FGBI "FEDERAL CENTRE FOR ANIMAL HEALTH" (FGBI "ARRIAH")

FEDERAL SERVICE FOR VETERINARY AND PHYTOSANITARY SURVEILLANCE (ROSSELKHOZNADZOR)

ISSN 2304-196X (Print)
ISSN 2658-6959 (Online)

VETERINARY SCIENCE TODAY

SCIENTIFIC JOURNAL

ВЕТЕРИНАРИЯ СЕГОДНЯ

НАУЧНЫЙ ЖУРНАЛ

JUNE | ИЮНЬ VOL. 14 No. 2 2025

veterinary.arriah.ru/jour
DOI 10.29326/2304-196X

PORCINE DISEASES

AIMS AND SCOPE

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FEDERAL SERVICE FOR VETERINARY
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VETERINARY SCIENCE TODAY

Veterinariia segodnia

FREQUENCY: 4 times a year

JUNE VOLUME 14 No. 2 2025
Published since 2012

ВЕТЕРИНАРИЯ СЕГОДНЯ

ПЕРИОДИЧНОСТЬ: 4 раза в год

ИЮНЬ ТОМ 14 № 2 2025
Основан в 2012 г.

Scientific Journal "Veterinary Science Today" is included in the scientometric system – Russian Science Citation Index (RSCI), Directory of Open Access Journals (DOAJ), as well as in the RSCI database.

Full-text e-versions of the Journal are published on the website of the scientific electronic library eLIBRARY.RU, DOAJ, and https://veterinary.arriah.ru/jour

Научный журнал «Ветеринария сегодня» входит в «Перечень рецензируемых научных изданий, в которых должны быть опубликованы основные научные результаты диссертаций на соискание ученой степени кандидата и доктора наук» по научным специальностям:

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Электронные версии журнала размещаются в полнотекстовом формате на сайте Научной электронной библиотеки (НЭБ) eLIBRARY.RU, в каталоге DOAJ и по адресу https://veterinary.arriah.ru/jour

Veterinary Science Today. 2025, Vol. 14, No. 2
Veterinariia segodnia

Published 4 times a year since 2012

Design and composition: Maria Bondar
Managing Editor: Elena Guseva
Coordinating Editor: Iana Vlasova
Content editors:
Julia Nurmukhambetova-Mikhailova, Maria Ryaguzova
Proof-reader: Karina Tulaeva
The Journal "Veterinary Science Today" is registered in the Federal Service for Supervision of Communications, Information Technology, and Mass Media Federal Service, Registration Certificate No FS 77-49033, March 21, 2012.

Circulation: 1175. Price: unregulated
Veterinary Science Today Journal can be subscribed through the Ural-Press subscription agency: Subscription code – 83862; 127015, Moscow, Novodmitrovskaya str., 5a, str. 4; +7 (499) 700-05-07, fax: 789-86-36 add. 3777; e-mail: moscow@ural-press.ru

Founder: 600901, Vladimir, Yur'evets, Federal Centre for Animal Health
Publisher: Veinard, 129626, Moscow, 102 Prospect Mira, bld. 31, office 12
Editorial Staff Office: 600901, Vladimir, Yur'evets, Federal Centre for Animal Health
Printing Office: Grand Prix, 152900, Yaroslavl Oblast, Rybinsk, Lugovaya str., 7
Approved for print: June 11, 2025
Issued: June 30, 2025

16+

Главный редактор: Груздев Константин Николаевич – д-р биол. наук, профессор, ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *https://orcid.org/0000-0003-3159-1969; AuthorID: 304722; Scopus Author ID: 6506731135; e-mail: gruzdev@arriah.ru*; тел.: 8 (4922) 45-37-96

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Фото на обложке: dusanpetkovic / GettyImages

Редакционная коллегия:

Дизайн и верстка: Бондарь Мария
Ответственный редактор: Гусева Елена
Редактор-координатор: Власова Яна
Редакторы-корректоры: Нурмухамбетова-Михайлова Юлия, Рягузова Мария
Корректор: Тулаева Карина
Журнал «Ветеринария сегодня» зарегистрирован в Федеральной службе по надзору в сфере связи, информационных технологий и массовых коммуникаций, свидетельство о регистрации № ФС 77-49033 от 21 марта 2012 г.

Тираж 1175 экземпляров. Цена свободная
Подписку на научный журнал «Ветеринария сегодня» можно оформить через Агентство по подписке ООО «УРАЛ-Пресс Стандарт»: Подписной индекс – 83862; 127015, г. Москва, Новодмитровская ул., дом 5а, строение 4; 8 (499) 700-05-07, факс: 789-86-36 доб. 3777; e-mail: moscow@ural-press.ru

Учредитель: 600901, г. Владимир, мкр. Юрьевец, ФГБУ «ВНИИЗЖ»
Издатель: ООО «Вейнард», 129626, г. Москва, проспект Мира, д. 102, стр. 31, комн. 12
Адрес редакции: 600901, г. Владимир, мкр. Юрьевец, ФГБУ «ВНИИЗЖ»
Типография: ООО «ГРАН ПРИ», 152900, Ярославская область, г. Рыбинск, ул. Луговая, 7
Подписано в печать: 11 июня 2025 года
Дата выхода в свет: 30 июня 2025 года

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Содержание

https://doi.org/10.29326/2304-196X-2025-14-2-114-122

Modern approaches to diagnosis and prevention of porcine reproductive and respiratory syndrome (review)

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ABSTRACT

Introduction. Porcine reproductive and respiratory syndrome (PRRS), caused by a virus from the family *Arteriviridae*, is one of the most economically significant porcine diseases in many countries. The disease is mainly manifested by reproductive disorders in sows, i.e. abortions in late pregnancy, early or delayed farrowing, birth of weak or non-viable piglets, irregular estrus; pathologies in early and middle pregnancy are less often reported. Piglets and fattening pigs have respiratory distress syndrome: coughing, sneezing, dyspnea and stunted growth. In addition, infection with PRRS virus undermines respiratory immunity, which makes the infected pigs more susceptible to secondary infections and increases mortality in the herd. This review provides up-to-date information on the current laboratory diagnostic tools and recent data on specific PRRS prevention and gives information on the promising biotechnological platforms that can be used to design new-generation vaccines.

Objective. To consider and summarize modern approaches to diagnosis and prevention of porcine reproductive and respiratory syndrome.

Materials and methods. Scientific publications of foreign and domestic authors served as the material for the research.

Results. The paper presents nosological characteristics of the disease, explores distinctive features of its clinical manifestations and epizootiology; analyzes structure of the pathogen's genome. This review describes and evaluates laboratory diagnostic techniques (both conventional and modern); currently available anti-PRRS vaccines and novel biotech platforms enabling to design safer and more effective next-generation vaccines. There are three major challenges in vaccine development at the current stage of PRRS pathogenesis research: insufficient understanding of immune protection mechanisms, the virus's ability to induce negative regulatory signals for the immune system, and the pathogen's high antigenic variability.

Conclusion. PRRS virus strains exhibit significant genetic and antigenic heterogeneity and frequently undergo recombination, which exacerbates the challenges of epizootiology, disease prevention, and control. Further in-depth study of host immune response characteristics, along with identification of T- and B-cell epitopes in the pathogen structure, will enable rational design of genetically engineered vaccines.

Keywords: review, porcine reproductive and respiratory syndrome, epizootiology, vaccination, diagnosis

Acknowledgements: The study was conducted as part of a state-funded research program of the Federal Center for Toxicological, Radiation and Biological Safety for 2025–2026 on topic 4.2.5 "Development of technological approaches for production of modern vaccines against viral and bacterial infections in animals".

For citation: Nikolaeva Yu. A. Modern approaches to diagnosis and prevention of porcine reproductive and respiratory syndrome (review). *Veterinary Science Today.* 2025; 14 (2): 114–122. https://doi.org/10.29326/2304-196X-2025-14-2-114-122

Conflict of interests: The author declares no conflict of interests.

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УДК 619:616.98:578.833:636.4:616-076:616-085.371

Современные подходы к диагностике и профилактике репродуктивно-респираторного синдрома свиней (обзор)

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РЕЗЮМЕ

Введение. Репродуктивно-респираторный синдром свиней (РРСС), вызываемый вирусом из семейства *Arteriviridae*, является одной из наиболее экономически значимых болезней свиней во многих странах мира. Основные проявления заболевания включают репродуктивную дисфункцию у свиноматок, которая проявляется абортами на поздних сроках беременности, ранними или отсроченными опоросами, рождением слабых или нежизнеспособных поросят, нерегулярным эструсом; реже сообщается о патологиях на ранних и средних сроках беременности. У поросят и откормочных свиней наблюдается респираторный дистресс-синдром: кашель, чихание, одышка, задержка роста. Кроме того, заражение вирусом РРСС приводит к снижению

респираторного иммунитета, что делает инфицированных свиней более восприимчивыми к вторичным инфекциям и повышает смертность среди поголовья. В настоящем обзоре представлена актуальная информация о текущем состоянии лабораторной диагностики и специфической профилактики РРСС, а также рассмотрены перспективные биотехнологические платформы для конструирования вакцин нового поколения.

Цель исследования. Рассмотреть и обобщить современные подходы к диагностике и профилактике репродуктивно-респираторного синдрома свиней.

Материалы и методы. Материалом для аналитического исследования послужили научные публикации зарубежных и отечественных авторов.

Результаты. Приведена нозологическая характеристика заболевания, рассмотрены особенности клинических проявлений, эпизоотологии, организации генома возбудителя. Описаны и обсуждены применяемые в ветеринарной практике классические и современные методы лабораторной диагностики, а также коммерчески доступные препараты для специфической профилактики РРСС и перспективные биотехнологические платформы для создания вакцин нового поколения, которые позволят достичь оптимального баланса между безопасностью и эффективностью. На текущем этапе изучения патогенеза РРСС существуют три основные проблемы в разработке вакцин: недостаточность сведений о механизмах иммунной защиты, способность вируса индуцировать негативные регуляторные сигналы для иммунной системы и значительная антигенная изменчивость возбудителя.

Заключение. Штаммы вируса РРСС демонстрируют значительную генетическую и антигенную гетерогенность и часто подвергаются рекомбинациям, что усугубляет проблемы эпизоотологии, профилактики и контроля заболевания. Дальнейшее углубленное изучение особенностей иммунного ответа организма-хозяина, а также идентификация Т- и В-клеточных эпитопов в структуре возбудителя позволит обеспечить рациональный дизайн генно-инженерных вакцин.

Ключевые слова: обзор, репродуктивно-респираторный синдром свиней, эпизоотология, вакцинация, диагностика

Благодарности: Исследование выполнено в рамках госзадания ФГБНУ «ФЦТРБ-ВНИВИ» на 2025–2026 гг. по теме 4.2.5 «Разработка технологических подходов к производству современных вакцин против вирусных и бактериальных инфекций животных».

Для цитирования: Николаева Ю. А. Современные подходы к диагностике и профилактике репродуктивно-респираторного синдрома свиней (обзор). *Ветеринария сегодня*. 2025; 14 (2): 114–122. https://doi.org/10.29326/2304-196X-2025-14-2-114-122

Конфликт интересов: Автор заявляет об отсутствии конфликта интересов.

Для корреспонденции: Николаева Юлия Александровна, младший научный сотрудник лаборатории вирусных антропозоонозов ФГБНУ «ФЦТРБ-ВНИВИ», Научный городок-2, г. Казань, 420075, Республика Татарстан, Россия, *yulia.nikolaeva111@mail.ru*

INTRODUCTION

Porcine reproductive and respiratory syndrome (PRRS) caused by the porcine reproductive and respiratory syndrome (PRRS) virus (*Betaarterivirus* types 1 and 2) is one of the most economically significant porcine diseases in many countries of the world: the global annual damage associated with this infection is estimated at more than 600 million US dollars. First outbreaks of the unknown disease were reported in the USA and Western Europe in the late 1980s and early 1990s, turning into a pandemic a few years later [1, 2]. Sows exhibited such reproductive failures as abortions, fetal mummification, stillbirths or birth of non-viable offspring, and growing piglets – respiratory manifestations (dyspnea, coughing and hyperthermia) [3] As it was established in the Netherlands in 1991, and later in the USA (in 1992), the disease was caused by the previously unknown RNA-containing virus. The disease came to be known as "porcine reproductive and respiratory syndrome" [4]. Retrospective research suggested that antibodies to PRRS pathogen had been detected before 1979 in Eastern Canada and in the mid-1980s in Iowa [5], but the viruses themselves were not identified. Presumably there were several critical epizootic milestones in the history of PRRS virus (PRRSV) dissimination, and therefore the origin of some strains, in particular from the cluster associated with MN184 strain [6], causing "acute PRRS" or "abortion storm" [7], and some highly pathogenic Chinese strains, remains unknown [8]. In Russia, the first PRRS outbreak was reported in 1991 following abortions in sows on the farms of the Kursk Oblast [9]. In 2007, during a PRRS outbreak in the Irkutsk Oblast, American genotype PRRSV-2 was isolated [10].

The causative agent is PRRSV, which is a small, enveloped positive-sense single-stranded RNA virus belonging to the genus *Betaarterivirus*, family *Arteriviridae*, order *Nidovirales* [11]. PRRSV strains are classified as PRRSV type 1 (European genotype – EU-like) and PRRSV type 2 (North American genotype – NA-like). The virus genome is characterized by high variability even if compared to other RNA viruses. Since the virus RNA-dependent RNA-polymerase lacks proofreading activity, the virus undergoes frequent mutations and recombination events resulting in occurrence of new virus isolates worldwide [12]. Having a length of about 14.9–15.5 kb, the viral genome contains at least 11 open reading frames (*ORFs*) with a 5' cap and 3' polyadenylated tail [13]. Non-structural proteins (nsp 1–12), which have the functions of protease, replicase, regulation of gene expression of the host cell and are responsible for the viral RNA synthesis, are encoded by *ORF1a* and *ORF1b,* which occupy approximately two thirds of the genome [14]. Structural proteins – capsid protein (N), membrane protein (M), glycoproteins GP2, GP3, GP4, GP5, and envelope protein (E) – are expressed by subgenomic RNA and encoded by *ORF2–7* [15]. Differences in nucleotide sequences of most conserved (*ORF7* gene encoding capsid protein N) and variable (*ORF5* gene encoding major glycoprotein GP5) form the basis of the current PRRSV genotyping system [16].

Despite multiple sequences deposited in databases, none of the existing classification systems covers diversity of the existing PRRSV variants [17]. Incomplete coverage of the available data and lack of reference sequences are the main shortcomings of the applied genotyping techniques [18]. In 2010, a phylogenetic lineage-based PRRSV

Table 1
PRRS virus genotypes and their known representatives [24, 25, 26, 27, 28]

Genotype	Known representatives, GenBank ID
PRRSV-1 (European genotype – EU-like)	
Subtype 1 (global)	strain Lelystad (NC_043487.1), Netherlands
Subtype 1 (Russian)	strain WestSib13 (KX668221.1), Russia
Subtype 2	strain Bor (JN651734.1), Belarus
Subtype 3	strain SU1-Bel (KP889243.1), Belarus
PRRSV-2 (North American genotype – NA-like)	
Lineage 1	strain NADC30 (MH500776.1), China
Lineage 3	strain QYYZ (JQ308798.1), China
Lineage 5	strain VR-2332 (AY150564.1), USA
Lineage 8	isolates JXA1 (AY032626.1), CH-1a (EF112445.1), China

typing system was proposed [19]. According to this system, PRRSV-1 strains are grouped into four subtypes (subtype 1 – global, subtype 1 – Russian, subtypes 2 and 3), and PRRSV-2 strains are grouped into nine lineages (lineage 1 – lineage 9) based on phylogenetic relationships in the *ORF5* region [20, 21]. Both genotypes, divided into clades, lineages, and sub-strains, exhibit high genetic diversity and possess approximately 60% nucleotide sequence identity [22, 23] (Table 1).

The objective of this analytical study was to review and summarize current approaches to the laboratory diagnosis and specific prevention of PRRS.

EPIDEMIOLOGY OF PRRS IN THE RUSSIAN FEDERATION

In the nomenclature of the World Organisation for Animal Health, PRRS is classified as a socially and economically significant disease [10]. According to the information provided, infection caused by PRRSV-2 is of greater epidemic importance, since viremia in animals infected with the strains of this genotype was more pronounced and prolonged than in those ones infected with PRRSV-1 [29]. PRRSV-1-1 isolates, including the so-called Russian group of viruses, PRRSV-1-2 and PRRSV-1-3 differ significantly in pathogenicity [3]. The phylogenetic analysis indicates that the European type of virus, mainly belonging to subtype 1 (Russian), is predominantly prevalent in Russia [29]. Most PRRSV-1 strains can be attributed to the Russian group; a small number of circulating strains homologous to Lelystad strain are probably associated with the use of attenuated vaccines based on PRRSV-1 [30]. However, during the PRRS outbreak in the Central Federal District in 2020, in addition to the viruses from the Russian group previously detected in these regions, Lelystad-like viruses were also detected [9, 31]. A virus phylogenetically closely related to this type was identified in Poland in 2010 [32]; this indicates that new PRRSV variants from Europe are still introduced into Russia. Until the mid-2000s, the North American PRRSV genotype had not been registered in Russia, but in 2007 an outbreak was recorded in the Irkutsk Oblast caused by high pathogenicity PRRSV-2, presumably brought in from China [33]. In addition, there is information about detection of PRRSV-2 in the Republic of Mordovia, Belgorod and Kemerovo Oblasts [3, 9, 34]. The origin of American strain introduction to the Russian territory has not been identified, but it is assumed that they could have been introduced, for example, from Denmark, where PRRSV-2 circulates and from where breeding animals are imported [9].

NOSOLOGICAL PROFILE OF PRRS

The disease is mainly manifested by reproductive disorders in sows, i.e. abortions in late pregnancy, early or delayed farrowing, birth of weak or non-viable piglets, irregular estrus; pathologies in early and middle pregnancy are less often reported [35, 36]. The primary cause of the reproductive disorders is virus-induced damage to the placenta and endometrium. Piglets and fattening pigs have respiratory distress syndrome: coughing, sneezing, dyspnea and stunted growth. In addition, PRRSV infection undermines respiratory immunity, which makes infected pigs more susceptible to secondary infections; as a result, bacterial pathogens manifest themselves in association with the viruses, thus, increasing livestock mortality [37]. The young animals are more susceptible to PRRSV than the adults are, while replacement boars and sows often suffer from subclinical infection [38].

LABORATORY DIAGNOSTICS

The main methods used for PRRS diagnostics are given in Table 2.

SPECIFIC PREVENTION

No ideal anti-PRRS vaccine has been developed so far. According to the modern requirements for a new generation of vaccines against PRRS, they shall demonstrate high efficacy, safety, and at the same time ensure cross-protection against different genotypes of the virus [44]. Due to the exceptional ability of PRRS to mutate and generate significant genetic variations, development of a broadly protective vaccine is particularly crucial for combating constantly emerging disease outbreaks [45].

The first commercially available modified live attenuated anti-PRRS vaccine (PRRSV-MLV) was released in the USA, in 1994. This event became a starting point for the vaccine large-scale safety and efficacy tests [46]. A significant

Table 2
Methods for diagnosing PRRS [3, 37]

Method	Principle of diagnosis	Peculiarities
Virus isolaton		
Culture method	Use of alveolar macrophage cell cultures.	Virus isolation may not be effective, since not all isolates (especially PRRSV-1) are capable of infecting MARC-145 and CL-2621 cells-clones derived from MA-104 monkey kidney cell line [39]
Serological methods		
Enzyme-linked immunosorbent assay (ELISA)	Based on detection of virus-specific antibodies using a diagnostic antigen. Most commonly used antibody detection method has been adapted to detect IgG, IgM, and IgA [40]	Commercial kits are available to determine serological status of pigs both in blood serum and in oral fluid used as a test object (test kits for detecting antibodies to PRRSV: "PRRS-SEROTEST", "PRRS-SEROTEST plus", Vetbiohim, Russia)
Immunofluorescence assay (IFA)	Based on detection of the viral antigen using specific antibodies labeled with a fluorescent dye. Specific fluorescence shall be observed in infected cells with the positive control serum. It is also designed to detect IgG, IgM and IgA [41]	IFA effectiveness depends on the quality of the labeled diagnostic antibodies and the test conditions. It is important to properly prepare samples and control tests that ensure reliability of the results
Virus neutralization test (VNT)	Based on the neutralization of the virus by antibodies of a specific serum. Used to detect functional antibodies related to the immune defense	According to the published sources, virus-neutralizing antibodies can be detected only on day 45 after infection, because antibody synthesis takes time. At early infection stages, antibody levels may be insufficient for detection. Thus, VNT may be ineffective at the initial stages of infection. The test has high specificity and sensitivity, which makes it one of the most reliable tests for detecting virus-neutralizing antibodies
Immunoperoxidase monolayer assay (IPMA)	Based on the use of fixed permissive line cells infected with the corresponding virus to detect specific antibodies. Used for detection of IgG isotype antibodies [42]	Can recognise a number of PRRSV variants, including field and vaccine strains; its sensitivity and specificity are comparable to those of RT-PCR. The most suitable method for early detection and monitoring of virus circulation
Molecular and genetic methods		
Real-time reverse transcription polymerase chain reaction (RT-PCR)	Based on detection of viral genome fragments. The advantages of RT-PCR are high sensitivity and specificity, as well as rapid assessment of the current infection status	This method does not differentiate inactivated virus from infectious virus. Available commercial test kits: "Test system 'PRRS' for detecting RNA and genotyping the porcine reproductive and respiratory syndrome virus (PRRSV) using polymerase chain reaction (PCR)" (developed by the Central Research Institute of Epidemiology, Rospotrebnadzor, Russia; "PCR-PRRS-FACTOR" (VET FACTOR, Russia); "AmpliPrime® PRRS" (NextBio, Russia)
ORF5 sequencing	Based on the molecular and genetic typing of PRRS virus isolates. Analysis of the *ORF5* fragment nucleotide sequences revealed significant genetic variability of the pathogen [43]. In 2010, a method for PRRSV typing based on phylogenetic relationships in the *ORF5* region was proposed [22], which later became conventional	No reliable data are available on correlation between the phylogenetic groups based on *ORF5* sequences and pathogenicity or cross-protection, therefore this approach is not suitable for assessing virulence of the virus strains
ORF7 sequencing	The *ORF7* sequence is widely used to determine genetic variations and phylogenetic relationships between different strains of PRRSV, which indicates the important role of *ORF7* in the pathogen evolution [23]	The reason for selecting *ORF7* as the sequencing region is the conserved nature of this gene. The method has several advantages: it can detect both genotypes of the virus, is fast, inexpensive, sensitive, and can detect new sublineages and subgenotypes. Thus, the method is a promising tool for diagnosis and epizootological surveillance
Morphological techniques		
Immunohistochemical method	Based on detection of specific antigens in formalin-fixed tissues. Allows visualization of the antigen alongside with histological lesions	This method enables virus identification at lesion sites; establishes cause and effect relationship, detects varying viral concentrations. It is less sensitive than PCR; there are certain requirements for sample preparation
Fluorescent *in situ* hybridization (FISH)	It is based on the use of DNA probes that bind to complementary targets in a sample. It is suitable for screening virus-infected tissues containing a relatively small number of affected cells	Although *in situ* hybridization is rarely used for diagnostic purposes, it is capable of detecting and differentiating PRRSV genotypes in formalin-fixed tissues. Sensitivity and specificity of this method for detecting PRRSV genome may be insufficient due to high genetic diversity of the virus, especially PRRSV-1. The method is useful for studying viral persistence and for routine diagnosis of PRRS

