia, endocarditis), *S. intermedius* (causative agent of mastitis, skin infections), *S. lentus* (responsible for abscess, sepsis).

The data on the microbial load in the production environment of livestock facilities allowed us to identify the places of highest bacterial contamination. The highest microbial load was detected on floor, walls and stall partitions in dairy cow facility as well as floor and milking machine fences located in milking hall. The detected microorganisms demonstrated high species diversity. The lowest microbial load was detected in calving facility and calf facility where small number of animals are kept.

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