Table 3
Commercial vaccines against PRRS

Vaccine name (developer)	Region where it is used	Genotype (strain)	Efficacy
Live vaccines [47, 48]			
Ingelvac® PRRS MLV (Boehringer Ingelheim, Germany)	Africa, Asia, Europe, North America, South America	PRRSV-2 (VR-2332)	Induces protection against homologous isolates, but limited cross-protection against heterologous strains. The efficacy of these vaccines is considered insufficient to eradicate the disease on farms: large-scale PRRS outbreaks were reported on farms where vaccination is practiced. Use of live modified anti-PRRS vaccines can be a problem, since the vaccine virus can be excreted for 2 weeks and may revert to a virulent form
Ingelvac® PRRS ATP (Boehringer Ingelheim, Germany)	Asia, Europe, North America	PRRSV-2 (JA-142)	
Fostera® PRRS (Zoetis, USA)	Africa, Asia, Europe, North America	PRRSV-2 (P129)	
Prime Pac® PRRS (MSD Animal Health, Netherlands)	Africa, Asia, Europe, North America	PRRSV-2 (Neb-1)	
Prevacent® PRRS (Elanco Animal Health Inc., USA)	Asia, Europe, North America	PRRSV-2 (RFLP 184)	
Unistrain® PRRS (Laboratorios Hipra, S.A., Spain)	Africa, Asia, Europe	PRRSV-1 (VP-046 BIS)	
ReproCyc® PRRS EU (Boehringer Ingelheim, Germany)	Africa, Asia, Europe	PRRSV-1 (94881)	
Pyrsvac-183® (Laboratorios Syva S.A., Spain)	Asia, Europe	PRRSV-1 (ALL-183)	
Suvaxyn® PRRS MLV (Zoetis, USA)	Europe	PRRSV-1 (96V198)	
ARRIAH-PRRS (Federal Centre for Animal Health, Russia)	Russia	PRRSV-2 (attenuated strain BD-DEP)	
ARRIAH-ResursVak (Federal Centre for Animal Health, Russia)	Russia	PRRSV-1 (attenuated strain Borz)	
Resvak (Shchelkovo biocombinat, Russia)	Russia	PRRSV-1 (strain PRRS-1SBC)	
Inactivated vaccines [49, 50]			
SUIPRAVAC® PRRS (Laboratorios Hipra, S.A., Spain)	Europe	PRRSV (VP-046 BIS)	Inactivated vaccines induce a weaker and shorter immune response and are often ineffective against heterologous strains, but they are more stable and less sensitive to storage conditions, and are safe for use in pregnant sows
PROGRESSIS® (Merial, France)	Europe	PRRSV-1 (P120)	
SUIVAC® PRRS-INe / SUIVAC® PRRS-IN (Dyntec, Czech Republic)	Europe	PRRSV-1 (VD-E1/VD-E2/VD-A1)	
Biosuis PRRS inact Eu+Am (Bioveta, Inc., Czech Republic)	Europe, Russia	PRRSV-1 (European strain MSV Bio-60, American strain MSV Bio-61)	
ARRIAH-PRRS Inact (Federal Centre for Animal Health, Russia)	Russia	PRRSV-1 (strain KPR-96)	
PRRS-FREE (Reber Genetics, Co. Ltd, China)	Asia, Russia	PRRSV-1, PRRSV-2 (antigens PE-PQAB-K13, PE-RSAB-K13, PE-DGD-K13, PE-M12-K13)	
VERRES-PRRS (Vetbiochim LLC, Russia)	Russia	PRRSV-1 (strain OB); recombinant proteins M and GP-5 PRRSV-1 (strain Tyu16)	
ARRIAH-RePovak (Federal Centre for Animal Health, Russia)	Russia	PRRSV-1 (strain KPR-96)	
ARRIAH-Aujeszky's+PRRS (Federal Centre for Animal Health, Russia)	Russia	PRRSV-1 (strain KPR-96)	

Table 4
Candidate vaccines against PRRS

Name of the vaccine candidate	Method of preparation, protective characteristics
Deletion mutant vCSL1-GP5-N44S	Obtained by substituting the 44th amino acid in ectodomain of GP5 protein, serine-to-asparagine substitution. In an *in vivo* trial, no side effects were observed in piglets immunized with vCSL1-GP5-N44S; the vaccine induced high levels of neutralizing antibodies post infection [54]
Attenuated strain A2MC2-P90	Obtained after *in vitro* attenuation of PRRSV-A2MC2 after 90 serial passages in MARC-145 cells. The resulting strain A2MC2-P90 retained its ability to induce IFN in cell culture. A2MC2-P90 ensured 100% protection for vaccinated piglets against lethal infection with extremely virulent HP-BPCC-XJA1 strain, while non-vaccinated piglets demonstrated 100% mortality rate by day 21 post infection [55]
Chimeric virus vCSL1-GP5-N33D	Chimeric vaccine candidate based on PRRSV-2 expressing hypoglycosylated GP-5. It was used on PRRSV-affected farms; it induced neutralizing antibodies in high titers 8 weeks after the vaccination [56]
Chimeric virus VR2385-S3456	S3456 fragment contains full-length gene sequences encoding structural proteins (*ORF3-6*) embedded in PRSSV strain VR2385 genome. Induced a high level of neutralizing antibodies against two heterologous strains [57]
Chimeric virus K418DM1.1	A chimeric virus with genomic basis of FL12 infectious clone of highly virulent American PRRSV, containing genes of structural proteins of PRRSV-2 strain LMY. K418 was further modified by deglycosylation of GP5 and exhibited strong immunogenicity. No reversion to the virulent state was observed [58]
Chimeric virus rJS-ORF2-6-CON	The backbone consisted of a consensus sequence *ORF2-6* (ORF2-6-CON), encoding all enveloped proteins, developed on the basis of 30 currently circulating PRRSV Chinese isolates. Chimeric virus rJS-ORF2-6-CON was created using avirulent infection clone HP-PRRSV2 JSTZ1712-12. *In vivo* test results have shown that the virus is not pathogenic to piglets and provides cross-protection against heterologous strains [45]
Chimeric virus rTGEV-GP5-N46S-M	The backbone was the porcine transmissible gastroenteritis virus co-expressing GP5 proteins (except for the first glycosylation site) and M. After double immunization of piglets, virus neutralizing antibodies were found; the *in vivo* efficacy of the vaccine was also confirmed following challenge with the PRRSV/Olot91 strain. The disadvantage is instability of the recombinant virus: GP5 expression decreased during 8–10 passages [59]

number of conventional (live and inactivated) vaccines have been developed by now; their brief description is given in Table 3.

Studies demonstrating circulation and persistence of the vaccine virus, in turn, raise concerns about its safety: viremia implies potential transmission of the vaccine virus to non-infected animals. In addition, the vaccine virus can cross the placental barrier in pregnant sows and infect developing fetuses, resulting in the pathogen transmission to uninfected newborn piglets during lactation. It has also been shown that vaccine strains are able to recombine with field strains, creating potentially new genetically distinct variants of PRRSV on individual farms [51]. For these reasons, efficacy of live attenuated vaccines is somewhat controversial, and it is generally recognized that their safety needs to be improved. In this context, DIVA strategy (differentiation of infected from vaccinated animals) will be of great importance for control and possible eradication of PRRS [52, 53]. Epizootological and regulatory considerations indicate the need to develop anti-PRRS DIVA vaccine, which will be characterized by a negative marker (that is, a marker absent in the vaccine strain, but permanently present in wild-type strains). Similar candidate vaccines have been developed on the platform of large DNA viruses such as pseudorabies virus (PRV) and bovine herpesvirus type 1 (BHV-1) by deleting genes encoding some structural proteins. However, in case of a small RNA virus such as PRRSV, which encodes only a few proteins with basic functions, the creation of a mutant virus with a deletion of the immunodominant and conserved protein segments (or with a combination of deletions within a single protein or even in different proteins) seems to be a more difficult task. Nevertheless, this approach may become a promising alternative for development of a live attenuated marker vaccine against PRRS [8].

PROMISING BIOTECHNOLOGICAL PLATFORMS FOR CREATING CANDIDATE VACCINES

Table 4 provides main characteristics of some candidate vaccines against PRRS, developed on various biotechnological platforms.

CONCLUSION

Thus, at the current stage of PRRS pathogenesis study, three major challenges in developing more efficient next-generation vaccines can be identified: insufficient understanding of immune protection mechanisms, the virus's ability to induce negative regulatory signals for the immune system and its substantial antigenic variability [59]. In particular, the last factor is the reason behind poor efficacy of the existing vaccines against heterologous infection. Further in-depth analysis of the host's immune response, as well as the identification of T- and B-cell epitopes in PRRSV structure, will ensure rational design of genetically engineered vaccines and ultimately attaining the optimal safety-efficacy profile.

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Received 17.01.2025
Revised 18.02.2025
Accepted 25.03.2025

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https://doi.org/10.29326/2304-196X-2025-14-2-123-132

Artificial intelligence-integrated drones used for detection of live wild boars, wild boar carcasses and remnants in the context of African swine fever control

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ABSTRACT

Introduction. Effective measures for African swine fever outbreak prevention and early detection are required in view of global spread of African swine fever, fatal viral hemorrhagic disease of domestic pigs and wild boars. Wild boar population managing and search for the wild boars died of African swine fever and being the virus source are considered priority measures for the disease control in wildlife.

Objective. Generalization of currently available knowledge about advanced technologies for the use of unmanned aerial vehicles (drones) in combination with artificial intelligence-based methods in the wild.

Materials and methods. Analytical research methods including search in the following databases were used: PubMed, Springer, Wiley Online Library, Google Scholar, CrossRef, Russian Science Citation Index (RSCI), eLIBRARY, CyberLeninka.

Results. Potential of using unmanned aerial vehicles (drones) and artificial intelligence (neural network) for detection of wild boars and their remnants in the context of combating African swine fever is described in the review. The role of wild boars in the disease spread and the need for wild boar population regulation are discussed in detail. Also, the importance of timely wild boar carcass removal and use of modern technologies for wild boar population recording and its density estimation are underlined. Data on the use of drones equipped with various technical devices for study of large animal populations in the wild are analyzed, advantages and peculiarities of unmanned aerial vehicle use are indicated. Experience gained in using neural networks-based techniques for automatic processing of animal images acquired from drones is also summarized.

Conclusion. Artificial intelligence-integrated unmanned aerial vehicles appear to be a key tool for managing wild boar populations and the rapid detection of African swine fever dead wild boars that allows improvement of overall effectiveness of the measures taken against this disease.

Keywords: review, wild boar, African swine fever, animal recording techniques, monitoring, aerial photography, unmanned aerial vehicles, UAVs, drones, artificial intelligence, neural network

Acknowledgements: The study was supported by the Ministry of Education and Science of the Russian Federation within the state assignment for the Federal Research Center for Virology and Microbiology (No. FGNM-2022-0004). The authors thank the reviewers for peer reviewing of this paper.

For citation: Bespalova T. Yu., Korogodina E. V., Mikhaleva T. V. Artificial intelligence-integrated drones used for detection of live wild boars, wild boar carcasses and remnants in the context of African swine fever control. *Veterinary Science Today*. 2025; 14 (2): 123–132. https://doi.org/10.29326/2304-196X-2025-14-2-123-132

Conflict of interests: The authors declare no conflict of interests.

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УДК 619:639.1.053:616-036.22:639.111.14:623.746.4-519:004.8

Интеграция применения дронов и искусственного интеллекта для обнаружения диких кабанов, туш и их останков в связи с африканской чумой свиней

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РЕЗЮМЕ

Введение. Глобальное распространение африканской чумы свиней, смертельно опасного вирусного геморрагического заболевания домашних свиней и диких кабанов, диктует необходимость применения эффективных мер предупреждения и раннего выявления вспышек. Контроль численности популяции, а также поиск туш диких кабанов, погибших от африканской чумы свиней и являющихся источником передачи вируса, считаются приоритетными мерами в управлении заболеванием в дикой природе.

Цель исследования. Обобщение имеющихся в настоящее время знаний о передовых технологиях применения беспилотных летательных аппаратов (дронов) в условиях дикой природы в сочетании с методами искусственного интеллекта.

Материалы и методы. При выполнении работы применялись аналитические методы исследований с использованием баз данных PubMed, Springer, Wiley Online Library, Google Scholar, CrossRef, РИНЦ, eLIBRARY, CyberLeninka.

Результаты. В данном обзоре рассматривается возможность применения беспилотных летательных аппаратов (дронов) и искусственного интеллекта (нейронных сетей) для обнаружения диких кабанов и их останков в контексте борьбы с африканской чумой свиней. Подробно обсуждается роль диких кабанов в распространении заболевания и необходимость контроля их популяции, значение своевременного удаления трупов кабанов, при этом подчеркивается важность использования современных технологий для учета численности и плотности популяции дикого кабана. Проанализирована информация о применении дронов, оснащенных различными техническими средствами, при изучении популяций крупных видов животных в условиях дикой природы, отмечены преимущества и особенности использования беспилотных летательных аппаратов. Также обобщен опыт применения нейронных сетей в контексте автоматической обработки полученных с помощью дронов изображений животных.

Заключение. Интеграция беспилотных летательных аппаратов и искусственного интеллекта, вероятно, может стать ключевым инструментом в контроле популяции дикого кабана и быстром обнаружении туш кабанов, погибших вследствие африканской чумы свиней, что в целом позволит повысить эффективность мер, направленных на борьбу с данным заболеванием.

Ключевые слова: обзор, дикие кабаны, африканская чума свиней, методы учета животных, мониторинг, аэрофотосъемка, беспилотные летательные аппараты, дроны, искусственный интеллект, нейронная сеть

Благодарности: Работа выполнена при поддержке Минобрнауки России в рамках Государственного задания ФГБНУ «Федеральный исследовательский центр вирусологии и микробиологии» (тема № FGNM-2022-0004). Авторы благодарят рецензентов за их вклад в экспертную оценку данной работы.

Для цитирования: Беспалова Т. Ю., Корогодина Е. В., Михалева Т. В. Интеграция применения дронов и искусственного интеллекта для обнаружения диких кабанов, туш и их останков в связи с африканской чумой свиней. *Ветеринария сегодня.* 2025; 14 (2): 123–132. https://doi.org/10.29326/2304-196X-2025-14-2-123-132

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

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INTRODUCTION

African swine fever (ASF), deadly viral hemorrhagic disease affecting both domestic pigs (*Sus scrofa domestica*) and wild boars (*Sus scrofa*), remains a critical global threat to pig industries [1]. Wild boars are now commonly recognized as key reservoirs and vectors for ASF transmission; infected migratory populations have been found to introduce the virus to multiple European countries [2, 3, 4, 5]. Monitoring of Eurasian wild boar populations – including assessments of population size, density, and dynamics – constitutes a critical component of ASF management strategies for the disease outbreak containment in wild populations. Early outbreak detection including systematic searching for carcasses, a primary source of direct and indirect ASF virus transmission, represents one of the most effective measures for ASF eradication in the wild. Rapid detection and safe disposal of dead wild boars can prevent further infection spread, since the virus is found to persist in ASF dead wild boars for several months [6, 7, 8, 9]. Searching for wild boar carcasses and remnants is a laborious and time – consuming work, which strongly depends on the outbreak size, season, terrain, vegetation density as well as other factors. According to researchers, most of the wild boar carcasses are often missed by traditional ground-based walking methods [10]. Therefore, alternative modern methods and technologies are required for reliable wild boar population size and density assessment and optimization of the process of searching for wild boar carcasses and remnants.

Currently, unmanned aerial vehicles (UAVs), known as unmanned aircraft system, copters, or drones controlled by one or more pilots using communication channels at remote piloting points (ground control stations), are becoming increasingly popular. Unmanned aerial vehicles are widely used in absolutely different fields, including wildlife monitoring. Moreover, various UAV systems, together with developing artificial intelligence (AI) technologies, are used for wild animal censusing, animal behaviour and movement analysis [11, 12, 13]. In the last decade, numerous studies of populations and natural habitats of both wild birds [14, 15, 16] and various wild large animal species (primates, elephants, hippos, ungulates) were carried out using drones as a part of environmental protection measures [17, 18, 19, 20, 21, 22, 23]. However, there are almost no data on their use for wild boar searching and for wild boar population size assessment in peer-reviewed sources. UAVs are one of the promising options to be added to the set of traditional monitoring methods. Some studies have shown that drones allow for more rapid and accurate estimation of wild animal populations in vast territories as compared with ground-based methods (walking monitoring, camera traps, etc.) [17, 24]. Previously, large-scale aerial photography of wildlife was carried out using manned aircrafts, but use of UAVs for aerial photography is much cheaper. UAVs can work under cloud cover in contrast to satellites [25]. Artificial intelligence and machine learning (ML) are revolutionizing wildlife monitoring by improving data quality for population estimates, streamlining data collection, and automating routine data processing. Neural networks – trained on extensive datasets from drone imagery, camera traps, and video cameras – can now achieve species-level identification and even distinguish individual animals. ML algorithms process visual data orders of magnitude faster than manual analysis, with demonstrated capability to filter tens of thousands of files in minutes to select animal-containing images, dramatically increasing research efficiency [18].

Given the ongoing ASF panzootic, testing of modern UAV-based approaches, firstly, as an observational tool

for wild boar population size and density assessment, and, secondly, as a tool for efficient searching for wild boar carcasses and their remnants is of importance for the infection management in the wild. A review of published literature identified a critical research gap: no comprehensive studies exist on the UAVs application for searching for live wild boars and wild boar carcasses. Our review of AI-integrated UAV systems successfully deployed for other animal species appears to be helpful for their adaptation to programs on wild boar population monitoring.

This review synthesizes current knowledge on advanced AI-integrated UAV (drone) technologies applied for wildlife monitoring. The review addresses the following aspects: role of wild boar in ASF spread and importance of prompt removal of dead wild boars; use of UAVs and neural networks for wild large animal population monitoring with focus on drone-based approach advantages and features as compared to traditional methods.

MATERIALS AND METHODS

Analytical methods and searching in the following databases: PubMed, Springer, Wiley Online Library, Google Scholar, CrossRef, RSCI, eLIBRARY, CyberLeninka were used for the work.

ROLE OF WILD BOARS IN ASF EPIZOOTY

Since genotype II ASF virus detection in Eastern Europe (2007) the disease has spread to many European countries and far beyond its borders (to Asia, America and Oceania). According to the World Organization for Animal Health, ASF has been reported in 64 countries, more than 934 thousand pigs and more than 31 thousand wild boars have been infected over the past three years. Eurasian wild boars are believed to play the main role in the disease spread in Europe where more than 19 thousand outbreaks have been reported in wild boar population[1]. In most European countries, ASF spread has been facilitated for many years by factors potentially associated with wild boar ecology, infection management strategies in the wild (for example, an efficient search for dead wild boars), as well as with the long-term ASF virus persistence in animal carcasses and in the environment [1]. Monitoring of wild boar populations in Europe shows a steady increase in the population size and expansion of the population habitat over the past decades that hampers ASF management in the infected areas [26]. In Central European countries, Eurasian wild boar population density is high, 1.15–5.31 animals per 100 ha [27, 28]. The population density is known to be one of the important factors associated with ASF spread among wild boars, the higher the density, the higher the probability of pathogen transmission by direct contact [29]. For example, in Poland, ASF cases were reported mainly in the areas where the wild boar population was more than 1 animal per 1 km^2, but statistical and mechanistic models did not show a clear and consistent effect of wild boar density on ASF epizootology [1, 30]. Wild boars living in close proximity to both private and commercial farms pose a risk of ASF outbreaks in domestic pigs that becomes higher with the relatively high number of wild boars [31]. Therefore, ASF management requires the most reliable information on wild boar population size and density in each region in the context of various measures. However, it is actually quite difficult to obtain data close to absolute ones. This is the most challenging for remote areas and vast territories.

When studying the wild boar population in the context of ASF control, it is important to take into account their biological behaviour peculiarities, seasonal and landscape factors, as well as the virus persistence in the environment. Recently, a lot of studies has been carried out to examine various factors that ultimately affect the effective search for wild boars, their carcasses and remnants. The search can be improved by target searching for preferred habitats for both healthy and infected animals. Wild boars are known to be very mobile, hide in dense vegetation, and to be predominantly nocturnal with peak activity in the late evening (at sunset), at midnight and in the morning hours at sunrise throughout most of the year. Reduced activity at temperatures above 15 °C is their behavioural adaptation mediated by physiological characteristics. Wild boars are less active in the forest than in open areas, and they choose reeds in swampy areas as a safe resting place [32, 33]. ASF-diseased wild boar preferences should be taken into account to find the places where they die. Such animals display changes in their behaviour, they prefer solitude with sufficient shelter, silence, coolness, and plenty of water, which is associated with the condition caused by the infection (depression, fever, dyspnoea) [34]. During the studies, the vast majority (71%) of infected carcasses were found in forests, especially in young woodlands, as well as in places remote from roads and settlements, in places of transition from woodlands to sparsely wooded areas and shrubs, near trails, waterbodies and forest edges with tall grass [34, 35, 36]. The space-time clustering in detected ASF-positive wild boar carcasses was most prominent at a distance of 2 km and within 1 week after the outbreak reporting [37]. Moreover, seasonal features of ASF spread should be considered when planning carcass search activities. In most European countries there was an evident seasonality in ASF incidence in wild boars that increased in winter (December – February) and peaked in summer (July). According to Russian researchers, ASF outbreaks in wild boars reported in the Russian Federation regions in 2007–2022 also occurred mostly in November – December and February, with peaks in the summer months (July – August) [38, 39].

Natural behaviour of wild boars – digging roots, rolling on the ground and exploring various objects – may be a risk factor for the infection if they live in the virus-infected environment. Some researchers have shown that ASF virus transmission in wild boar habitats can occur not only through direct contact with infected conspecifics, but also through indirect contact with carcasses, secretions, soil, water, grass, or agricultural crops [28, 40, 41], while physical contact with pathogen-positive carcasses or the substrates beneath them poses an equal risk of ASF virus infection [42]. The carcass and remnant (bones and skin) decomposition sites remain attractive to wild boars for a long period of time [40]. The carcass decomposition process depends on the season and can take several days in summer to several months in winter [43]. ASF dead wild boars are a permanent source of infection for other animals, as the virus is highly resistant to environmental conditions and persists for a long time in various organs, tissues and secretions. It has been reported that a frozen carcass

[1] WOAH. African swine fever. https://www.woah.org/en/disease/african-swine-fever/#ui-id-2

can maintain infectious ASF virus for several months enabling the virus to overwinter and to initiate a new outbreak when the defrosted carcass is visited the following spring by a susceptible wild boar [37]. In the study carried out in Germany, it was noted that wild boars rummaged on decomposition sites, sniffing and poking on the conspecifics' carcasses, chewing on their bare ribs, the contact was observed in 30% of all visits by wild boars to such sites and the wild boars were especially "interested" in rooting on the soft soil that had formed under and around decomposed carcasses [8]. Later, it was found that more than 50% of cases of transmission in Eastern Poland were associated with indirect contact with infected carcasses that contributed to ASF virus persistence in wild boar populations [44]. In a recent study in the Czech Republic, a two-year monitoring using camera traps was conducted to assess the attractiveness of wild boar carcasses to their live conspecifics. It was shown that the number of visits by wild boars to the sites with experimentally placed carcasses during the year was more than five times higher than to control sites (without carcasses). Wild boars found the carcass relatively quickly, on average in 2 days in spring and summer, 6 days in autumn and 8 days in winter. The earliest visits were recorded in the spring, when the decomposition process was accompanied by a strong odour. Also, number of direct contacts with the carcass that varied depending on the season was determined. In autumn, wild boars came into direct contact with the carcass during 340 out of 541 visits (62.8%), in spring – during 71.2% of visits, in summer – during 74.5% of visits. The largest number of direct contacts was recorded in winter – 84.1% [33]. These findings are of great importance, since infected tissues (muscles, skin, subcutaneous fat) and organs of decomposing carcasses can be sources of ASF infection for several months, especially at low temperatures [9, 45]. Stability of the pathogen in the soil depends on the temperature: under experimental conditions at +4 °C, the virus retained its infectivity for up to 112 days [46], in the soil under the carcass – up to 2 weeks [47, 48]. The virus survival rate is found to depend on the soil type and pH level: the virus persists for a week in the forest and meadow soils, for 3 days in the soil of swampy areas, for at least 3 weeks in sand, and quickly dies in acidic forest soils [49].

Wild boars are omnivorous animals, just like domestic pigs, they are characterized by cannibalism. Tissues of other animals, including their conspecifics, were found in the stomach contents of wild boars [50]. In the study performed by J. Cukor et al. [51], direct contact of wild boar with carcasses was observed in 81% and cannibalism was observed in 9.8% of all reported visits of wild boars. Therefore, deliberate or accidental consumption of carcasses (cannibalism) or invasive contact with carcasses (with infected blood, tissues, or biological materials) can be considered as decisive factors in the chain of ASF virus transmission among wild boars [52]. Furthermore, infected carcasses can also maintain indirect virus spread by potential vectors – arthropods [42], as well as scavengers. According to J. Rietz et al. [53], some scavengers, in particular foxes, do not consume wild boar carcasses on site, but can move (scatter) their remnants over rather long distances in 6–10 days. Carcass parts are scattered over 400 m in 75% of cases, and maximum over 1.2 km. This should be considered for effective carcass searching as a part of ASF outbreak management. At the same time, such remnants scattering distances make a ground search by humans almost impossible.

Thus, wild boar carcasses and the surrounding soil are a reservoir for the long-term ASF virus persistence, and therefore early, rapid and effective search for potentially infected carcasses and their timely and safe removal from the environment are extremely important for minimization of the risk of the disease spread in the population. In ASF endemic areas the special attention should be paid to these measures using the accumulated knowledge about diseased wild boar behaviour and environmental factors that increase the likelihood of carcass detection.

For the purpose of ASF control wild boar population should be regulated and its density should be maintained at the lowest possible level in each region [6]. Existing methods of animal censusing are based on their direct counting during direct field observation with naked eye or binoculars, as well as on-site images obtained at fixed points using camera traps, as well as sampling, surveying, or analysis of various indirect evidence of animal life [54, 55]. The methods differ in the territory coverage, counting techniques, objects to be counted, used technical devices, etc. For example, the widely used method of winter route counting determines the correlation between the number of animals detected in a selected area (along the route), the number of tracks (left during one day) and the daily animal movement length (provided that the snow cover is appropriately thick). Today, this basic method is considered simple and universal, it is relatively low cost since used technical tools are cheap, but it is not suitable for censusing of elusive animals [55]. The significant disadvantages of conventional methods for wild animal censusing (complete snow cover, low accuracy, dependence on weather conditions, etc.) dictate the need for improvement of monitoring technologies. Combined methods are more useful for obtaining reliable data on the animal population size and migration. Currently, simultaneous use of several methods with specialized equipment, such as camera traps, video or infrared (IR) cameras, has been proven effective. However, according to some researchers, aerial monitoring is the most effective method of animal censusing as compared with field methods [19, 54, 55, 56].

USE OF DRONES FOR WILDLIFE MONITORING

Remotely piloted aircraft systems (UAVs) – commonly known as drones – have been gaining increasing popularity worldwide over the past few years. UAV system includes three main components: the aircraft itself (drone), which performs tasks in the air; the ground station where the drone takes off and lands and where the communication and control equipment is installed; and the operator who directly controls the drone during flight. UAVs have many advantages that make them a powerful tool for exploring wildlife. Until the past decade, it has been challenging to gather data on number of the animals located in particular area and at particular time because aircraft missions and satellite images are expensive, and ground-based surveys in many cases are limited by accessibility to sites, the areas that could be covered [11]. UAVs now provide transformative capabilities for field research, dramatically decreasing manpower demands, survey durations, and project expenditures. UAVs can be successfully used in remote areas and under harsh climate conditions. The selection of the UAV for wild animal monitoring and

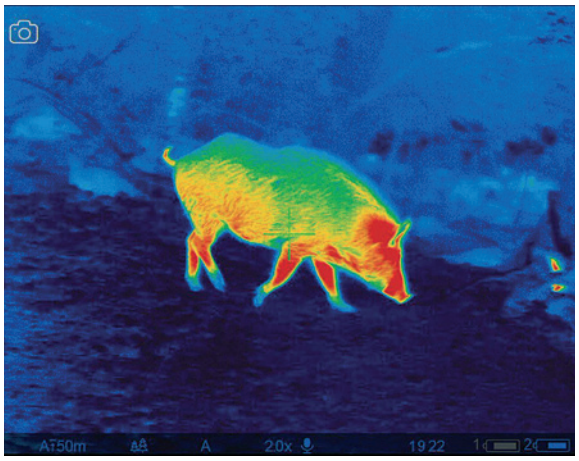

Fig. 1. Thermal image of a wild boar (https://pulsarvision.com/journal/calm-alert-hungry-getting-to-know-animals-through-thermal)

Fig. 2. Wild boar monitoring using drone technology (AI-generated image)

animal population size estimation critically depends on its payload specifications and installed sensors. UAV systems may incorporate machine vision sensors and AI-powered analytical capabilities. AI coupled with neural networks facilitates population census, real-time spatial monitoring as well as migratory pattern analysis and automated species classification [17, 57]. UAVs equipped with advanced electronic payloads – including digital sensors, night/thermal imaging cameras, communication systems, and GPS/GLONASS (Global Navigation Satellite System, GNSS) positioning units – exhibit significantly enhanced operational capabilities. Sensors based on machine vision enable visual perception of the UAV environment by creating a captured scene image, for example, a thermal imaging camera captures and registers IR radiation emitted by surrounding objects. There are practically no hard-to-reach places for UAVs with photo and thermal imaging cameras. Infrared cameras facilitate biotic/abiotic differentiation while maintaining diurnal/nocturnal operational capacity under varying environmental conditions. Their ability to detect thermally distinct targets enables wildlife monitoring through dense canopy cover and during crepuscular/nocturnal periods. In thermal IR waves, an animal looks like a bright object, provided that the animal's body temperature is higher than the ambient temperature (with a difference of up to 30–40 °C). Thermal images of the best quality are obtained at sunrise, late evening and at night (Fig. 1) [19, 54, 55, 57, 58].

Current UAV capabilities demonstrate significant potential for search for wild boars, their carcasses and remnants. Drones are capable of flying slowly at low altitudes, exploring areas that are hard-to-reach during ground-based surveys, such as dense forests or wetlands, as well as detecting moving and stationary objects without risk to humans (Fig. 2). The latter is important when searching for both living individuals at rest and animal carcasses. It has been found that images of the area covered by one frame made at altitude of 150 m are optimal for accurate counting of large groups of animals.

UAVs offer apparent advantages over traditional manned aircraft, owing to lower operational costs with minimal space requirements for their taking off. In addition, drones are relatively quieter than the latter, they may present less disturbance risk to animals due to noise, and could reduce the risk of biased counts because animals are less likely to flee and hide [20]. Regarding search for wild boar carcasses and remnants with UAVs, it should be taken into account that experiments have shown the high attractiveness of the places with wild boar carcasses and remnants for their fellow wild boars [33]. Since drones can easily detect live wild boar gatherings in these areas, this technology can also be used for wild boar remnant searching. Moreover, UAV thermal imaging can directly detect wild boar remnants by capturing heat signatures from fly larvae clusters and/or microbial activity during carcass decomposition. The heat generated by feeding larvae can be detected during their peak activity – between the 6th and 29th day of carcass decomposition – at ambient temperatures of 15–27 °C, when insect populations on the remnants are highest. Analysis of image resolution at varying flight altitudes revealed that thermal contrast between remnants and background was highest in noon recordings at 4 m altitude. A 15 m altitude proved optimal for balancing survey speed and detection efficacy during long-term monitoring, and objects became frequently overlooked beyond 30 m. All these factors should be taken into account when planning flights in order to maximize the chances for the remnant detection [59, 60].

UAV systems are continuously improving. Computer vision-integrated drone systems providing new capabilities and expanding UAV functionality are increasingly applied for object detection and recognition. Using computer vision, drones can autonomously process visual information, identify objects and make environment-dependant decisions. Currently, modern advanced technologies include the so-called FPV drones (First Person View) with Betaflight software and real-time video transmission. These systems enable high-speed, precise spatial data acquisition and long-range video signal transmission. FPV drones differ from conventional GPS drones in their smaller size and weight, which makes them easy to maneuver and move quickly (flight speeds can reach 100 km/h or more). FPV drones equipped with high-resolution cameras and video transmitters allows the user to see the real-time image on special glasses or monitor, feel the effect of their own presence in the airspace and remotely control the drone movements while adjusting the speed, altitude and angle of inclination of the device to control flight over a given

Fig. 3. Hugging-wing robot [62]

terrain. For the purpose of the environment monitoring, FPV drones, like other UAVs, are used to collect data and explore vast, new or remote territories, detect and track moving objects, wildlife habitats, and provide high-quality geo-referenced images[2] [61]. Also, application of universal robotic systems, hugging-wing robots, that can both hover in the air and perch on vertical supports such as tree trunks and poles is one of the promising methods for wild animal behaviour monitoring and collecting data on their habitats (Fig. 3). Remote autonomous navigation enables precise landing site determination for such robotic systems, achieving positioning accuracy within several meter ranges [62].

When planning UAV operations, some critical factors must be taken into account as they significantly impact both data quality and collection efficiency. These factors include: low resolution of the camera or sensor image, battery charge duration (which therefore determines the range and area covered in a single drone flight), weather conditions (strong wind, rain, snow), operator's control skills and experience, etc. [21]. Drone management and maintenance require special training for ground operators and compliance with security measures. In our country, use of any UAV is allowed only upon obtaining all required official documentation and permits in compliance with unmanned aerial vehicle regulations in place in the Russian Federation.

SOFTWARE AND ARTIFICIAL INTELLIGENCE USED FOR PROCESSING OF THE DATA COLLECTED BY DRONES

Conventional methods include visual analysis of photographs but manual photo analysis becomes increasingly labor-intensive and time-consuming when processing large photographic datasets. This method is inherently susceptible to human error factors including fatigue, inattention, etc. This disadvantage can be minimized by involving several specialists in the work or using software enabling automatic information processing [58]. Images obtained by UAV-mounted sensors are typically stitched together into an assembled digital map by using software programs. This digital map can then be uploaded into GIS (Geographic Information System) software, which can be geographically referenced using GPS data automatically gathered by the UAV in flight. When a UAV lack an on-board GPS, geographic coordinates can be manually obtained by reference to Ground Control Points (physical landmarks with known coordinates). Image processing of the digital map may be performed manually by the user, or automatically by image processing software that classifies objects. Digital files associated with drone images may be very large (up to 70 terabytes), particularly with the high resolution required for accurate object recognition [63]. Currently, domestic and foreign researchers use various software programs for processing data collected during wildlife monitoring [17, 58]. Longmore S. N. et al. [64] combined astronomical detection software with existing ML algorithms for automatic decrypting thermal images of animals, this pipeline contributed to effective detection of animals in the images. Currently, up to 30 software programs are being developed in Russia for different animal species identification, which count the number of animals both in a single image and in a series of images, some of software programmes enable simultaneous processing of thermal images and video materials[3] [55]. For example, the Thermal Infrared Object Finder (TIOF) software developed on the Python platform is capable of processing a large amount of infrared image data for specific animal identification [65].

Convolutional neural networks (CNNs) represent a state-of-the-art approach facilitating animal detection and counting in aerial imagery. CNNs are one of the main types of neural networks used for image recognition and classification that are composed of two main parts: feature extraction and classification. Feature extraction is aimed at creating maps of objects through utilizing processes called convolutions. CNN model contains three types of layers: convolutional layer, pooling layer and fully connected layer. The first two perform feature extraction, and the fully connected layer displays the extracted features and performs classification. Deep learning models offer a significant advantage in processing accuracy over conventional classification methods when trained and tested with large datasets, so the use of neural networks enables creation of accurate models of animal populations, tracking migration routes, and estimating population size [17]. Neural network-based flight control expands the UAVs capabilities. Neural networks demonstrate dynamic adaptability through continuous learning from operational data, enabling real-time optimization of both flight parameters and image acquisition settings in response to unpredictable environmental variables. They can combine data from various sensors mounted on the drone to improve perception and situational awareness, which allows the drone to make more informed

[2] https://sky-space.ru/blog/fpv-dron (in Russ.)

[3] https://ru.rt.com/qo5p (in Russ.)

decisions. In addition, neural networks allow UAVs to move autonomously, easily maneuver around obstacles during flight and that is very important for monitoring remote territories. Neural networks can optimize trajectories for drones, which is useful for the applications such as aerial photography or surveillance, where certain trajectories must be followed for optimal data collection [61]. The use of neural network algorithms minimizes the time required for task implementation (from a few seconds to several minutes), but the neural network training can take tens of hours. At the same time, the user should have programming skills in environments such as Python or Java, and the computer on which the ML will be performed must be equipped with appropriate equipment [15].

Outcomes of AI application for animal monitoring are presented in some studies and reports posted on the Internet resources. Zhou M. et al. tested two deep learning neural network models: CNN and deep residual networks (ResNet), for their efficacy for the classification of four animal species: cattle (*Bos taurus*), horses (*Equus caballus*), Canadian geese (*Branta canadensis*) and white-tailed deer (*Odocoileus virginianus*). The results have showed that visible images collected at a distance of 60 m or less are sufficient for accurate classification, and that the most effective algorithm can be the ResNet model with 18 layers (ResNet 18), since the overall accuracy rate for animal identification was 99.18% [66]. The experiment conducted by D. Marchowski on counting populations of 33 waterfowl species demonstrated successful use of AI-integrated UAVs in 96% of 343 cases. ImageJ/Fiji software and ML methods with neural network algorithms such as DenoiSeg were used for automated counting [15]. Krishnan B. S. et al. used fusion approach for ML, combining several pairs of thermal and visible images acquired from drones. It was interesting that for white-tailed deer, which were typically cryptic against their backgrounds and often in shadows in visible images, the added information from thermal images improved detection and classification in fusion methods from 15 to 85%. It has been found that image fusion in combination with two models of deep neural networks is ideal for photographing animals that are cryptic against the background [23]. Combining images were taken from 75 and 120 m above ground level, a faster region-based CNN (Faster R-CNN) was trained using annotated images labelled "adult caribou", "calf caribou" and "ghost caribou" (animals moving between images and blurring individuals during processing of photogrammetric data). The model accuracy, precision, and repeatability was 80, 90, and 88%, respectively [17]. In Hortobágyi Nemzeti Park (Hungary), AI technologies are used for preservation of endangered Asian wild Przewalski horses. Researchers are using drones to monitor the horse herd behaviour. The acquired high-resolution footage is processed on the Microsoft Azure platform and analysed using AI, which is able to distinguish horses from other animals[4]. The first tests of the software developed by specialists of the Moscow Institute of Physics and Technology in cooperation with the Ministry of Natural Resources and Environment of the Russian Federation were conducted in the Land of the Leopard National Park (Primorsky Krai, Russia). The software program enables recognition of Amur leopards, Amur tigers and other wild animals[5]. Also, AI-based wild animal recognition system developed by NtechLab company is currently tested in Russia. The system is currently integrated with videos containing bear images, but in the future it is planned to expand its functionality to cover other wild animal species[6]. The Ministry of Natural Resources and Environment staff-members are performing aerial surveys using drones and neural networks in some Russian regions to search for ungulate aggregations[7].

Finally, it is worth noting that in 2024, a team of American researchers created the Aerial Wildlife Image Repository (AWIR), which is a dynamic interactive database with annotated images acquired from drones equipped with conventional and thermal imaging cameras. AWIR provides the first open-access repository for users to upload, annotate, and curate images of animals acquired from drones. The AWIR also provides benchmark datasets that users can download to train AI algorithms to automatically detect and classify animals. The AWIR contains 6,587 animal objects in 1,325 visible and thermal images of predominantly large birds and mammals [67].

CONCLUSION

Reliable data on population size and density are required for ASF spread prevention in wild boars and risk assessment. Animal carcass searching serves as a critical tool for early ASF detection. The combination of modern UAVs with neural network algorithms is a highly effective method of obtaining accurate and timely information about the natural environment, which, in particular, opens up new opportunities in the field of wild boar population monitoring. In the era of the active AI development and widespread UAVs use, application of innovative technologies in combination with traditional methods appears to contribute to enhancing the efficiency of searching for live wild boars and their carcasses as well as the reliability of the obtained data, that can improve animal health control as a part of ASF management strategies. Close cooperation of programmers, wildlife researchers and veterinarians are required for successful implementation of such approaches. Since AI-integrated UAV is a cutting-edge technique used in wildlife research field, it requires ongoing evaluation.

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Received 28.02.2025
Revised 08.04.2025
Accepted 05.05.2025

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Вклад авторов: Авторы внесли равный вклад на всех этапах работы и написания статьи.

https://doi.org/10.29326/2304-196X-2025-14-2-133-139

Bovine respiratory syncytial virus infection: clinical manifestations, pathogenesis and molecular epidemiology (review)

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ABSTRACT

Introduction. Bovine respiratory syncytial infection is widespread in all countries of the world, including the Russian Federation. The etiologic agent is *Orthopneumovirus bovis*, it belongs to the family *Pneumoviridae*, genus *Orthopneumovirus*. Cattle are the main reservoir of the virus.

Objective. This literature review aims to summarize and give analysis of the published data on clinical manifestations, pathogenesis and molecular epidemiology of the causative agent of bovine respiratory syncytial infection.

Materials and methods. The study is based on publications from the most authoritative domestic (eLIBRARY.RU) and foreign (Web of Science, Scopus, PubMed) sources, as well as the results of our own studies published in the literature.

Results. Animals of all ages are susceptible to the disease, the infection is most severe in calves under 6 months of age. The incidence of the herd is on average 60–80%. The nature of the infection varies from asymptomatic and mild to severe lower respiratory tract disease, including emphysema, pulmonary edema, interstitial pneumonia and bronchopneumonia, while the mortality rate among calves can reach 20%, and in adult animals the subclinical form is more often recorded. The virus has a powerful immunomodulatory effect. Severe damage to the respiratory tract is mediated mainly by hyperactivity of the immune response, and not by the replication of the virus itself. The virus increases the susceptibility of calves to secondary infections and promotes colonization of the lower respiratory tract by bacteria. Currently, ten genetic subgroups of the virus (I–X) have been identified using phylogenetic analysis of the nucleotide sequences of the G and N genes, between which there is a geographical correlation. In regions such as the Urals, Siberia, and the Republic of Kazakhstan, isolates of the virus of genetic subgroups II and III circulate among cattle.

Conclusion. The review presents current data on the etiology, pathogenesis features and clinical manifestations of bovine respiratory syncytial infection, as well as the genetic diversity of the pathogen in the world, in the Russian Federation and the Republic of Kazakhstan.

Keywords: review, respiratory syncytial infection, BRSV, cattle, pathogenesis, molecular epidemiology

Acknowledgements: The study was funded from the budget as part of the fulfillment of state task No. 0533-2021-0018 (Siberian Federal Scientific Centre of Agro-BioTechnologies, Russian Academy of Sciences).

For citation: Koteneva S. V., Glotov A. G., Glotova T. I., Nefedchenko A. V. Bovine respiratory syncytial virus infection: clinical manifestations, pathogenesis and molecular epidemiology (review). *Veterinary Science Today.* 2025; 14 (2): 133–139. https://doi.org/10.29326/2304-196X-2025-14-2-133-139

Conflict of interests: Glotov A. G. is a member of the editorial board of the "Veterinary Science Today" journal since 2020, but was not involved into the decision making process related to this article publication. The manuscript has passed the review procedure accepted in the journal. The authors did not declare any other conflicts of interests.

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УДК 619:616.98:578.831.31(048)

Респираторно-синцитиальная инфекция крупного рогатого скота: особенности клинического проявления, патогенеза и молекулярной эпизоотологии (обзор)

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РЕЗЮМЕ

Введение. Респираторно-синцитиальная инфекция крупного рогатого скота широко распространена во всех странах мира, в том числе и в Российской Федерации. Этиологический агент – *Orthopneumovirus bovis*, относящийся к семейству *Pneumoviridae*, роду *Orthopneumovirus*. Крупный рогатый скот – основной резервуар вируса.

Цель исследования. Целью данного обзора литературы являлось обобщение и анализ опубликованных данных об особенностях клинического проявления, патогенеза и молекулярной эпизоотологии возбудителя респираторно-синцитиальной инфекции крупного рогатого скота.

Материалы и методы. Информационной базой для проведения исследования служили публикации из наиболее авторитетных отечественных (eLIBRARY.RU) и иностранных (Web of Science, Scopus, PubMed) источников, а также результаты собственных исследований, опубликованных в литературе.

Результаты. Заболеванию подвержены животные всех возрастов, наиболее тяжело протекает инфекция у телят в возрасте до 6 месяцев. Заболеваемость поголовья составляет в среднем 60–80%. Характер течения инфекции варьирует от бессимптомного и легкого до тяжелого заболевания нижних дыхательных путей, включая эмфизему, отек легкого, интерстициальную пневмонию и бронхопневмонию, при этом уровень смертности среди телят может достигать 20%, а у взрослых животных чаще регистрируют субклиническую форму. Вирус оказывает мощное иммуномодулирующее действие. Тяжелые повреждения дыхательных путей опосредованы в основном гиперактивностью иммунного ответа, а не самой репликацией вируса. Вирус повышает восприимчивость телят к вторичным инфекциям и способствует колонизации нижних дыхательных путей бактериями. В настоящее время с помощью филогенетического анализа нуклеотидных последовательностей генов G и N выявлено десять генетических подгрупп вируса (I–X), между которыми существует географическая корреляция. В таких регионах, как Урал, Сибирь, а также в Республике Казахстан среди крупного рогатого скота циркулируют изоляты вируса генетических подгрупп II и III.

Заключение. В обзоре представлены актуальные данные об этиологии, особенностях патогенеза и клинического проявления респираторно-синцитиальной инфекции крупного рогатого скота, а также генетическом разнообразии возбудителя в мире, Российской Федерации и Республике Казахстан.

Ключевые слова: обзор, респираторно-синцитиальная инфекция, BRSV, крупный рогатый скот, патогенез, молекулярная эпизоотология

Благодарности: Исследование выполнено за счет бюджетных средств в рамках выполнения государственного задания № 0533-2021-0018 (СФНЦА РАН).

Для цитирования: Котенева С. В., Глотов А. Г., Глотова Т. И., Нефедченко А. В. Респираторно-синцитиальная инфекция крупного рогатого скота: особенности клинического проявления, патогенеза и молекулярной эпизоотологии (обзор). *Ветеринария сегодня.* 2025; 14 (2): 133–139. https://doi.org/10.29326/2304-196X-2025-14-2-133-139

Конфликт интересов: Глотов А. Г. является членом редколлегии журнала «Ветеринария сегодня» с 2020 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

Bovine respiratory syncytial infection (BRSI) is an acute, highly contagious viral disease that primarily affects the lower respiratory tract in cattle. BRSI ranks among the most significant infectious respiratory diseases in bovine populations [1, 2, 3, 4].

According to the current classification, the causative agent of the infection is *Orthopneumovirus bovis* (formerly Bovine respiratory syncytial virus, BRSV) belonging to the *Pneumoviridae* family, genus *Orthopneumovirus* [5]. To maintain consistency with the published literature the virus will be hereinafter referred to by its historical designation (BRSV).

BRSV was first isolated from calves during an outbreak of severe respiratory disease in Switzerland in 1969. Several surveys in the early 1980s and later confirmed that the virus is enzootic in calf populations worldwide. According to L. E. Larsen, the agent has the highest pathogenic potential of all viruses circulating in cattle [6].

BRSV is related to human respiratory syncytial virus (HRSV), and they exhibit similar epidemiological, clinical and pathological manifestations [7].

Cattle are the natural hosts and reservoirs of BRSV, but small ruminants may also contribute to the virus transmission [8]. The infectious virus or antibodies against it were also detected in sheep, goats, alpine chamois, bison, and camelids [9, 10, 11].

BRSI has a wide geographical distribution and is reported in many countries on all continents [12, 13, 14]. The virus spreads via airborne transmission. The seroprevalence varies greatly in different geographical regions and averages 30–70%, but may reach 100% [1, 15].

BRSV has been registered since 1975 in our country [16]. According to the Russian researchers, retrospective studies across 16 regions of the Russian Federation detected seroconversion to the virus in calves of different age groups, indicating its role in respiratory pathology: one month of age (4.0% of cases), 3–4 months of age (37.5%), 4–6 months of age (52.6%) and 7–9 months of age (50.0%) [17]. In Siberian farms, the BRSV seropositivity in animals averages 20–70% [18]. A recent study analyzing biomaterial samples collected during mass outbreaks of acute respiratory disease in 8 regions of the Ural and Siberian Federal Districts (Russia) and the Republic of Kazakhstan found the BRSV genome in 20% of cows and 14.3% of heifers. Additionally, the virus was detected in 3.05% samples from calves under one month old and 6.7% samples from calves aged 1–6 months [19].

The BRSV incidence in cattle ranges from 60 to 80%, with mortality in severe calf cases reaching up to 20% [20]. Infection rates exhibit seasonal patterns, peaking during winter months. The BRSV key characteristic is its capacity to infect hosts despite the presence of virus-neutralizing antibodies, leading to recurrent infections throughout animal lifetime [21].

BRSV affects cattle of all ages and breeds, though the most severe clinical manifestations typically occur in calves aged 1–6 months. In adult cattle, outbreaks primarily develop following either initial introduction of the pathogen into a seronegative herd or during reinfection events. The observed age-related resistance pattern, where adult animals demonstrate greater viral resistance than calves, likely reflects acquired immunity through repeated antigenic exposure. Clinical presentation patterns differ by herd im-

munity status: when BRSV is introduced to immunologically naive herds, cattle of all ages typically display clinical signs; in contrast, in herds with endemic viral circulation, clinical disease is predominantly observed in calves [22].

Risk factors affecting the prevalence of infection include animal age, herd size, animal density per unit area, introduction of new animals, seasonality, high milk production, reduced natural resistance in animals and zootechnical factors [23]. However, severe outbreaks may occur even in herds with optimal housing conditions, suggesting that BRSV can induce disease independently of predisposing environmental factors [24].

The mechanisms enabling BRSV persistence in cattle populations remain incompletely understood. Clinically affected animals are regarded as the primary infection sources, suggesting that recurrent outbreaks most commonly result from the reintroduction of the virus into herds prior to new disease events. However, BRSV can also be isolated from asymptomatic carriers, where it may persist for months, establishing latent infections that could explain outbreaks in relatively isolated calves. The virus can also circulate at minimal levels among seropositive cows, with periodic reactivation [1].

VIRAL GENOME CHARACTERISTICS

Orthopneumovirus bovis is an enveloped virus containing single-stranded negative RNA approximately 15,000 bp in length [1]. Virions may be spherical, but are usually filamentous or pleomorphic in shape, approximately 200 nm in diameter. The viral genome encodes nine structural proteins and two non-structural proteins. The structural proteins include three enveloped glycoproteins (F, G, SH), nucleocapsid proteins (N, P, L), nucleocapsid-associated proteins (M2–1 and M2–2) and matrix protein (M) [25].

The G protein mediates viral binding to host cells, while the F protein facilitates viral entry into cells, systemic spread within the host organism and formation of characteristic syncytia [21].

The F protein is involved in the immune response by stimulating the production of virus-neutralizing antibodies and facilitates the penetration of viral particles into host cells, as well as mediates the fusion of infected cells to form syncytia – multinucleated giant cells. The G protein is mainly involved in receptor binding and adsorption process [12]. The F and G genes play an important role in viral infectivity and are the main targets of the immune system [6, 20]. The F gene is highly conserved, and its nucleotide sequence variation is lower among BRSV isolates compared to the G gene [26]. Due to its high genetic variability, the G gene can be used for the evolutionary analysis of virus strains [7].

The SH protein is a short integral membrane protein. It plays an important role in inhibiting apoptosis during infection, promoting viral replication. This protein is not essential for viral replication, but is involved in evading the host immune response [27].

The nucleoprotein (N) plays an important role in viral transcription and replication, acting as a scaffold for the assembly of the viral ribonucleoprotein complex. It can be expressed on the surface of infected cells early in the viral replication cycle [28].

The phosphoprotein (P) acts as a regulatory factor for viral transcription and replication. The polymerase L is an RNA-dependent RNA polymerase responsible for viral transcription and replication [20].

The M protein is located on the inner surface of the viral envelope and plays a role in virion assembly. Unlike other viral mRNAs, M2 mRNA is translated into two distinct proteins, M2–1 and M2–2, through a ribosome termination-dependent reinitiation mechanism. The M2, M2–1, and M2–2 gene products serve as key regulatory proteins that modulate the BRSV replication cycle. M2–1 incorporates into the ribonucleoprotein complex to facilitate viral mRNA transcription, while M2–2 regulates the transition from transcription to replication [28].

The nonstructural proteins NS1 and NS2 modulate the innate immune response early in the viral replication cycle by interfering with interferon induction/signaling, dendritic cell maturation, and T-lymphocyte activation. Additionally, NS1 and NS2 inhibit apoptosis, thereby prolonging the survival of infected cells and enhancing viral production [28].

Currently, BRSV is classified into four antigenic subgroups (A, B, AB, non-typeable) [1] and ten genetic subgroups [7, 26, 29].

PATHOGENESIS

Orthopneumovirus bovis demonstrates cytopathic effects in cell cultures and induces extensive bronchial epithelial damage *in vivo*. The virus initially infects upper respiratory tract epithelial cells, then rapidly disseminates via cell-to-cell transmission to the lower respiratory tract, where it replicates in bronchioles [30]. Primary cellular targets include bronchial epithelial ciliated cells and alveolar type I pneumocytes [31]. BRSV has also been reported to infect intraepithelial dendritic cells and basal epithelial cells of the conductive airways, using *in vitro* cultures [32]. This broad cellular tropism within the respiratory tract enables efficient viral replication and systemic dissemination.

The direct pathological consequences of lytic viral replication include sloughing of necrotic epithelial cells, resulting in ciliostasis and impaired mucociliary clearance, and accumulation of exudate in the bronchioles and alveoli. The initial influx of polymorphonuclear neutrophils into the airways is rapidly replaced by a predominantly lymphomononuclear infiltration of peribronchiolar tissues and increased microvascular permeability, resulting in submucosal edema. The loss of ciliated epithelium increases the amount and viscosity of mucous secretions. Bronchiolitis, characterized by inflammation, necrosis, and obstruction of the bronchioles, leads to airway narrowing, airflow impairment, and respiratory distress. Lung consolidation occurs due to accumulation of inflammatory cells and fluid in the alveoli and bronchioles, resulting in additional respiratory distress. Interstitial pneumonia, another common pathological manifestation, develops from inflammation and thickening of the pulmonary interstitial tissue. In severe cases, the virus causes bronchiolar obstruction and alveolar damage, severely compromising the calf's respiratory function [25, 30].

The severity and duration of the disease depend primarily on the host immune response rather than on viral replication. Innate immune mechanisms provide the respiratory tract with the first barrier against establishment of productive infection. Subsequently, specific humoral and cellular immunity play a decisive role in eliminating

the infection and mitigating its course [30]. It has been established that severe BRSV disease begins at low viral load or after viral elimination and is associated with a hyperreactive immune response [33]. The detection of hyaline membranes and eosinophils in the caudal lung regions, even in areas without detectable virus, further confirms the role of immune-mediated pathological processes in BRSI pathogenesis [6].

The progression of infection and the immune response profile are primarily shaped by cytokine regulation patterns. BRSV employs multiple mechanisms to inhibit both innate and adaptive immune responses, negatively impacting immunological memory formation. During infection, dendritic cell functionality becomes compromised, resulting in dysregulated adaptive immunity: T-helper 1 (Th1) responses are delayed or suppressed, while Th2 cytokine production is upregulated [25].

The Th1-mediated immune response involves production of type I interferons (IFNs), particularly IFN-*α* and IFN-*β*, that play a critical role in inhibiting viral replication and dissemination. Various defense mechanisms are then activated, including the expression of antiviral proteins, to interfere with viral replication and dissemination. In addition to type I IFNs, innate immune cells secrete proinflammatory cytokines and mediators such as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-*α* (TNF-*α*), which promote inflammation and recruitment of immune cells to the site of infection, and mediate the systemic clinical features associated with infection.

Severe infection is associated with modulation of the Th2 immune response with increased expression of Th2-promoting cytokines and elevated concentrations of BRSV-specific IgE antibodies in lymphatic fluid [34]. Pathogenesis features of severe BRSV infection in calves include rapid neutrophil infiltration, excessive mucus production, delayed T cell response, expression of IL-4, IL-5, IL-10, IL-13 and IL-17 cytokines [28, 35, 36].

NS1 and NS2 proteins play a crucial role in immunosuppression by inhibiting the type I IFN response and other immune system components [33]. This leads to reduced antiviral immunity and diminished phagocytic activity in the lungs of infected animals, contributing to the development of bronchopneumonia [4].

Despite these protective mechanisms, cattle are subject to numerous reinfections with BRSV. Subsequent infections are generally less severe but maintain circulation of the virus in the population, facilitating infection of susceptible animals [25].

BRSV induces secondary bacterial infections in the lower respiratory tract, leading to the development of severe pneumonia [37]. The virus enhances the adhesion of bacteria (*Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Trueperella pyogenes*) to the epithelial cells of the respiratory tract [18, 33, 36, 38, 39, 40, 41, 42, 43]. Studying the mechanism of bacterial superinfection caused by *Pasteurella multocida* after BRSV infection, P. E. Sudaryatma et al. found that bacterial adhesion to epithelial cells of the lower respiratory tract of cattle is enhanced by increased expression of the platelet activating factor receptor (PAFR) [42]. Infection of bronchial and lung epithelial cells with BRSV increased adhesion of *Pasteurella multocida* to these cells, but did not affect the enhancement of adhesion to tracheal epithelial cells [41]. The results of the studies confirmed the ability of the virus to preferentially replicate in the lower respiratory tract. McGill J. L. et al. found that coinfection with BRSV and *Mannheimia haemolytica* in calves results in increased expression of IL-17, IL-21 and IL-22 in the lungs and peripheral blood [36].

CLINICAL SIGNS

The incubation period for BRSI in cattle is 2 to 5 days. In experimental studies, the onset and duration of clinical disease varied significantly, but symptoms were usually present between day 2 and 8 after infection [44]. Virus replication is detected at day 2–3 after infection and continues until day 7–10 after infection. Under natural conditions, the disease can manifest in various forms – ranging from subclinical cases with minimal clinical signs to severe forms featuring pronounced respiratory lesions, dyspnea, and even animal death.

The infection may be asymptomatic, limited to the upper respiratory tract, or affect both the upper and lower respiratory tract. In mild cases involving upper respiratory tract lesions, clinical signs include cough, serous-mucous nasal discharge (rhinitis) and conjunctivitis, mildly to moderately increased respiratory rate, fever, anorexia, and lethargy. In moderate cases, affected calves exhibit a respiratory rate exceeding 80 breaths per minute, tachypnea, harsh lung sounds across most of the pulmonary fields, and a pronounced cough.

Severe infection is characterized by high fever, profound depression and marked dyspnea. Affected animals may develop acute respiratory failure accompanied by grunting expiration, open-mouth breathing with protruding tongue, neck extension, head lowering, and salivary discharge. Pulmonary emphysema and edema are consistently observed in these cases, with occasional development of subcutaneous emphysema [6, 20, 34, 45].

Pathological changes are confined to the lungs. At necropsy, characteristic findings include interstitial pneumonia with cranioventral lung consolidation. The bronchial tree contains abundant mucopurulent exudate. Caudodorsal pulmonary regions frequently exhibit overdistension due to interlobular, lobular, and subpleural emphysema. The lungs appear grossly enlarged, with notable tissue friability. Tracheobronchial and mediastinal lymph nodes often show enlargement, edema, and occasional hemorrhage. In cases of bacterial superinfection, the parenchyma demonstrates increased edema and consolidation, with potential development of fibrinous or suppurative bronchopneumonia [6, 20].

BRSV MOLECULAR EPIZOOTOLOGY

Orthopneumovirus bovis, as most RNA viruses, exhibits significant genomic heterogeneity and low replication fidelity, facilitating the development of diverse viral subpopulations within a single host [1, 46].

Molecular genetic studies of BRSI outbreaks have demonstrated the circulation of identical viral strains among animals within single herds. During recurrent outbreaks, genomic divergence between viral strains can reach 11%, with emerging genetic variants becoming dominant [47].

Molecular epizootiological studies of BRSV have revealed significant geographic correlation between viral variants and emergence of new genetic lineages [46].

Currently, ten genetic subgroups of BRSV have been identified through phylogenetic analysis of G and N gene nucleotide sequences [14]. Subgroup I comprises strains isolated in Europe prior to 1980 [48]. This group strains were last recorded in cattle in Belgium in 1997.

Subgroup II strains circulate predominantly in Denmark, Sweden, Norway, and Japan [7, 14, 49]. Subgroup III incorporates strains originating from the USA, Italy, China, and Turkey [14, 46, 50]. Chang Y. et al. confirm the dominance of these strains in China [12]. Subgroup IV contains two distinct subclasses: IA and IB. Subgroup IV IA includes strains isolated in England in 1971 and 1976, and IB includes those isolated in the Netherlands in the 1980s [7]. Subgroups V and VI were identified in France and Belgium [7, 49], while subgroups VII and VIII were detected in Croatia (2018) and Italy [29, 46]. Recent surveillance has revealed two additional subgroups: IX (identified in Brazil [51] and Japan) and X (found in Japan) [26].

Until recently, there was no information on the genotypes of virus strains circulating in Russia. Glotov A. G. et al. were the first in our country to sequence the complete nucleotide sequence of the glycoprotein G gene of five virus isolates circulating among high-yield dairy cattle in Siberia and two vaccine strains. Based on phylogenetic analysis, it was established that the population of Siberian BRSV isolates is represented by two subgroups and one independent clade. Thus, NSO1 and NSO2 isolates recovered from calves in the Novosibirsk Oblast, were assigned to subgroup II of BRSV strains. The nucleotide similarity of these isolates with the Croatian strain was 99.09%, with the Swedish strain – 98.44%, with the Italian strain – 98.31%, and nucleotide mutations were found in the G gene sequence relative to other strains of subgroup II, leading to a number of unique amino acid substitutions. Alt3 and Alt4 isolates recovered from animals in the Altai Krai, were assigned to subgroup III. The nucleotide similarity of the Altai isolates with the Chinese strains was 98.73–97.34%. Unique amino acid substitutions were found in the sequences of isolate Alt3. A separate clade was formed by isolate K18, recovered from diseased heifers imported from Canada during an outbreak of mass respiratory disease after mixing them with local cattle, as well as the attenuated strain 375, included in the composition of two vaccines. The complete nucleotide sequences of the G glycoprotein gene obtained from BRSV isolates were deposited in the GenBank database under accession numbers OR426499–OR426505 [19].

CONCLUSION

The analysis of the presented data allows us to conclude that BRSI is widespread in many countries worldwide, including the Russian Federation. The infection causes significant economic losses in dairy and beef cattle production due to morbidity, mortality, and treatment and prevention costs. Cattle serve as the main reservoir of BRSI. BRSV replication is restricted exclusively to the respiratory tract. The virus increases susceptibility of calves to secondary infections and facilitates bacterial colonization of the lower respiratory tract, resulting in severe pathological manifestations that progress to bronchopneumonia or fibrinous pneumonia. A characteristic feature of BRSV is its capacity to induce immunopathology. The pathogenic effect of the virus stems from an imbalanced immune response skewed toward Th2-dependent processes. The pathogen exerts potent immunosuppressive effects, which contribute to disease complications and recurrent infections.

The relatively rapid evolutionary rate leads to significant genetic and antigenic heterogeneity among field virus strains. The identification and characterization of genetically distinct BRSV subgroups circulating within regional farms, along with comprehensive studies of their antigenic properties, are crucial for implementing effective infection control measures. This includes developing precise diagnostic methods and effective vaccines to reduce economic impacts.

Given BRSV's pronounced genetic variability, investigations into its molecular epizootology are of particular importance. Continued research on the genetic diversity of circulating BRSV strains and their pathogenic potential within our country remains essential for formulating effective immunoprophylaxis strategies against this infection.

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Received 15.01.2025
Revised 23.02.2025
Accepted 18.03.2025

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https://doi.org/10.29326/2304-196X-2025-14-2-140-147

Factors contributing to ocular pathologies in fish

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ABSTRACT

Introduction. With the decline in industrial salmon catches, fish hatcheries play a crucial role in replenishing stocks of these commercially valuable fish species. In aquaculture conditions, salmonids often demonstrate eye lesions, which reduce their adaptability in natural environments. Diagnosing these pathologies enables their classification by causative factors and development of therapeutic and preventive measures.

Objective. To search for and summarize scientific publications on ocular pathologies in salmonids at facilities engaged in industrial breeding, commercial farming or reproduction in Asia, America, Europe, and the Russian Federation.

Materials and methods. A search for Russian- and English-language articles in PubMed, Scopus, Web of Science, and eLIBRARY.RU databases was conducted. To prepare the review, 44 research papers published between 1975 and 2024 were used.

Results. The study demonstrates that eye lesions in Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*), and rainbow trout (*Oncorhynchus mykiss*), such as non-parasitic cataracts (lens opacity), keratopathy (corneal opacity), and unilateral or bilateral exophthalmia (eye protrusion), are reported at fish hatcheries and aquaculture facilities in the Northwestern region of the Russian Federation, as well as in several foreign countries. Eye lesions lead to decline in immunophysiological state and growth rates in aquaculture, reduction in the number of healthy fish, increased feed costs, and release of substandard fish from hatcheries into natural water bodies, sometimes resulting in their mortality. Basic information on factors contributing to the development of ocular pathologies in salmonids is presented. An analysis of therapeutic and preventive measures for eye lesions is provided, highlighting the importance of a differentiated and causative factor-dependent approach.

Conclusion. In global veterinary practice and fish pathology, the problem of eye protrusion in fish remains understudied, with limited research on the topic. This review analyzes and differentiates the key factors contributing to the development of ocular pathologies in salmonids. Identifying these factors will enable early diagnosis, determination, and development of preventive measures or effective treatment regimens, ultimately preserving fish health, improving the productive capacities of aquaculture establishments, and reducing economic losses.

Keywords: review, ocular pathology, exophthalmia, cataract, keratopathy

For citation: Bychkova L. I., Karaseva T. A., Pylnov V. A. Factors contributing to ocular pathologies in fish. *Veterinary Science Today*. 2025; 14 (2): 140–147. https://doi.org/10.29326/2304-196X-2025-14-2-140-147

Conflict of interests: The authors declare no conflict of interests.

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УДК 619:617.7:639.3

Факторы, способствующие развитию патологических изменений в глазах у рыб

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РЕЗЮМЕ

Введение. При сокращении промышленных уловов лососевых огромное значение принадлежит рыбоводным заводам по воспроизводству запасов этих видов промысловых рыб. В условиях искусственного выращивания лососевых часто отмечают поражения глаз, которые приводят к снижению уровня адаптации рыб в естественных условиях. Диагностика патологий позволяет классифицировать их по воздействующему фактору и разработать лечебные и профилактические мероприятия.

Цель исследования. Поиск и обобщение научных публикаций по проблеме патологии глаз у лососевых на предприятиях, занимающихся промышленным разведением и их товарным выращиванием или воспроизводством, в странах Азии, Америки, Европы и в Российской Федерации.

Материалы и методы. Проведен поиск русско- и англоязычных статей в наукометрических базах данных PubMed, Scopus, Web of Science, eLIBRARY.RU. Для подготовки обзора была использована информация из 44 научно-исследовательских работ, опубликованных в период с 1975 по 2024 г.

Результаты. Показано, что поражение глаз у атлантического лосося, кумжи, радужной форели в виде непаразитарной катаракты (помутнение хрусталика), кератопатии (помутнение роговицы), одно- или двухстороннего выпадения глазного яблока регистрируется на заводах по воспроизводству водных биологических ресурсов и на объектах аквакультуры в Северо-Западном регионе Российской Федерации, а также в ряде зарубежных стран. Отмечено, что поражение глаз влечет за собой снижение иммунофизиологического статуса и темпов роста в условиях аквакультуры, уменьшение количества

полноценной рыбы, увеличение кормовых затрат и выпуск неполноценной рыбы в естественные водоемы с рыбоводных заводов, а иногда ее гибель. Представлена основная информация о факторах, способствующих развитию глазных патологий у лососевых. Проведен анализ лечебно-профилактических мероприятий, применяемых при поражении глаз, показана значимость дифференцированного подхода к данной проблеме в зависимости от действующего фактора.

Заключение. В мировой ветеринарной и ихтиопатологической практике проблема выпадения глаз у рыбы недостаточно изучена, количество исследований на эту тему ограниченно. В данном обзоре проанализированы и дифференцированно представлены основные факторы, способствующие развитию глазных патологий у лососевых, выявление которых позволит осуществить раннюю диагностику, определить и разработать меры профилактики или эффективные схемы лечения, что, в свою очередь, приведет к сохранению здоровья рыб, повышению продуктивности рыбоводных предприятий и снижению экономических потерь.

Ключевые слова: обзор, патология глаз, экзофтальмия, катаракта, кератопатия

Для цитирования: Бычкова Л. И., Карасева Т. А., Пыльнов В. А. Факторы, способствующие развитию патологических изменений в глазах у рыб. *Ветеринария сегодня*. 2025; 14 (2): 140–147. https://doi.org/10.29326/2304-196X-2025-14-2-140-147

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

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INTRODUCTION

Growth and survival of wild and aquacultured fish largely depends on visual capacities as well as on prey detection and capture efficiency. The eye is an extremely important sensory organ for most fish species and one of the most vulnerable to negative environmental impacts. In aquaculture, there are many factors that can cause temporary or permanent changes in the cornea, lens, eyeball, or conjunctiva. In this regard, the eye condition is of diagnostic value and is often used as an indicator of fish health [1, 2].

The clinical signs of eye diseases in fish include cataracts, keratopathy, and exophthalmia. Cataract is a lens opacity that occurs due to pathological changes in the underlying epithelium or the lens fiber composition and structure [3]. Keratopathy is a complex of degenerative changes resulting in the compromised cornea protective function and its opacity. Exophthalmia is an ocular protrusion in fish resulting from mycotic infections or toxic environment.

In our country, the nonparasitic cataracts and other ocular lesions in salmonids were brought to notice by A. M. Marchenko in the 1980s. The disease was detected at the Maysky salmon hatchery in the Kabardino-Balkarian ASSR: lens opacity, hemorrhage in the postorbital region, unilateral or bilateral exophthalmia were reported in juvenile Terek trout. The same disease was later detected in young Caspian salmon in the Chaikend fish hatchery in the Azerbaijan SSR. The causes of the pathological changes in the eyes were carefully studied and analyzed [4].

In the following years, as the level of intensification of biotechnological processes and the volume of fish aquacultured in Russia and in the world increased, the specialists studied a wide range of eye pathologies in the aquacultured fish. However, the number of published studies documenting eye pathologies in fish remains limited compared to the extensive scientific literature on other organ studies. The diagnosis of an eye disease is established on the basis of epizootological data, deviations in the behavior of the diseased fish, clinical signs and laboratory test results. The histopathological tests allow classification of the eye conditions from acute inflammation to cataracts, keratitis, retinopathy and other changes.

The objective of this work was to summarize and review scientific papers on diseases of parasitic and nonparasitic etiology associated with ocular pathology in aquacultured fish, as well as factors causing ocular pathology.

SITUATION IN THE RUSSIAN FEDERATION AND IN THE WORLD

In 1990s, eye lesions were reported in juvenile Atlantic salmon and trout at salmon hatcheries in the Murmansk Oblast (Taibolsky, Umbsky, Kandalakshsky, Knyazhegubsky), Karelia (Petrozavodsky, Kemsky, Vygsky) and Arkhangelsk Oblast (Onezhsky and Solzensky). Such pathologies as exophthalmia and nonparasitic cataracts were reported. A specific sign of exophthalmia involved lesions of the cornea and periorbital skin fold characterized by dense white papule-like structures (1–2 mm diameter) with broad bases and tapered tips. As a result of the disintegration of these structures, the cornea and the periorbital skin fold were destroyed, which resulted in the leakage of the eyeball contents and its prolapse from the orbit in fish of all ages. Wild Atlantic salmon (*Salmo salar*) broodstock captured in the Kola River (Murmansk Oblast) demonstrated eyeball deformity, opacity and corneal thickening with perforations in the papule-like structure sites. Despite clinical presentation suggestive of infectious etiology, the causative agent of this eye pathology remained unidentified. In fish hatcheries in the Murmansk Oblast, eye lesions were more often observed in salmonids of all age groups: fry, underyearlings and two-year-olds. Cases of nonparasitic cataracts were reported in salmon hatcheries in Karelia [5].

Various types of ocular pathologies due to infectious and non-infectious agents and, as an exception, a parasitic agent, resulted in the need to analyze all available literature on ocular pathologies in fish. The number of publications on this problem in the world is limited, however the analysis of the literature demonstrated that the disease is widespread in the Scandinavian countries, USA, Canada, Japan and causes great economic damage, which is expressed in the decreased growth rate and increased feed costs, compromised immunity and higher susceptibility of

the fish to bacterial pathogens in the aquaculture environment [6, 7, 8]. In 1960s and 1970s, specific eye lesions were first reported in Japan and the USA as pale nodules and granulomas of varying morphology in aquacultured rainbow trout, yellowtail (*Seriola quinqueradiata*), as well as in salmonids of the genus *Salmo* and *Oncorhynchus* demonstrating bacterial renal disease, tuberculosis and streptococcal infection [9, 10, 11].

Ocular pathologies causing vision loss in fish have emerged as a critical global challenge for hatcheries and aquaculture farms [12, 13].

FACTORS OF OCULAR PATHOLOGY DEVELOPMENT IN FISH

For more than 100 years, the publications on ocular pathologies have been mainly related to cataracts of parasitic etiology associated with infection with larval *Diplostomum* spp. trematodes. Parasitic cataract, or black spot disease, occurs in both wild and aquacultured fish. In aquaculture, fish grown in lakes, ponds, and mesh cages are susceptible to the disease. The infected fish may demonstrate exophthalmia, hemorrhages, cataracts, retinal detachment, decreased growth rate, and cachexia [7, 10, 14].

Among the numerous factors resulting in visible ocular disorders in fish, the three key ones include the following:

– effects of bacteria and viruses;

– unbalanced diet (alimentary diseases);

– poor water supply, contamination of the water with toxic and chemical substances.

Infectious diseases. Bacteria and viruses often induce pathological changes in the eyes of fish [15]. Along with other clinical signs of infection, exudative or exudative-hemorrhagic inflammation in fish can be manifested as unilateral or bilateral exophthalmia. In this condition, the fish's eye abnormally protrudes from the orbit due to pressure from inflammatory exudate accumulating behind the eyeball (Fig. 1).

Exophthalmia and hemorrhages in the eyes of aquacultured rainbow trout were indicated by D. W. Bruno et al. in cases of acute viral hemorrhagic septicemia (VHS). VHS is widespread in Europe, North America, Japan, and Taiwan [10]. Similar pathology is observed in plasmocytoid leukemia of chinook salmon (*Oncorhynchus tshawytscha*) along the western coasts of America and Canada. The disease causative agent in salmonids is a retrovirus (salmon leukemia virus, SLV). Exophthalmia and subsequent blindness are typical for fish infected with viral encephalopathy and retinopathy (VER, or viral nervous necrosis, VNN) [14, 16].

Fig. 1. Infectious exophthalmia and ocular hemorrhagic lesions in farmed rainbow trout (photo by T. A. Karaseva)

In 1980s, an outbreak of a new disease was reported in juvenile Caspian trout (*Salmo trutta caspius*) at the Chaikend fish hatchery, Azerbaijan SSR, when specific eye lesions were observed. Clinical examination demonstrated dense white papule-like structures (1–2 mm in diameter) with broad bases and tapered tips on the cornea and periorbital skin fold. As a result of their disintegration, the cornea and the periorbital skin fold degraded, which resulted in the discharge of the eye contents and eyeball prolapse. It was histologically determined that papule-like structures consist of epithelium, Bowman's membrane and the corneal stroma. Eosinophilic and small basophilic inclusions were observed in the cytoplasm of the epithelial cells. Clusters of virus-like particles (30–40 nm) were detected using electron microscopy. The experts suggested these particles to be a virus of the *Picornaviridae* family [4, 17].

In the early 1990s, fish pathologists A. M. Marchenko and T. E. Rodina described a coinfection in Caspian trout underyearlings and broodstock, which was caused by *Renibacterium salmoninarum* bacterium (causative agent of bacterial kidney disease) and an unknown filterable agent, presumably a virus. The diseased underyearlings demonstrated papule-like formations in the eyes similar to those detected in fish at the Chaikend fish hatchery, pale gills, sandy-colored liver, and gray edematous kidneys. The highest mortality rate (25%) was reported in fish with the specified clinical signs. Thorough disinfection of fish tanks and equipment, selection of clinically healthy broodstock, and feeding therapeutic feed with erythromycin helped to stop the fish mortality. However, for a long time, the ocular pathology was reported in singular individuals [4].

It is important that in the coming years, these diseases were not reported in the Caspian trout, and the suspected viral pathogens did not spread over the territory of the Russian Federation and were not reported in salmonids in other regions. Perhaps their spread was limited to the Caspian Sea basin. The viruses are known to lack epizootic potential in the ecosystem due to the rare contact of individuals of the same species, but with a high density of fish in aquaculture they can acquire pronounced pathogenic properties and trigger a disease outbreak [18].

Exophthalmia, hemorrhages in the eyeball and a wide range of signs of chronic pathology are reported among the symptoms of bacterial diseases [8, 19, 20, 21]. For example, in 1986, at the Taibolsky fish hatchery in the Murmansk Oblast, a specific ocular pathology was first detected in juvenile Atlantic salmon (*Salmo salar*). Uncontrolled fish transportation resulted in rapid disease spread over the salmon hatcheries in the Murmansk and Arkhangelsk Oblasts and in Karelia [22, 23]. In aquaculture practice, this pathology was reported as exophthalmia, nonparasitic cataract, or mechanical injury. The disease was observed in salmonids of all age groups raised in fish hatcheries: fry, underyearlings and two-year-olds [5, 22, 23]. The disease-typical signs were also detected in rainbow trout (*Parasalmo mykiss*) when grown in marine net cages,

Eurasian river perch (*Perca fluviatilis*), common minnow (*Phoxinus phoxinus*) and ninespined stickleback (*Pungitius pungitius*), which inhabit freshwater lakes – water sources of the salmon hatcheries [22, 23].

The bioassay results indicated that exophthalmia in Northwest fish hatcheries was caused by gram-positive cocci bacteria, which were initially identified as *Streptococcus* sp. In terms of biochemical properties, 93 of the obtained serotypes were homogeneous and in terms of antigenic properties they were close to the causative agent of streptococcal infection in yellowtail [22]. The disease was called streptococcal infection in salmonids, respectively. Later, in the 9th edition of "Bergey's Manual of Determinative Bacteriology" (1993), these bacteria, pathogenic for salmonids and yellowtail, were assigned to the species *Enterococcus seriolicida* [24]. Thus, it was found that pathological processes in the eyes of fish developed in case of streptococcal infection. The disease cause is generally similar to septicemia throughout the year, so ocular pathology, which is only one of the enterococcal infection signs, develops gradually. At the beginning of the disease, the clinical signs are mainly manifested by unilateral exophthalmia and hemorrhages in the eyeball. Later, at different stages of the pathological process development, optic neuritis, corneal ulceration, vitreous prolapse and lens extrusion via pupillary rupture are reported, or leukoma is formed in fish. In the terminal disease stage, complete ocular prolapse occurs with conjunctival rupture [25]. Herewith, underyearlings salmonids do not survive, and in older fish the eye socket may become filled with pigmented connective tissue. Histological examination of the diseased fish revealed the fundus and iris hyperemia, corneal keratinization, delamination, erosion and necrosis, hyperemia and hemorrhages in the choroid, retinal deformity [22]. A number of authors find much in common in the epizootology of streptococcosis and such diseases as furunculosis and bacterial kidney disease, the etiological agents of which are closely associated with the hosts, and outside the body of fish can survive only for a limited time in water and bottom sediments [26].

At the same time, in the 1980s and 1990s, the juvenile salmonids demonstrated cataracts, the etiology of which could not be established. Thus, at the Petrozavodsky fish hatchery, singular cases of ocular lesions were reported in the juvenile salmon and lake salmon (*Salmo salar morpha sebago*); at the Vygsky fish hatchery, cataracts were observed in 8% of fish, and at Kemsky fish hatchery – in 9% of the total number of fish grown at the hatchery. No mortality was reported in the diseased fish. The microbiological test results did not confirm the infectious nature of cataracts in fish on Karelian aquaculture farms, though it was assumed against the background of widespread streptococcal infection [5].

Another bacterial disease that often affects fish's eyes is vibriosis. It is a widespread disease of wild and aquacultured fish in marine and brackish waters [27, 28, 29, 30]. Cold water vibriosis usually refers to *Listonella* (*Vibrio*) *anguillarum*-associated septicemia (Bergman, 1909). This *Vibrio* species represents aquatic saprotrophic microflora, it can be found in water, soil, mollusks and other marine inhabitants [9, 31, 32]. The main route of the infection transmission is with water and through contact with the diseased fish. On marine farms, *Listonella anguillarum* is released into the marine environment from the intestines, kidneys, ulcers and damaged eyes of the diseased and recovering fish. Serous hemorrhagic inflammation and tissue necrosis are typical at all stages of vibriosis. Of all the salmonids aquacultured in Europe, rainbow trout is the most susceptible to the disease. In the 1970s and 1980s, during the period of intensive development of trout aquaculture, vibriosis was widespread in the Gulf of Finland and Gulf of Riga located in the Baltic Sea. The disease outbreaks were reported in the farmed trout nearly every year, averaging a 30% mortality rate [33]. In northern European Russia, a vibriosis outbreak was first reported in two-year-old rainbow trout at White Sea fish farms in July 2004, two weeks after their transfer to marine cages. The disease was acute, and the mortality rate exceeded 40% [34].

Fig. 2. Exophthalmia, corneal necrosis, and eyeball ulceration in rainbow trout with vibriosis caused by Listonella (Vibrio) anguillarum (photo by T. A. Karaseva)

Unilateral exophthalmia is generally observed in infected fish at the initial vibriosis stages. The subsequent ocular lesions are characterized by degeneration of all eye structures and tissues. They involve destruction of the cornea, dislocation of the lens, eyeball erosion and ulceration, and bleeding (Fig. 2). Less frequently, fish develop leukoma. In the survived individuals, the affected eye usually remains in place, with residual tissues staying within the eye socket [35].

Alimentary diseases. Since the early 1990s, the increase in the number of ocular pathologies in fish coincided with the introduction of granular salmon feeds and attempts to replace high-quality animal proteins in these feeds with plant proteins or low-quality animal protein substitutes. For the growth of fish, especially salmonids, use of balanced feed in the diet is very important. A deficiency of even one component in the feed leads to the development of changes, often irreversible, in the body of fish, including those in the eyes [36, 37]. When using unbalanced feeds, the body is deficient in vitamins, amino acids, and mineral elements, which causes various types of eye lesions: cataracts, keratopathy, and eyeball protrusion. The S. G. Hughes review on ocular diseases that occur due to imperfect salmon feeds examines six types of pathologies. They include deficiency of riboflavin (vitamin B_2), thiamine (vitamin B_1), vitamin A, sulfur-containing amino acids (methionine and cysteine), tryptophan and zinc. In case of ocular pathologies, common are three main signs:

cataracts, keratopathy, and exophthalmia. Furthermore, both eyes are affected [2].

Thus, in case of riboflavin (vitamin B_2) deficiency in feed, lens opacity and sometimes corneal rupture with adhesion of the lens to cornea were reported in fish. Histological examination revealed corneal thickening, as well as vascularization, hyalinization and degeneration of the lens subepithelial layers. All these changes led to the loss of its transparency. The consequences of thiamine deficiency (vitamin B_1) in salmonids involved corneal opacity and inflammation, blindness. Pathological changes in the eyes were noted in experimental conditions simulating lack of vitamin A in the diet of char and rainbow trout. Corneal and lens opacification as well as photophobia were reported in rainbow trout. The addition of beta-carotene to the feed prevented eye lesions only in warm water (above 12.4 °C), no such therapeutic effect was, however, observed at lower water temperatures [36]. Exophthalmia occurred with a lack of ascorbic acid and tocopherol (vitamin E) [37, 38].

Ocular pathologies in fish are caused by a deficiency of sulfur-containing amino acids in the feed: methionine, cysteine, tryptophan. Their deficiency contributes to the lens opacification and involves the adjacent ocular tissues in the degenerative process. Japanese scientists found that when fed a zinc-deficient diet, the rainbow trout developed cataracts. The cataract that had already appeared did not resolve even when the fish began to receive feed with a sufficient amount of zinc. The zinc requirement for fish is 15–30 mg/kg of feed [36, 39].

Corneal and lens opacification was reported when mold fungi were detected in the eyes of juvenile Atlantic salmon. The source of infection was substandard granular feed, and the disease was systemic. Moreover, the mold fungi formed mycelia in the fish eyes [40].

Since early 21st century, solving the problem of adequate feeding in the aquaculture of valuable fish species (salmon, whitefish) has significantly reduced the number of fish with ocular damage and improved the disease situation in salmon hatcheries in the Murmansk Oblast and Republic of Karelia.

Water toxicoses. The aquatic environment is often polluted by petroleum products, pesticides, chemical dyes, nitrates, and heavy metal salts [41]. In aquaculture, the fish lens is used as a test organ to assess the toxicity of chemical and other compounds [42, 43]. Experimental studies were conducted in this area, which demonstrated that the fish lens was highly sensitive to anthropogenic factors. The effects of such industrial toxicants as trichlorobenzene, nitrobenzene, 3-naphthol and salts of heavy metals (lead, copper, zinc) on fish were examined. It was found that these toxic compounds caused a change in proliferative activity in the lens epithelium [44]. The effect of toxicant exposure on cytodifferentiation, as well as on changes in the biochemical composition and optical properties in the lens nucleus and cortex (decreased optical density of the lens nucleus) was established. The peculiarities of the reaction of various cytodifferentiation zones of the fish lens epithelium to a number of toxic compounds (benzene, inorganic and heavy metal salts) are also reported, which consist in the inhibition or stimulation of mitotic activity in the germinal zone of the lens epithelium [41]. The formation and proliferation of tissue fibers further leads to cataracts.

Thus, the toxic effects of the aquatic environment also contribute to the development of pathological processes in the eyes of fish.

In the North-West of the Russian Federation, the Onezhsky fish hatchery was the most affected in terms of ocular pathologies for several years (2017–2022). According to the results of our long-term research, the main cause of the emerging pathologies in salmonids at this farm was the quality of the incoming water. Clinical investigation revealed exophthalmia in underyearling and two-year-old Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*). The highest level of eye lesions was reported in two-year-old Atlantic salmon – from 8 to 20%. In 2023, the percentage of fish with eye lesions was minimal (about 1%). The fish with unilateral or bilateral exophthalmia demonstrated gill pallor, pale and friable hepatic tissue, yellow bile in the gallbladder, and black discoloration of the posterior kidney. Microscopic examination of scrapings from the postorbital region of the protruded eyeball revealed a large number of coccoid and rod-shaped bacteria. After inoculation of the fundus contents on the nutrient media, the growth of colonies of bacteria of the genera *Staphylococcus*, *Flavobacterium* (*Flexibacter*), *Pseudomonas* was reported. The main source of water supply for the Onezhsky fish hatchery is lake Andozero. The meteorological conditions in summer lead to the lake shallowing, as a result of which the water entering the hatchery contains a large amount of inorganic suspensions. In summer, the water temperature rises and the oxygen level drops to 3.3–3.5 mg/L. It was perhaps these factors that contributed to the emergence and development of pathological processes in the eyes of salmonids with further exophthalmia, usually on the left side. To stabilize the water quality and temperature conditions in the pools, reconstruction of the water supply system with the installation of cooling equipment is essential.

CONCLUSION

When growing different types of fish in aquaculture conditions, there is a risk of various pathological processes. Among the factors contributing to the development of the pathological process in the eyes of fish, important are the following: pathogenic microorganisms, unbalanced feed, toxic substances. Despite the limited number of Russian and foreign publications on the problem of ocular pathology in salmonids, the literature review allows for the conclusion that the diseases leading to blindness in fish are an urgent problem for the fish hatcheries and farms all over the world.

In many descriptions of infectious and nutritional diseases, the term "exophthalmia" (eye protrusion) is used, so it is obvious that exophthalmia is a comprehensive term for many diseases and the most common pathological condition that can be observed in the eyes of fish.

In order to prevent any problems associated with ocular diseases, it is necessary to monitor the disease situation. An integrated approach is essential, which includes evaluation of the water source, monitoring of the incoming water quality during roe incubation and growing larvae to an adult, use of balanced feeds for salmonids, ichthyopathological monitoring of the immuno-physiological status of fish, and preventive measures to control infectious and parasitic diseases of fish. This will enable effective monitoring and minimize the risk of various ocular diseases in aquacultured salmonids.

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Received 20.11.2024
Revised 20.01.2025
Accepted 13.03.2025

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Вклад авторов: Бычкова Л. И. – работа с литературой, подготовка текста, анализ и обобщение; Карасева Т. А. – редактирование текста, подбор литературных источников, подготовка текста, анализ и обобщение; Пыльнов В. А. – администрирование, редактирование, анализ текста.

https://doi.org/10.29326/2304-196X-2025-14-2-148-155

Epizootic situation on contagious porcine diseases in the Republic of Burundi

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ABSTRACT

Introduction. In Burundi, where 80% of the population are engaged in livestock farming, industries with short reproduction cycles (pig farming, poultry farming) prevail. Despite government support measures and annually increasing pig population, the country has been unable to fully meet the demand for livestock products. This is due to numerous problems in the sector, with infectious animal diseases being the primary issue. Infectious disease outbreaks can have catastrophic consequences for the human population, including threats to food security, loss of access to animal protein, increased production costs due to the need for expensive disease control measures, and risks to human health in case of zoonotic diseases.

Objective. The aim is to study the nosological profile of porcine infectious diseases, identify factors contributing to animal infections and assess the swine erysipelas epizootic situation in the Republic of Burundi from 2018 to 2023.

Materials and methods. Data of annual reports of the General Directorate of Animal Health and test results of the National Veterinary Laboratory of Burundi (2018–2023) were used to analyze the epizootic situation on infectious porcine diseases. Retrospective and epizootiological analyses were conducted and variational statistical methods were applied.

Results. The analysis revealed a high prevalence of porcine parasitic diseases, which is attributed to the equatorial climate. Within the overall structure of infectious diseases, parasitic infestations ranked first, growing from 81.2% in 2018 to 92.8% in 2023. Bacterial infections were the second most widespread, rising from 3.6% in 2018 to 6.3% in 2023. A steady increase in swine erysipelas cases was observed: in 2023 the number of cases was 1.7 times higher than in 2022 and seven times higher than in 2020. Moreover, the number of provinces where the disease is detected is annually growing. Swine erysipelas is currently reported in 12 out of 18 Burundian provinces.

Conclusion. The Republic of Burundi suffers significant annual losses due to animal deaths caused by infectious disease outbreaks. In the absence of specific disease prevention measures (particularly for erysipelas) and weak veterinary control of animal movements between households, infections spread rapidly. Therefore, studying the epizootic situation and developing measures to stabilize it under local conditions is a crucial scientific and practical task for ensuring biological and food security.

Keywords: pig farming, socio-economic factors, climatic factors, parasitic diseases, bacterial infections, swine erysipelas, Republic of Burundi

Acknowledgements: This study was carried out as part of the research and development project "Improving methods of diagnosis, treatment and prevention of diseases in food-producing animals, poultry and fur animals" (Registration No. 121032300041-1). The authors express their deep gratitude to Dr. Ntakirutimana Désiré, Director of Animal Health Department of Burundi, for providing statistical data from annual livestock reports, and to Dr. Niyokwizera Pascal, Head of the National Veterinary Laboratory in Bujumbura, for access to laboratory test results.

For citation: Koshchaev A. G., Niyongabo H., Gorkovenko N. E., Nimbona C., Ntirandekura J.-B. Epizootic situation on contagious porcine diseases in the Republic of Burundi. *Veterinary Science Today.* 2025; 14 (2): 148–155. https://doi.org/10.29326/2304-196X-2025-14-2-148-155

Conflict of interests: The authors declare no conflict of interests.

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УДК 619:616.9:636.4:616-036.22(675.97)

Эпизоотическая ситуация по заразным болезням свиней в Республике Бурунди

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РЕЗЮМЕ

Введение. В Бурунди, где 80% жителей занимается животноводством, преобладают отрасли с коротким циклом воспроизводства (свиноводство, птицеводство). Несмотря на государственные меры поддержки и ежегодный прирост численности поголовья свиней, в стране не удается обеспечить население животноводческой продукцией в полной мере. Это связано с тем, что в отрасли существует немало проблем, среди которых первое место занимают заразные болезни животных. Вспышки инфекционных заболеваний могут иметь катастрофические последствия для населения страны, связанные

с подрывом продовольственной безопасности, потерей доступа к животному белку, повышением себестоимости животноводческого производства из-за необходимости применения дорогостоящих мер по борьбе с заболеваниями, последствиями для здоровья человека в случае возникновения зоонозов.

Цель исследования. Изучение нозологического профиля заразной патологии свиней, выявление причин, способствующих инфицированию животных, и оценка эпизоотической ситуации по роже свиней в Республике Бурунди за период с 2018 по 2023 г.

Материалы и методы. Для анализа эпизоотической обстановки по заразным болезням свиней использовали данные ежегодных отчетов Генерального управления животноводства, а также результаты исследований Национальной ветеринарной лаборатории Республики Бурунди за 2018–2023 гг. В процессе работы выполняли ретроспективный и эпизоотологический анализ, применяли методы вариационной статистики.

Результаты. Проведенный анализ показал широкое распространение паразитарных болезней свиней, что связано с особенностями экваториального климата. В общей структуре заразных болезней инвазии занимают лидирующее место с ростом от 81,2% в 2018 г. до 92,8% в 2023 г. На втором месте по распространению находятся инфекционные болезни бактериальной этиологии – от 3,6% в 2018 г. до 6,3% в 2023 г. Выявлено стабильно растущее число случаев рожи свиней: в 2023 г. зарегистрировано в 1,7 раза больше случаев по сравнению с 2022 г. и в 7 раз больше по сравнению с 2020 г. При этом с каждым годом возрастает количество провинций, где выявляют данное заболевание. В настоящее время в 12 из 18 провинций Бурунди регистрируется рожа свиней.

Заключение. Республика Бурунди ежегодно несет большие убытки от гибели животных в результате вспышек заболеваний инфекционной природы. При отсутствии в стране специфической профилактики заразных болезней, в частности рожи, слабом контроле со стороны ветеринарной службы за перемещениями животных между домовладениями инфекции быстро распространяются. Поэтому изучение эпизоотической ситуации и разработка мер для ее стабилизации в конкретных условиях является важной научной и практической задачей для обеспечения биологической и продовольственной безопасности.

Ключевые слова: свиноводство, социально-экономические факторы, климатические факторы, инвазионные болезни, бактериальные инфекции, рожа свиней, Республика Бурунди

Благодарности: Работа выполнена в рамках темы НИОКР «Совершенствование методов диагностики, лечения и профилактики болезней продуктивных животных, птиц и пушных зверей» (регистрационный номер 121032300041-1). Авторы выражают глубокую признательность директору Управления охраны здоровья животных Республики Бурунди Нтакирутимана Дезире за предоставленные статистические данные ежегодных отчетов по животноводству; начальнику Национальной ветеринарной лаборатории г. Бужумбуры Нийоквизера Паскалю за возможность использовать результаты лабораторных исследований.

Для цитирования: Кощаев А. Г., Нийонгабо Х., Горковенко Н. Е., Нимбона К., Нтирандекура Ж.-Б. Эпизоотическая ситуация по заразным болезням свиней в Республике Бурунди. *Ветеринария сегодня*. 2025; 14 (2): 148–155. https://doi.org/10.29326/2304-196X-2025-14-2-148-155

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

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INTRODUCTION

The Republic of Burundi is situated in East Africa between the Congo River basin and the eastern highlands. The country shares borders with Rwanda to the north, Tanzania to the east, and the Democratic Republic of the Congo to the west, with its southwestern frontier extending along the shores of Lake Tanganyika. Burundi's topography is characterized by a highland plateau featuring dramatic elevation variations ranging from 772 to 2,684 meters above sea level, the climate is equatorial.

Due to the relief features, Burundi has a wide variety of natural and climatic zones. The average annual temperature ranges from 17 to 23 °C. The country receives substantial precipitation, averaging 1,500 mm annually. Rainy seasons last from February to May and from September to December [1, 2].

With a population of 13.2 million (2023), Burundi has one of Africa's highest population densities at 442 people/km^2. The agricultural sector employs 80% of the workforce, with livestock production accounting for 14% of national gross domestic product (GDP) and 29% of agricultural GDP. However, the country faces growing pressure on land resources, particularly due to shrinking pasture areas. This has led to a shift toward livestock species with shorter reproduction cycles, and many farmers are showing interest in pig farming. Pig and poultry farming are gaining popularity as an alternative to cattle breeding, especially among low-income families. Over 79.2% of households own livestock, 12.9% of them have at least one pig [3].

Over the past few years, the Government of Burundi, in collaboration with international trade partners, has actively supported the pig breeding sector through several key initiatives. This bilateral cooperation has facilitated implementation of specialized breeding projects to develop improved pig breeds and establishment of modern animal breeding centers across the country.

Pursuant to Presidential Decree No. 100/087 of 26 July 2018 "On the Organizational Structure of the Ministry of Environment, Agriculture and Livestock, the Directorate General of Livestock" includes three divisions:

– Directorate of Animal Health, including the single service of the National Veterinary Laboratory (NVL);

– Directorate of Development of Livestock Farms, including the single service of the National Centre for Artificial Insemination (CNIA);

– Fisheries Development Authority, including the single service of the National Center for Research and Development of Fisheries and Aquaculture (CNDAPA).

The mission of the Directorate for Animal Health is to prevent spread of animal diseases in general and domestic animal diseases in particular [4].

Despite government support initiatives, the anticipated expansion of the national pig population has failed to achieve projected targets. This shortfall primarily stems from challenges confronting the sector, most notably – persistent outbreaks of infectious animal diseases, including zoonoses [5].

Swine erysipelas represents one of the most economically impactful diseases affecting pig production in Burundi. The infection primarily spreads among healthy pigs through consumption of contaminated feed and water [6, 7]. It is caused by *Erysipelothrix* spp., that are rod-shaped gram-positive facultative anaerobic bacteria. Only eight species belong to this genus, among which is the most frequently isolated *Erysipelothrix rhusiopathiae* [8, 9, 10]. While pigs serve as the main reservoir for this bacterium, *E. rhusiopathiae* has also been identified in various domestic animals, fish and birds [11, 12]. In humans, the pathogen causes erysipeloid disease, predominantly affecting individuals in high-risk occupations including veterinarians, butchers, farmers, and fishermen [13, 14, 15]. Although human *E. rhusiopathiae* infections remain relatively uncommon, surveillance data indicate a rising trend in human cases in recent years [10, 16]. Due to its zoonotic potential, swine erysipelas is currently recognized by the World Health Organization as a significant zoonotic disease [17].

The aim of the study was to investigate the nosological characteristics of swine infectious pathology, identify the factors contributing to animal infection, and assess the swine erysipelas epizootic situation in the Republic of Burundi for the period from 2018 to 2023.

MATERIALS AND METHODS

The study was carried out at the Department of Microbiology, Epizootology and Virology at the Kuban State Agrarian University named after I. T. Trubilin as part of the research and development project: "Improving methods of diagnosis, treatment and prevention of diseases in food-producing animals, poultry and fur animals" (Registration No. 121032300041-1).

To analyze the epizootic situation with regard to porcine infectious diseases in the Republic, we used data from the annual reports of the Directorate General of Livestock, as well as the study results of the National Veterinary Laboratory of the Republic of Burundi for 2018–2023. In the course of the work we performed a retrospective and epizootiological analysis and applied methods of variational statistics.

RESULTS AND DISCUSSION

An analysis of statistical data on pig farming development in the Republic of Burundi revealed a consistent upward trend in livestock numbers during the study period (Fig. 1). This growth was facilitated through collaborative efforts between public and private organizations to establish supportive socio-economic conditions for pig-rearing households [18]. The country has implemented a "solidarity chain" system, where breeding centers distribute pigs free of charge to small farms and households, with recipients subsequently providing an equivalent number of offspring to animal-deprived households.

However, a significant number of pigs die due to inappropriate conditions, such as inadequate sanitation in pig houses, poor-quality feed and infectious diseases.

The indoor pig-rearing system predominates in Burundian households. Notably, over 70% of household pig facilities are constructed without adhering to World Organization for Animal Health (WOAH) recommendations [19], a key factor facilitating the emergence and spread of infectious and parasitic diseases. Most traditional pig houses feature wooden structures with clay-filled cracks, lack proper roofing or have fragile roof coverings, and typically receive no regular cleaning. In contrast, a minority of modernized facilities include designated feeding areas, replaceable bedding, proper liquid manure drainage, concrete floors, brick walls and durable roofing materials.

Figure 2 shows the quantitative data for traditional and modernized pig-rearing facilities.

While the total number of pig housing facilities has increased, the proportion of those meeting modern standards has declined steadily from 29.85% in 2018 to 25.35% in 2023, reaching the minimum in 2019 (20.54%). Consequently, over 70% of pig production facilities in the country fail to meet basic zoohygienic requirements, which may significantly increase the risk of infectious and parasitic disease outbreaks. Another contributing factor

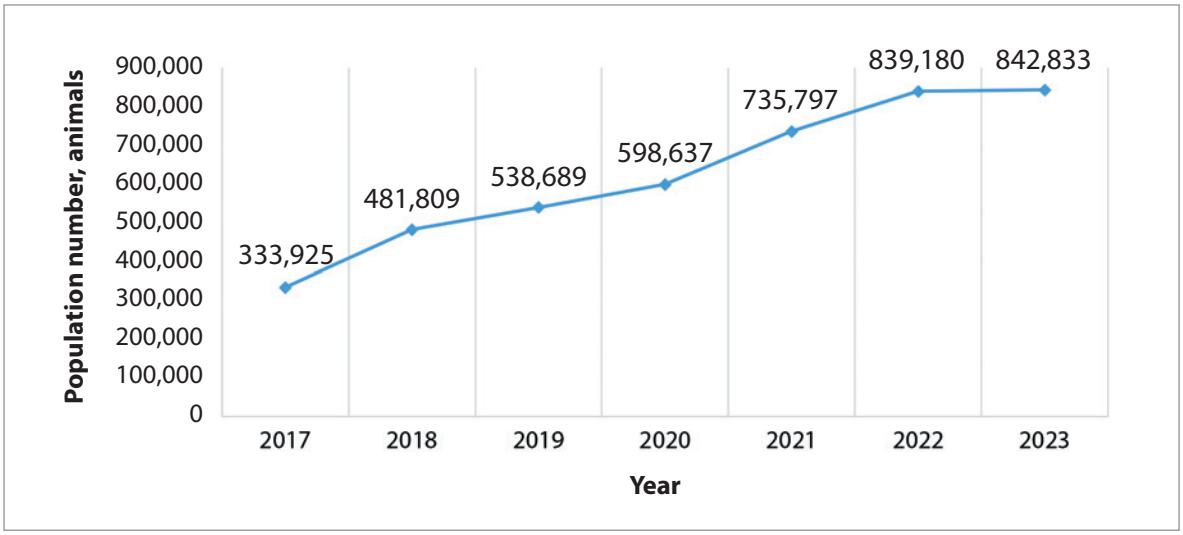

Fig. 1. Dynamics of pig population in the Republic of Burundi in 2017–2023

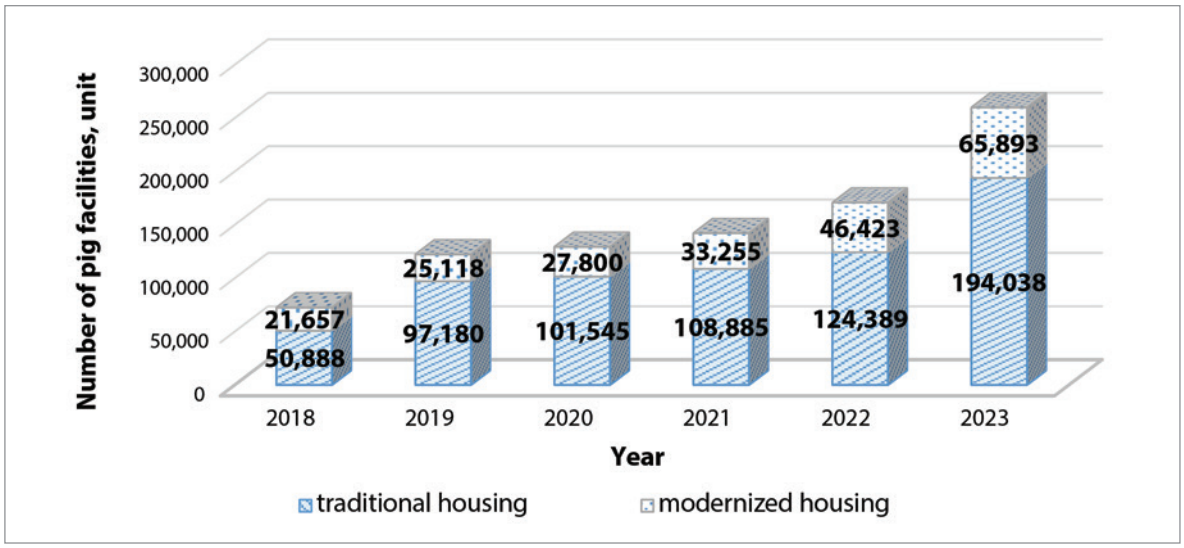

Fig. 2. Proportion of traditional to modernized pig housing in Burundi households from 2018 to 2023

to contagious disease transmission is the inadequate monitoring system for disposal of animal carcasses and slaughterhouse waste, as many pig owners dispose of biological waste in open landfills accessible to scavengers.

Burundi's geographical location and tropical climate create favorable conditions for numerous infectious animal diseases (Table 1), including several zoonoses with epizootic and epidemic manifestations that occasionally occur.

The animal disease situation in the country is impacted by the equatorial climate, which accounts for the overwhelming predominance of parasitic infections among swine contagious diseases [20, 21]. Between 2018 and 2023, the reported cases of swine invasive diseases remained consistently high, representing approximately 92% of all swine infectious pathologies (Fig. 3).

It is important to note that veterinary services in Burundi do not routinely conduct epizootiological monitoring to detect swine infectious disease pathogens or confirm their absence. Diagnostic testing to determine the cause of animal deaths is performed only in cases of mass mortality. Currently, infectious disease diagnosis in Burundi relies on microbiological (microscopy, culturing), serological (ELISA, agglutination, microagglutination tests), and molecular genetic (PCR) methods, but only when clinical symptoms or mass mortality events occur. In case of suspected parasitic diseases, clinical and microscopic examinations are primarily used, with post-mortem diagnostics occasionally performed during necropsies of deceased or emergency-slaughtered animals. Table 2 presents registered and laboratory-confirmed cases of swine infectious diseases in Burundi over five years, based on annual reports from the General Directorate of Animal Health.

The analysis of swine infectious disease prevalence in Burundi revealed that African swine fever (ASF) accounted for the highest case numbers until 2021. However, reported ASF cases declined by over 3.5-fold beginning in 2020, with no cases recorded in 2023. The ASF-freedom has been achieved through import and export restrictions on pigs. The movement of pigs within the borders of Burundi has been limited due to the unfavorable epizootic situation in neighboring countries [22, 23]. Currently, all international swine imports are exclusively processed through the Burundi Institute of Agricultural Sciences (ISABU), with mandatory diagnostic testing and quarantine measures.

It should be emphasized that among viral swine diseases only ASF has been reported in the Republic, which is likely to reflect limited laboratory capacity for viral diagnosis. While classical swine fever outbreaks were recorded before 2018, no cases have been detected in recent years. Since 2023, bacterial infections have become predominant in swine pathology (Fig. 4), with erysipelas (33.8%) and salmonellosis (32.2%) representing the most prevalent diseases. Other frequently diagnosed conditions include colibacillosis (15.0%) and ringworm (13.4%) in domestic pig populations.

Table 1
Prevalence of contagious porcine diseases in the Republic of Burundi from 2018 to 2023 (according to the General Directorate of Animal Health)

Disease group by agent type	Reported cases by year, %				
	2018–2019	2019–2020	2020–2021	2021–2022	2022–2023
Bacterial	3.60	3.20	5.20	4.80	6.30
Parasitic	81.20	93.70	91.30	92.16	92.80
Viral	14.50	2.50	2.70	1.70	0
Fungal	0.63	0.54	0.76	1.40	0.97

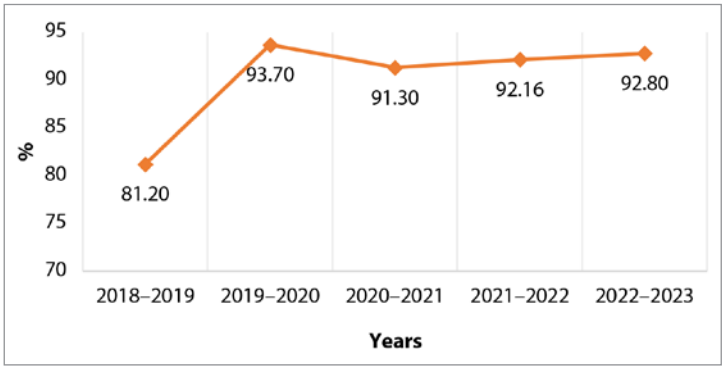

Fig. 3. Dynamics of reported cases of porcine parasitic diseases in the Republic of Burundi from 2018 to 2023

Table 2
Nosological profile of infectious porcine diseases in the Republic of Burundi from 2018 to 2023 (according to the General Directorate of Animal Health)

Nosological unit	Number of registered cases by year									
	2018–2019		2019–2020		2020–2021		2021–2022		2022–2023	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%
African swine fever	4,093	77.5	1,164	40.0	1,034	31.4	598	21.3	–	–
Salmonellosis	753	14.3	965	33.2	694	21.1	853	30.4	902	32.2
Erysipelas	31	0.6	130	4.5	486	14.8	552	19.6	948	33.8
Colibacillosis	218	4.1	392	13.5	743	22.6	274	9.8	422	15.0
Pasteurellosis	8	0.15	4	0.14	45	1.4	38	1.4	158	5.6
Ringworm	178	3.4	253	8.7	290	8.8	495	17.6	375	13.4

n – number of cases.

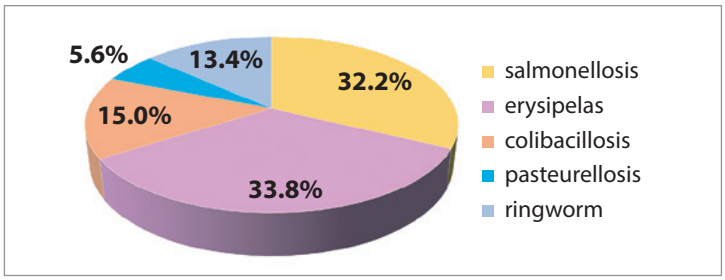

Fig. 4. Reported cases of porcine bacterial infectious diseases in the Republic of Burundi in 2023

A graphical representation of five-year trend of swine bacterial disease number in the Republic of Burundi clearly shows cyclical fluctuations in salmonellosis and colibacillosis prevalence in pigs. At the same time, the swine erysipelas occurrence level is steadily increasing. Notably, erysipelas cases increased 1.7-fold in 2023 as compared to 2022, and surged more than 7-fold relative to 2020 levels (Fig. 5). Of particular concern is the absence of erysipelas vaccination programs in Burundi. Diagnostic capabilities have improved significantly since 2019, transitioning from purely clinical diagnosis to laboratory confirmation using bacteriological culturing and enzyme immunoassay methods.

A study of the territorial distribution of swine erysipelas in the Republic showed that the number of provinces where the disease is registered is growing every year (Table 3).

Between 2018 and 2020 swine erysipelas was confined to a single province. However, the disease expanded to 5 provinces by 2022 and reached 9 provinces by 2023. Cumulatively, outbreaks were documented in 12 of Burundi's 18 provinces over this five-year period (Fig. 6).

The provinces where swine erysipelas has not been detected are located in southern regions (Bururi, Rutana, Makamba), the extreme eastern province (Cankuzo), and northwestern areas (Bubanza and Bujumbura Mairie). Swine erysipelas outbreaks have been confirmed in all central provinces of Burundi. Despite movement restrictions between provinces and districts, reported erysipelas cases continue to rise steadily, that may be attributed to growth

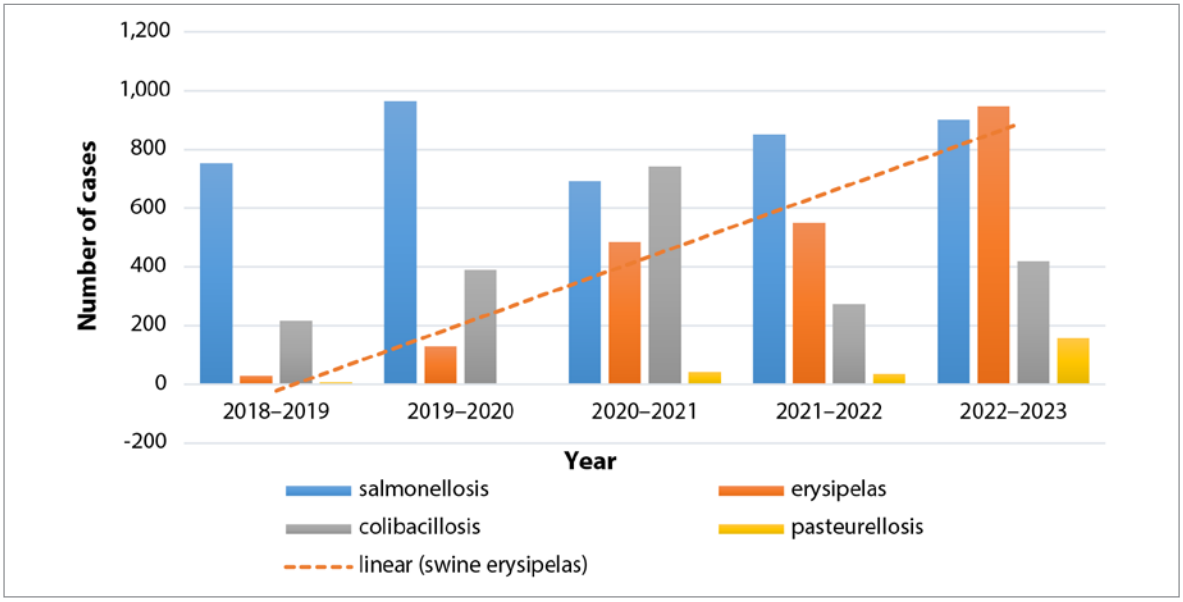

Fig. 5. Dynamics of reported cases of porcine bacterial infectious diseases in the Republic of Burundi from 2018 to 2023

in pig populations, inadequate livestock farming practices, illegal movement of animals and/or animal products across or within the borders and improved diagnostic efforts by the National Veterinary Laboratory.

The escalating incidence of swine erysipelas raises significant concerns among competent authorities and veterinary specialists in Burundi. The pathogen demonstrates prolonged environmental persistence in soil and other reservoirs due to the country's humid equatorial climate, facilitating the establishment of endemic disease outbreaks. Moreover, many farmers tend to conceal outbreaks and animal deaths, and improperly dispose of carcasses in open landfills accessible to scavengers, exacerbating transmission risks. In the absence of erysipelas vaccination programs, the current epizootic situation poses a serious threat.

CONCLUSION

For the Republic of Burundi, pig farming (whether on private, small-scale, or semi-commercial farms) is a crucial component of food security, ensuring the population's access to animal protein. The government has taken substantial efforts to supply farms with livestock and promote pig farming as a key industry with a short production cycle. However, the communities suffer significant annual losses due to infectious disease outbreaks. These losses stem from multiple factors, including unfavorable climatic conditions, on the one hand, and low livestock farming standards, on the other. A major contributing factor is inadequate epizootic control by the state veterinary service, as well as lack of vaccination programs, particularly those against swine erysipelas. The incidence of swine erysipelas has risen sharply in recent years. In 2023, reported cases increased by over 1.7 times compared to 2022 and more than seven-fold compared to 2020. This trend has led to the establishment and maintenance of persistent, endemic outbreaks across the country. Between 2018 and 2023, the disease was recorded in 12 of Burundi's 18 provinces. Given these findings, Burundi's veterinary authorities must prioritize systematic action in three key areas: 1) epizootiological monitoring of swine infectious diseases, which will enable early outbreak detection, control and containment of infectious diseases, particularly swine erysipelas, in the country; 2) training livestock owners in essential biosecurity practices, following WOAH guidelines (separation, cleaning and disinfection); 3) development of

Table 3
Distribution of swine erysipelas in the provinces of the Republic of Burundi from 2018 to 2023 (according to the National Veterinary Laboratory)

Provinces	Period (years)				
	2018–2019	2019–2020	2020–2021	2021–2022	2022–2023
Bujumbura Rural	–	–	–	–	62
Gitega	–	–	11	35	198
Karuzi	–	130	–	–	–
Kayanza	–	–	–	–	76
Kirundo	–	–	–	–	204
Mwaro	–	–	–	–	4
Muyinga	–	–	–	–	48
Muramvya	–	–	–	7	–
Ngozi	–	–	–	–	143
Ruyigi	–	–	160	160	–
Rumonge	–	–	315	124	145
Cibitoke	31	–	–	226	68
Total for the country	31	130	486	552	948

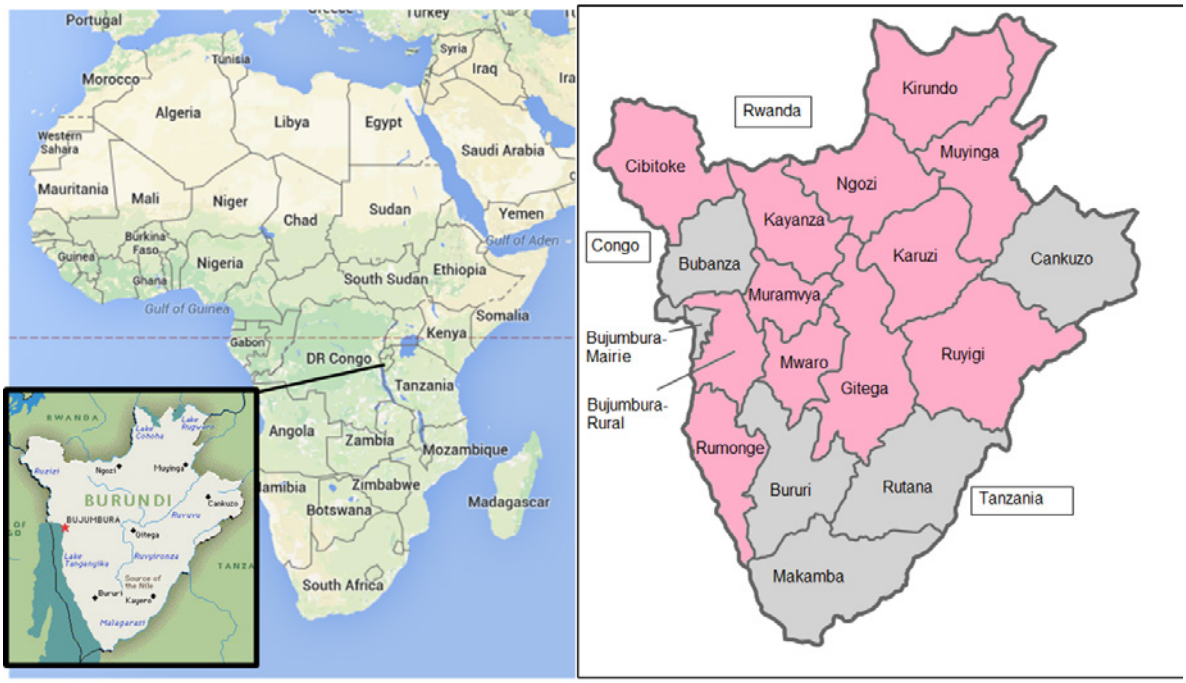

Fig. 6. Administrative divisions of the Republic of Burundi (provinces with reported cases of swine erysipelas are shown in pink)

a national policy for modernization of pig farming through strategic policies, including measures to ensure affordable feed supplies.

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Received 06.12.2024
Revised 17.01.2025
Accepted 09.04.2025

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Вклад авторов: Кощаев А. Г. – принятие ответственности за все аспекты работы, целостность всех частей статьи и за ее окончательный вариант; Нийонгабо Х. – сбор, анализ и интерпретация полученных данных, создание рисунков и таблиц, составление черновика рукописи; Горковенко Н. Е. – формирование идеи, формулировка ключевых целей и задач, критический пересмотр черновика рукописи с внесением ценных замечаний интеллектуального содержания, участие в научном дизайне; Нимбона К. – участие в научном дизайне; Нтирандекура Ж.-Б. – участие в научном дизайне.

https://doi.org/10.29326/2304-196X-2025-14-2-156-163

Virucidal activity of disinfectants against African swine fever virus

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ABSTRACT

Introduction. The most effective strategy to control African swine fever is to implement a set of anti-epizootic measures aimed at preventing introduction and spread of the disease pathogen. Currently, there is a wide range of commercially available disinfectants used at the facilities subject to veterinary control. Their effectiveness against African swine fever virus is unknown and is only confirmed by the manufacturers, who do not always provide substantiated evidence.

Objective. The objective of the research is to test virucidal activity of various disinfectants against African swine fever pathogen in the laboratory.

Materials and methods. Twelve samples of disinfectants with different chemical compositions were tested. The first *in vitro* assessment stage was carried out using suspension method, i.e. working solutions of the tested disinfectants in experimental concentrations and exposure times were added to the liquid-phase virus-containing material. During the second stage, swabs from concrete test plates contaminated with African swine fever virus were tested following treatment of surfaces with the working disinfectant solutions. Each stage was performed in two variants: without organic contamination and with its imitation (application of inactivated bovine serum on the test surface). The samples were tested using virus isolation in a sensitive porcine spleen cell culture. Results were assessed and interpreted in hemadsorption test. The disinfectant sample was considered to exhibit virucidal activity, if no reproduction of African swine fever virus was observed.

Results. Nine out of twelve tested disinfectants demonstrated a virucidal effect against reference African swine fever virus Arm 07 strain (genotype II), when tested on test surfaces. Such results suggest the need to evaluate further the efficacy of various disinfectants against this pathogen.

Conclusion. The fact that such disinfectant products that are incapable of inactivating African swine fever virus under the conditions specified in their instructions are potentially marketed underlines the need to improve regulatory framework in order to ensure effectiveness of general disease prevention and control measures.

Keywords: African swine fever virus, disinfectants, chlorine-containing compounds, glutaraldehyde, potassium peroxymonosulfate

Acknowledgements: The study was funded by the Federal Centre for Animal Health as a part of its research activities in "Veterinary Welfare".

For citation: Lavrentiev I. A., Igolkin A. S., Shevtsov A. A., Kolbin I. S., Puzankova O. S., Gavrilova V. L., Chernyshev R. S. Virucidal activity of disinfectants against African swine fever virus. *Veterinary Science Today.* 2025; 14 (2): 156–163. https://doi.org/10.29326/2304-196X-2025-14-2-156-163

Conflict of interest: Igolkin A. S. is a member of the editorial board of the "Veterinary Science Today" journal, but was not involved into the decision making process related to this article publication. The manuscript has passed the review procedure accepted in the journal. The authors did not declare any other conflicts of interests.

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УДК 619:578.842.1:614.48

Вирулицидная активность дезинфицирующих препаратов в отношении возбудителя африканской чумы свиней

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РЕЗЮМЕ

Введение. Наиболее эффективной стратегией борьбы с африканской чумой свиней остается проведение комплекса противоэпизоотических мероприятий, направленных на предотвращение заноса и распространение возбудителя данной болезни. В настоящее время существует широкий спектр коммерческих дезинфицирующих средств, применяемых на объектах ветеринарного надзора, эффективность которых в отношении вируса африканской чумы свиней неизвестна и подтверждается только заверениями производителей, которые не всегда предоставляют обоснованные доказательства.

Цель исследования. Лабораторные испытания вирулицидной активности различных дезинфицирующих препаратов в отношении возбудителя африканской чумы свиней.

Материалы и методы. Исследовано 12 образцов дезинфицирующих средств с различным химическим составом. Первый этап по оценке свойств *in vitro* проводили суспензионным методом путем добавления к жидкофазному вируссодержащему материалу рабочих растворов испытуемых препаратов в экспериментальных концентрациях и при различном времени экспозиции. Второй этап осуществлялся посредством тестирования смывов с контаминированных вирусом африканской чумы свиней тест-пластин из бетона после их обработки рабочими растворами дезсредств. Каждый этап проводили в двух вариантах: без органического загрязнения и с его имитацией (экспозиция инактивированной сыворотки крови крупного рогатого скота

на тест-поверхности). Образцы исследовали методом вирусовыделения в чувствительной культуре клеток селезенки свиньи. Учет и интерпретацию результатов проводили в реакции гемадсорбции. Считали, что образец препарата обладал вирулицидной активностью при отсутствии репродукции вируса африканской чумы свиней.

Результаты. Вирулицидным эффектом в отношении референтного штамма Arm 07 вируса африканской чумы свиней (II генотип) при испытаниях на тест-поверхностях обладали 9 из 12 испытуемых препаратов, что свидетельствует о необходимости проведения дальнейших исследований по оценке действенности различных дезинфицирующих средств в отношении данного возбудителя.

Заключение. Возможность присутствия в коммерческом обороте дезсредств, неспособных при заявленных в инструкции условиях инактивировать вирус африканской чумы свиней, подчеркивает необходимость совершенствования нормативно-правовых актов в целях обеспечения эффективности мер общей профилактики и борьбы с болезнью.

Ключевые слова: вирус африканской чумы свиней, дезсредства, дезинфектанты, хлорсодержащие препараты, глутаровый альдегид, пероксомоносульфат калия

Благодарности: Работа выполнена за счет средств ФГБУ «ВНИИЗЖ» в рамках тематики научно-исследовательских работ «Ветеринарное благополучие».

Для цитирования: Лаврентьев И. А., Иголкин А. С., Шевцов А. А., Колбин И. С., Пузанкова О. С., Гаврилова В. Л., Чернышев Р. С. Вирулицидная активность дезинфицирующих препаратов в отношении возбудителя африканской чумы свиней. *Ветеринария сегодня.* 2025; 14 (2): 156–163. https://doi.org/10.29326/2304-196X-2025-14-2-156-163

Конфликт интересов: Иголкин А. С. является членом редколлегии журнала «Ветеринария сегодня», но никакого отношения к решению опубликовать эту статью не имеет. Рукопись прошла принятую в журнале процедуру рецензирования. Об иных конфликтах интересов авторы не заявляли.

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INTRODUCTION

Despite efforts taken to prevent spread of African swine fever (ASF) in the Russian Federation, there are still high risks to introduce the infection into pig farms. The damage caused by the disease is significant. As a result, Russia's total losses from ASF in 2018–2020 amounted to 32,571.6 million rubles [1].

Under the current conditions, it is especially important to ensure biological security for all categories of pig farming enterprises, i.e. a set of administrative and physical measures taken to reduce the risk of the disease introduction, its rooting and spread [2, 3].

Failure to comply with or imperfect biosecurity regulations increase the likelihood of introducing pathogens of dangerous diseases, including African swine fever virus (ASFV). Therefore, it is important to implement effective protective measures (i.e. segregation, cleaning, disinfection) on all pig farms, pig slaughterhouses and pig processing plants, taking into account the possibility of virus introduction via virus sources (infected animals) and contaminated objects (vehicles, personnel attire, consumables, and farm equipment).

Relevant veterinary rules are implemented in case of ASF to define the structure of veterinary posts that shall treat vehicles at the exit from the outbreak and the protection zone, and shall conduct three-stage disinfection in the outbreak to prevent the infection spread [4]. As point 49 of the mentioned rules reads, that chlorine-containing disinfectants (minimum 25% active chlorine) or equivalent disinfecting agents with high virucidal activity against the pathogen shall be used for disinfection, according to the instructions for use. However, not all instructions for commercial disinfectants provide evidence-based data on their efficacy against target pathogens and proper application protocols.

All of the above indicates that in order to ensure protection against introduction and spread of ASF, effective disinfection measures are required that take into account the pathogen stability in the environment and pig products, as well as the virus tolerance to certain types of disinfectants resulting from a complex virion structure.

The temperature range of ASFV resistance is 5 °C – up to 7 years, 18–20 °C – 18 months, 37 °C – 30 days, 50 °C – up to 1 hour. The virus can persist for 6 years in serum at a temperature of 5 °C, in smoked ham – up to 180 days, in frozen meat – up to 155 days [5, 6].

Data on survival of ASFV in external environments and in various excreta from the infected animals are given in Table 1.

It should be noted that ASFV is not mentioned in SanPiN 3.3686-21, since it does not belong to zoonotic pathogens [12]. Pursuant to point 2.13.2 of the "Rules for disinfection and decontamination at the facilities under state veterinary surveillance", ASFV is classified as stable (stability group 2 out of four groups specified in the Rules) [13]. The document also includes disinfection requirements based on the pathogen stability characteristics. However, the listed disinfectants do not include a wide range of currently used products (based on acids, alkalis, aldehydes, chlorine compounds, iodine, phenols, quaternary ammonium compounds) with the following claimed advantages: relatively short exposure time, absence of pronounced corrosive and toxic effects, enhanced effect due to the synergy of components at low concentrations of active substances [14, 15, 16].

As specified in "Methodological guidelines for quality control of veterinary disinfection in livestock facilities" (see Appendix 3 to the above mentioned rules), in order to control the quality of the on-going disinfection, tests are specified for indicator microorganisms (for stability group 2: *Staphylococcus aureus*, *S. epidermatis*, *S. saprophiticus*) [13]. Although stability of ASFV and staphylococci is comparable, it is not equivalent, due to the difference in the pathogen structure and the specific mechanisms

Table 1
ASFV resistance to the environmental factors, based on findings from independent researchers

Environmental object	Storage conditions	Observation period	Reference
Faeces	+4 °C	5–280 days	[7, 8]
	+20 °C	3–11 days	[7, 9]
Manure	–20 °C	2 months	[10]
	+4 °C	30–145 days	[8, 10]
	+20 °C	21 days	[10]
Urine	–20 °C	3 months	[10]
	+4 °C	5–60 days	[7, 10]
	+20 °C	5–21 days	[7, 10]
Beach sand	+20 °C	14 days	[7]
Backyard soil	+20 °C	7 days	[7]
Bog mud	+20 °C	3 days	[7]
Soil	–20 °C	2 months	[10]
	+4 °C	45–650 days	[8, 10]
	+20 °C	30–132 days	[8, 10]
Moist soil	+4 °C	up to 3 days	[7]
	+20 °C	up to 3 days	[7]
Water	–20 °C	3 years	[11]
	+4 °C	2–33 months	[8, 10, 11]
	+20 °C	2–13 months	[8, 10, 11]

of resistance [17, 18]. For example, key components of the cell wall structure in Gram-positive bacteria, including *S. aureus*, are peptidoglycan and teichoic acids, while the ASFV virion core is surrounded by a dense protein layer, an inner lipid envelope, and a capsid, which serves as the outer layer in intracellular virions. The aforementioned factors also contribute to the differences in sensitivity to various pH levels. For example, the optimal range for staphylococcal growth is between 7.2 and 7.4, and in case of high acidity (pH < 4.5), bacterial growth slows down, unlike ASF pathogen, which is inactivated at pH ranging between < 3.9 or > 11.5 [19, 20].

Therefore, in order to assess disinfecting properties of the tested products or their components, it is more suitable to use different methods for different types of specific pathogens (separately for bacteria, viruses, fungi, etc.).

As part of the research work done by the Federal Centre for Animal Health Reference laboratory for ASF, the virucidal activity of commercial disinfectants against ASFV genotype II is tested. The experimental testing includes two stages: *in vitro* determination of the minimum effective concentration and exposure time; spraying test surfaces with working solutions of the disinfectants. Artificial organic soiling is applied at all stages to simulate near-real-life conditions.

The purpose of the experiment is to compare coded disinfectants (with the known chemical composition) based on their virucidal properties against ASF pathogen.

MATERIALS AND METHODS

Twelve disinfectants manufactured in Russia have been tested. The disinfectant samples were coded before the experiments.

Due to the absence of relevant internal regulations, the experiments were conducted according to a disinfectant test scheme similar to that given in GOST R 58151.4-2018 and R 4.2.3676-20, but after appropriate adjustment of the methods for disinfectants used for veterinary purposes and their adaptation to the current pathogen – ASFV [21].

African swine fever virus was handled and the obtained results were interpreted in accordance with the "Methodological guidelines for African swine fever virus isolation and titration in porcine spleen cell culture" [22].

Stage 1 (i.e. *in vitro* assessment stage) was carried out in two variants: without protein load and with the addition of inactivated bovine serum at a 40% concentration in the virus-disinfectant mixture. A 2-day subconfluent monolayer of primary porcine spleen cell culture supplemented with Eagle's minimal essential medium, as prescribed by the Federal Centre for Animal Health, containing 10% foetal bovine serum was used as a test object.

The cell culture was inoculated with liquid-phase hemadsorbing ASFV genotype II reference strain Arm 07 deposited into the Federal Centre for Animal Health collection of strains.

Working solution of the tested disinfectants was added at a ratio of 1:9 (i.e. 1 part of virus-containing material to 9 parts of the disinfectant) to the cell debris-free suspension containing ASFV Arm 07 strain at a minimum titer of 6.0 lg $HAdU_{50}/cm^3$.

Seventy percent-inactivated bovine serum was used to neutralize disinfectant effect and simulate organic soiling.

The resulting samples (both with serum and without) were kept at room temperature during the exposure time, and subsequently neutralized with bovine serum at a 1:1 ratio (1 part of sample and 1 part of the neutralizer).

Then the mixture (virus, disinfectant and neutralizer) samples were added into the plate wells with a monolayer of ASFV-sensitive porcine spleen cell culture, 30 minutes later the mixture was removed and replaced with the supportive medium. The cell culture was incubated in 5% CO_2 atmosphere for 7 days at 37 °C, results monitored daily.

Stage 2 of disinfection efficacy testing included spraying 10 × 10 cm concrete test plates with working solutions from a spray bottle. Before the experiment, all surfaces were mechanically cleaned (washed with soap and brush, rinsed with running water, then wiped several times with a sterile wet cloth) and autoclaved.

The test plates were placed horizontally; ASFV suspension was pipetted onto each plate (minimum titer of 6.0 lg $HAdU_{50}/cm^3$) at a rate of 0.5 cm^3/m^2; with 5% inactivated bovine serum added to cover 100 cm^2 area, and was evenly distributed over the surface. The virus-contaminated surfaces got dry at room temperature, and then were treated with the tested disinfectant solution at the minimum effective concentration and exposure time.

To simulate organic contamination (protein load), 40%-inactivated bovine serum was used, which was applied to virus-contaminated surfaces. Surfaces were then sprayed with the test product at the application rate of 0.3 L/m^2 (per manufacturer's protocol).

Control test plates were sprayed with sterile or boiled tap water at the same application rate (0.3 L/m^2) as in the experimental procedure. In order to determine completeness of ASFV inactivation, swabs were sampled from the test surfaces and then applied onto a sensitive porcine spleen cell culture (virus detection was performed through virus isolation with three blind passages conducted in cell culture for each sample).

Results were examined by the presence or absence of hemadsorption phenomenon – a qualitative specific indicator of ASFV replication. The disinfectant sample was considered to exhibit virucidal activity, if no hemadsorption was observed.

RESULTS AND DISCUSSION

Table 2 provides results of disinfectant efficacy testing (judged by the presence or absence of virucidal activity) for coded disinfectant samples during two consecutive *in vitro* stages.

It was found that nine out of twelve disinfectants were able to inactivate highly virulent ASFV reference strain Arm 07 at all concentrations and exposure times, prescribed by manufacturers, on sprayed concrete test surfaces.

Disinfectants coded as 1, 2 and 3 with potassium peroxymonosulfate as the main active ingredient (at concentrations of 0.55, 0.5 and 1.5%, respectively) exhibited activity against ASFV both when tested by suspension method and by spraying onto the concrete test surfaces, during 30 and 60 minute-exposure times. Potassium peroxymonosulfate is able to inactivate ASFV even with significant organic contamination, but in this case its concentration should be at least 1.0% [23]. The research findings demonstrate efficacy of potassium peroxymonosulfate-based disinfectants even at lower concentration (both with and without protein load), which can be taken into account when optimizing chemical composition of disinfectants.

The disinfectant coded as 4 (0.002% chlorine dioxide working solution) demonstrated sufficient virucidal efficacy at experiment stage 1, however, ASFV persisted on the concrete test surface after 3-minute exposure. The tested concentration of ClO_2 exceeds the recommended one (0.0012%) for inactivation of ASF pathogen; and the lack of activity is most likely caused by insufficient exposure time. To achieve optimal virus degradation efficacy, when applying chlorine dioxide to the surface, it is necessary to adhere to a strict time-temperature regimen (at least 50 min, 37 °C) [24].

Disinfectants coded as 5, 6, 7, 8, 9, 10 and 12 contain glutaraldehyde in their composition. Disinfectant 7 did not show pronounced virucidal activity in the experiment, despite sufficiently high concentration of the active substance (0.024%) in the working solution (as declared by the manufacturer) exceeding the efficacy rates of other disinfectants, for example, the one coded as 10 and containing only 0.015% glutaraldehyde, but proved effective against ASFV during the experiment. The available data from the few existing studies show the possibility to use glutaraldehyde at 0.1% concentration during 30-minute exposure [23]. The lack of efficacy of disinfectant No. 7 may be explained either by insufficient levels of excipients in its formulation or deviations in its manufacturing process. The critical lack of data on the minimum effective concentration of glutaraldehyde required for ASFV inactivation highlights the need for research to assess the virucidal efficacy of the disinfectants. It should be noted that glutaraldehyde concentration of more than 0.5% can increase cytotoxicity, which significantly limits the range of experiments [25].

Disinfectant No. 11, containing 0.012% sodium carbonate peroxosolvate, 0.75% sodium alkylbenzene sulfonate, 0.135% citric acid, 0.36% methylene blue, 0.00015% sodium carbonate, showed partial virucidal efficacy against the pathogen. The disinfectant exhibited no virucidial efficacy against ASFV during 30-minute exposure at the declared concentrations, however, with prolonged exposure time, namely 60 and 90 minutes, it was quite effective. The individual components in its composition, according published data, demonstrate activity against ASF pathogen at high concentrations and during prolonged exposure time [26]. Chlorine compounds are widely used as disinfectants due to their high efficacy resulting from the ability to denature proteins; however, they have a corrosive effect and are inhibited by organic substances. Sodium alkylbenzene sulfonate is classified as an anionic surfactant with well-pronounced detergent properties. The virucidal properties of citric acid were confirmed when used at 2% concentration during 30-minute exposure; the effect is achieved due to interaction of lipophilic structures with the virus membrane and decreased pH. Sodium carbonate also proved effective against ASFV at 1% concentration and after 30-minute exposure [26].

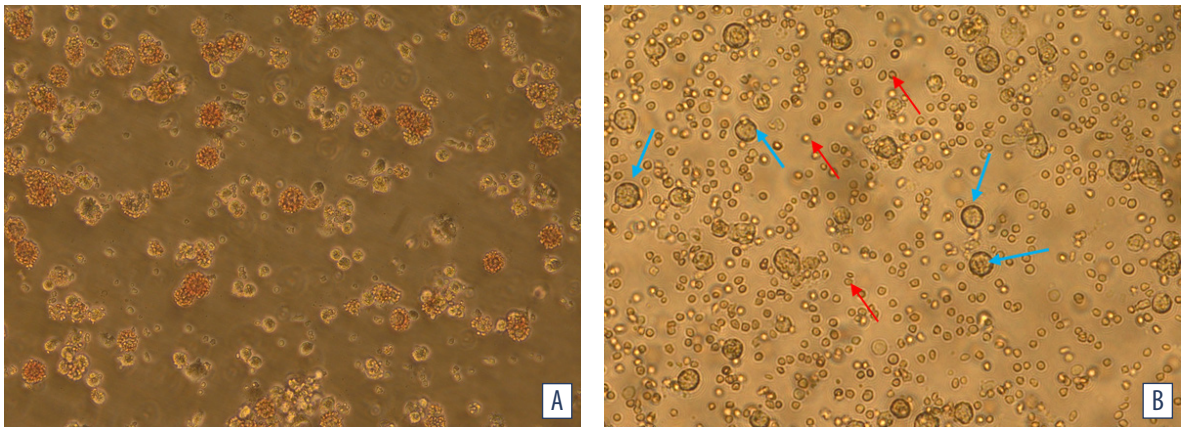

Fig. Porcine spleen cell culture: A – infected with ASFV;
B – intact (blue arrows indicate porcine spleen cells, red – red blood cells)

Table 2
Results of testing virucidal activity of disinfectants against ASFV in cell culture and on a concrete test surface

Product code	Components (active ingredients)	Concentration of the active substance, %	Exposure time, min	Stage 1 (suspension method)		Stage 2 (spraying)	
				without protein load	with protein load	without protein load	with protein load
1	potassium peroxymonosulfate	0.55	30	+	+	+	+
2	potassium peroxymonosulfate	0.5	30	+	+	+	+
3	potassium peroxymonosulfate	1.5	60	+	+	+	+
4	chlorine dioxide	0.002	3	+	+	–	–
5	glutaraldehyde	0.0375	15	+	+	+	+
	benzalkonium chloride	0.025					
6	alkyldimethylbenzylammonium chloride	0.03	30	+	+	+	+
	glutaraldehyde	0.05					
7	glutaraldehyde	0.024	15	+	+	–	–
	alkyldimethylbenzylammonium chloride	0.01					
8	didecyldimethylammonium chloride	0.0156	15	+	+	+	+
	alkyldimethylbenzylammonium chloride	0.034					
	glutaraldehyde	0.0214					
9	glutaraldehyde	2.0	10	+	+	+	+
	alkyldimethylbenzylammonium chloride	1.7					
	didecyldimethylammonium chloride	0.8					
	isopropyl alcohol	1.0					
10	alkyldimethylbenzylammonium chloride	0.018	5	+	+	+	+
	glutaraldehyde	0.015					
	formaldehyde	0.01					
	polyhexamethylene guanidine	0.0006					
	isopropyl alcohol	0.004					
11	sodium carbonate peroxosolvate	0.012	30	+	+	–	–
	sodium alkylbenzene sulfonate	0.75					
	citric acid	0.135	60	+	+	+	+
	methylene blue	0.36					
	sodium carbonate	0.00015	90	+	+	+	+
12	didecyldimethylammonium chloride	0.05	15	+	+	+	+
	alkyldimethylbenzylammonium chloride	0.15					
	glutaraldehyde	0.05					
	amino oxide	0.05					
	isopropyl alcohol	0.05					
	isotridecanol ethoxylated	less than 0.05					

"+" – virucidal effect in place (absence of hemadsorption);
"–" – no virucidal effect (presence of hemadsorption).

The conducted tests demonstrate that protein load (as a simulation of organic contamination), short exposure time, and relatively low temperatures (of the working solution, treated object, and environment) are all critical factors that can significantly reduce efficacy of disinfectants. Therefore, thorough mechanical pre-cleaning of surfaces and strict adherence to recommendations, including those on exposure time and temperature regime, is still of great importance for practice, which agrees with published literature [17].

CONCLUSION

During experiment stage 1 (in porcine spleen cell culture) all the 12 tested disinfectants exhibited virucidal activity against ASFV reference strain Arm 07, which confirms *in vitro* efficacy and absence of cytotoxic effects, if the recommended concentrations and exposure time are complied with.

During experiment stage 2 (on concrete test surfaces with or without simulation of organic contamination), only 9 out of 12 disinfectants were effective, which underscores the need for comprehensive studies to assess the virucidal activity of disinfectants using various test objects.

To optimize composition of disinfectants in order to increase their efficacy against ASF pathogen, we consider it advisable to use glutaraldehyde at a minimum concentration of 0.05%, potassium peroxymonosulfate at a minimum concentration of 0.5%.

The experimentally confirmed impact of organic contaminants on disinfectant virucidal efficacy shows that for practical purposes it is extremely important to thoroughly clean surfaces and select the most effective products for both disinfection and washing. In addition, it is necessary to account for other factors, including a composition and concentration of active ingredients that are effective against the specific pathogen, ambient air temperature, temperature of treated surfaces, temperature of the disinfectant working solution, exposure time, drying parameters and other relevant variables.

Application of those commercial disinfectants with the unknown virucidal activity against ASF pathogen increases the risk of undermining substantial efforts invested in preventive and eradication measures. Therefore, there is a need to introduce relevant regulatory framework on testing commercial disinfectants for their efficacy against currently circulating pathogens of infectious diseases listed in the instruction for use.

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Received 03.12.2024
Revised 17.01.2025
Accepted 09.04.2025

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Вклад авторов: Лаврентьев И. А. – формирование идеи, развитие ключевых целей и задач, подготовка текста рукописи, проведение статистического анализа; Иголкин А. С. – формирование идеи, развитие ключевых целей и задач, утверждение окончательного варианта рукописи; Шевцов А. А. – подготовка текста рукописи; Колбин И. С. – проведение экспериментов; Пузанкова О. С. – проведение экспериментов; Гаврилова В. Л. – проведение экспериментов; Чернышев Р. С. – формирование идеи, разработка методологии, проведение экспериментов, подготовка текста рукописи.

https://doi.org/10.29326/2304-196X-2025-14-2-164-170

Towards improved differential diagnostics of bovine tuberculosis in the Republic of Dagestan

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ABSTRACT

Introduction. Non-specific tuberculin reactions are among the most critical challenges in tuberculosis diagnosis, with their incidence increasing annually. Given the complex epidemiological challenges, improving bovine tuberculosis diagnostics is critically important.

Objective. Development of effective comprehensive differential bovine tuberculosis diagnosis and introduction of improved techniques for the infection detection in farms with different animal health statuses in the Republic of Dagestan.

Materials and methods. 1,670 cattle were subjected to tuberculin testing; 3,502 serum samples were used for serological testing, 112 samples for immunological testing, 57 samples of pathological material collected from animals and 76 environmental samples were used for bacteriological testing. *Mycobacterium bovis, Mycobacterium bovis* BCG, *Mycobacterium avium, Mycobacterium scrofulaceum* strains were used in the study.

Results. Nonspecific reactions in the farms of all categories were found to be widespread in the Republic. Diagnostic value of intradermal and intravenous tuberculin tests in tuberculosis-infected herds was determined (9.4% of extra-detected diseased animals). Complement fixation test is poorly sensitive and highly specific. Indirect haemagglutination assay results are not confirmed by conventional methods in most cases, which suggests their low specificity. 39 mycobacterial isolates were recovered from 57 biological samples and identified: 8 (20.5%) as *Mycobacterium bovis*; 31 (79.5%) as non-tuberculous mycobacteria (acid-fast species), among them 29 (93.5%) were identified as Runyon II organisms, 2 (6.5%) as Runyon III organisms. 43 isolates out of 76 environmental samples were recovered: among them 2 (4.6%) were identified as *Mycobacterium bovis*, 23 (53.5%) as Runyon II organisms and 18 (41.9%) as Runyon III organisms. Among culture media, Löwenstein-Jensen's egg-based medium provides the best growth performance and most effective suppression of competing microflora.

Conclusion. The obtained data provide a fundamental basis for developing an effective comprehensive method for differential diagnosis of bovine tuberculosis.

Keywords: tuberculosis, cattle, skin test, serological tests, immunological methods, mycobacteria, nutrient media, non-tuberculous species, disease-free farms, PPD tuberculin

For citation: Baratov M. O. Towards improved differential diagnostics of bovine tuberculosis in the Republic of Dagestan. *Veterinary Science Today*. 2025; 14 (2): 164–170. https://doi.org/10.29326/2304-196X-2025-14-2-164-170

Conflict of interests: The author declares no conflict of interests.

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УДК 619:616.98:579.873.21:636.22/.28:616.9-07

К совершенствованию дифференциальной диагностики туберкулеза крупного рогатого скота в условиях Республики Дагестан

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РЕЗЮМЕ

Введение. При диагностике туберкулеза неспецифические реакции на туберкулин являются одной из наиболее важных проблем, увеличивающихся с каждым годом. Учитывая сложную ситуацию, в том числе и эпидемиологическую, совершенствование методов диагностики туберкулеза крупного рогатого скота является весьма актуальным.

Цель исследования. Разработка эффективного комплексного метода дифференциальной диагностики туберкулеза крупного рогатого скота и внедрение усовершенствованной схемы выявления инфекции в хозяйствах с различным эпизоотическим состоянием в условиях Республики Дагестан.

Материалы и методы. Аллергическим исследованиям подвергли 1670 гол. крупного рогатого скота, серологическим – 3502 образца сывороток крови, иммунологическим – 112 проб, бактериологическим – 57 проб патматериала, отобранного от животных, и 76 проб – из объектов внешней среды. В исследовании использовали штаммы культур *Mycobacterium bovis, Mycobacterium bovis* БЦЖ, *Mycobacterium avium, Mycobacterium scrofulaceum*.

Результаты. Установлено широкое распространение неспецифических реакций во всех категориях хозяйств республики. Определена диагностическая ценность внутрикожной и внутривенной проб в неблагополучных по туберкулезу стадах, где число дополнительно выявляемых больных составило 9,4%.

Реакция связывания комплемента имеет низкую чувствительность и высокую специфичность. Результаты реакции непрямой гемагглютинации в большинстве случаев не подтверждаются классическими методами, что определяет ее низкую специфичность. Из 57 проб биоматериала было изолировано и идентифицировано 39 культур микобактерий: 8 (20,5%) – *Mycobacterium bovis*; 31 (79,5%) – нетуберкулезные кислотоустойчивые виды, из которых 29 (93,5%) относятся к II группе по классификации Раньона, 2 (6,5%) – к III группе. Из 76 проб объектов внешней среды изолированы 43 культуры, из которых 2 (4,6%) отнесены к *Mycobacterium bovis*, 23 (53,5%) – ко II группе и 18 (41,9%) – к III группе по классификации Раньона. Наилучшими ростовыми и ингибирующими постороннюю микрофлору свойствами обладает яичная среда Левенштейна – Йенсена.

Заключение. Полученные данные являются базисной основой для разработки эффективного комплексного метода дифференциальной диагностики туберкулеза крупного рогатого скота.

Ключевые слова: туберкулез, крупный рогатый скот, аллергическая диагностика, серологические тесты, иммунологические методы, микобактерии, питательные среды, нетуберкулезные виды, благополучные хозяйства, ППД-туберкулин

Для цитирования: Баратов М. О. К совершенствованию дифференциальной диагностики туберкулеза крупного рогатого скота в условиях Республики Дагестан. *Ветеринария сегодня*. 2025; 14 (2): 164–170. https://doi.org/10.29326/2304-196X-2025-14-2-164-170

Конфликт интересов: Автор заявляет об отсутствии конфликта интересов.

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INTRODUCTION

Animal tuberculosis control in the Caspian region, including in the Republic of Dagestan, has attained some success. At the same time, an analysis of the epizootic situation in this territory shows that the disease prevalence varies between the regions and the number of infected farms has remained almost unchanged in recent years [1, 2, 3, 4].

Practice shows that the implementation of measures to prevent the infection introduction to free farms and to achieve freedom from the disease on the infected farms must be constantly monitored. Uncontrolled movements of livestock, animal products and feed pose risks of introducing the pathogen into disease-free farms. All these circumstances necessitate the need to constantly improve measures for the prevention and control of bovine tuberculosis (bTB), taking into account the changing epizootic situation and the peculiarities of modern animal husbandry [5, 6].

One of the main issues in control system is qualified diagnostics, which often requires complex and targeted tests that are not covered by current regulations [7, 8].

As the incidence of animal tuberculosis declines, the issue of non-specific reactions unrelated to the disease is becoming increasingly pressing. At the same time, due to imperfect differentiation methods, such reactions bring great economic losses leading to slaughter of healthy livestock and costly animal health actions [9, 10].

Since many aspects of such reaction mechanisms remain understudied, multiplicity of concepts still exists [11, 12].

Despite extensive study, the etiology of nonspecific PPD-tuberculin reactions in animals has yet to be fully elucidated. Domestic and foreign literature data show that the main cause of nonspecific reactions in healthy animals are non-tuberculous mycobacteria and acid-resistant actinomycetes, which are morphologically, culturally, physiologically and genetically closely related to mycobacteria [13, 14].

There are reports that mycobacterium-like organisms (*Corynebacterium*, *Nocardia*, *Rhodococcus*), which share group-specific features with mycobacteria, may also sensitize the animal body to tuberculin [3, 4, 15, 16, 17].

However, it has been established that not all tuberculosis-infected animals react to the tuberculin test. It is also known that when animals are tested using various methods, only reactors to certain tests are identified, which is probably due to chronic multi-stage disease duration, environmental factors, physiological state of the animal, etc. [18, 19, 20, 21].

This certainly makes it difficult to diagnose tuberculosis and necessitates the use of a set of diagnostic tests, including tuberculin, serological, bacteriological and immunological ones. Each of these methods has advantages along with disadvantages, which increases the effectiveness of diagnosis [22, 23, 24].

It should be noted that a single diagnostic algorithm has not been developed yet, moreover, the role of serological and immunological methods, in our opinion, is often underestimated [25, 26, 27].

In this regard, further study of sensitization problems, spread of mycobacteria and related microorganisms in nature, their isolation rates from the biological samples from tuberculin-reacting animals and environmental samples, the ability to sensitize the body to tuberculin and their epizootic significance are of great scientific and practical value.

MATERIALS AND METHODS

The nature of allergic reactions was confirmed by intradermal and simultaneous tests using PPD-tuberculin for mammals and nontuberculous mycobacteria (NTM) complex in accordance with the "Veterinary rules for preventive, diagnostic, restrictive and other measures, the establishment and lifting of quarantine and other restrictions aimed to prevent and eradicate tuberculosis"[1],

[1] https://docs.cntd.ru/document/565721619 (in Russ.)

which has been effective since March 1, 2021. A total of 1,670 cattle of different age groups (cows, heifers) have been tested.

A comparative analysis of different test results was performed: based on tuberculin intradermal, intravenous, intrapalpebral, and ophthalmic tests for allergic reactions on bTB-infected cattle farms – 170 animals; simultaneous and intrapalpebral tests on bTB free farms – 386 animals; serological tests: CFT with MAC (complement fixation test with mycobacterial antigen complex) – 1,411 serum samples; IHA (indirect hemagglutination) test using RBC diagnostic reagents – 2,091 samples; immunological tests: rosette test, LTT (lymphocyte transformation test), LSLT (leukocyte specific lysis test) – 112 samples; bacteriological tests – 57 biological samples and 76 environmental samples.

The collection of pathological material (blood, lesions, lymph nodes), transportation, storage, pre-culture treatment, preparation of nutrient media were performed in compliance with "Handbook of Microbiological and Virological Tests" (ed. by M. O. Birger, 1982; in Russ.).

Mycobacterium bovis, *Mycobacterium bovis* BCG, *Mycobacterium avium*, *Mycobacterium scrofulaceum* field strain cultures isolated from homogenated pathological material and environmental samples and collection strains of these cultures stored in the laboratory were used in the study.

Biological samples from tuberculin-reacting cattle of bTB-infected farms were handled according to Alikayeva's method.

Pieces of pathological material were ground in a porcelain mortar with crushed glass. Then the homogenated material was poured into sterile vials with a 3% solution of $C_{12}H_{25}SO_4Na$ (sodium lauryl sulfate) in 1:1 ratio. After mixing, it was left at room temperature for 20 minutes. Then the vials were centrifuged for 20 minutes at 1,500 rpm, the supernatant was removed, the precipitate was washed twice with sterile distilled water and inoculated into Löwenstein – Jensen's, Finn II, Petraniani, Gelberg media and modified Shkolnikova medium to detect and exclude cell wall deficient mycobacteria (L-forms).

Environmental samples (hay, straw, scrapings from feeders, soil, manure) were crushed, mixed with saline solution, ground and dispensed into vials with a 5% solution of H_2SO_4 (sulfuric acid) in 1:1 ratio and left at room temperature for 30 minutes. Then the vials were centrifuged at 1,500 rpm for 20 minutes, the supernatant was removed, the precipitate was washed twice by centrifugation and used for inoculation. Each sample of the preciptate was inoculated in 8 tubes and incubated in a thermostat at 37–38 °C.

Table 1
Percentage ratio of tuberculin reactors and tuberculosis-infected cattle in the Republic of Dagestan in 2022–2023

Area	Cattle tested, animals	Tuberculin reactors		Diseased cattle identified, animals	Reactors / diseased animals, %
		animals	%		
Mountainous	167	45	26.9	2	4.44
Foothill	182	59	32.4	–	–
Lowland	201	66	32.8	4	6.06
Total	550	170	30.9	6	3.50

Tuberculosis agents and non-tuberculous mycobacteria were differentiated in accordance with GOST 26072-89 (ST SEV 3457-81) "Livestock and poultry. Methods of tuberculosis laboratory diagnosis"[2] and GOST 27318-87 (ST SEV 5627-86) "Livestock. Methods of nontuberculous mycobacteria identification"[3].

All experiments were carried out in strict accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (ETS No. 123).

RESULTS AND DISCUSSION

An analysis of the test results for allergic reactions indicates that reactions to PPD-tuberculin for mammals in the Republic of Dagestan are prevalent, regardless of the natural and climatic zones.

The ratio of animal reactors in all categories of farms was 30.9%, with a majority of reactions occurring on bTB free farms, which is indicative of widespread nonspecific reactions in healthy cattle.

It should be noted that the number of tuberculin reactors on free farms in mountainous and foothill areas is slightly less than on lowland farms (Table 1).

These figures significantly differ from previously published data, reflecting the correlation between the number of tuberculin reactors and vertical zonation. Comparative cartographic analysis shows that in the second half of the last century and in the beginning of the current century, the correlation between the number of both tuberculin-reactors and confirmed bTB cases and the number and density of animal populations and proximity to the lowland zone was recorded.

Analysis revealed no climate zone-dependent variations in tuberculin test reactivity or confirmed bTB incidence. The prevalence of reactors (45) and bTB cases (2) on mountainous zone farms (4.44% ratio), despite conditions favoring stronger immunity, appears attributable to: uncontrolled inter-farm interactions, substantial seasonal livestock transfers, and regular import of feed from lowlands.

While no bTB cases were confirmed among foothill zone reactors, the historical prevalence matches that of the lowland zone, precluding any conclusion of disease-free status. The data provided should be considered interim and require annual confirmation.

Tuberculin reactivity in cattle shows significant seasonal variation, with peak rates occurring during spring and autumn months. More than 80% of the reactors are detected during this period.

In order to compare the effectiveness of various tests for allergic reactions on bTB infected farms, 170 animals were tested with tuberculin intradermal, intravenous, intrapalpebral and ophtalmic tests (Fig. 1).

The diagnostic value of intradermal and intravenous tests and their role in bTB diagnosis were determined. It was established that most effective diagnostic approach for the animals from bTB infected herds is the combination of intradermal and intravenous tuberculin tests. The percentage of additionally detected diseased animals was 9.4%. This method proved to be effective both for the initial diagnosis and for the differentiation of tuberculin nonspecific reactions.

[2] https://docs.cntd.ru/document/1200025492 (in Russ.)

[3] https://docs.cntd.ru/document/1200025497 (in Russ.)

The comparative study of simultaneous and intrapalpebral tests of animals from bTB free farms for the purposes of initial diagnosis (386 animals were tested) gave inconclusivee results (Fig. 2): simultaneous test showed 19 PPD-tuberculin reacting animals (4.9%), 15 animals reacted to NTM complex (3.9%); 4 animals showed reactions to intrapalpebral administration of tuberculin (1.0%).

With Dagestan accounting for 21.3% of Russia's sheep and goat population (ranking first) and 5.3% of cattle (ranking third), predominantly in backyard farming systems, developing reliable methods to differentiate non-specific reactions has become a critical modern priority. The standard simultaneous testing protocol proved ineffective under these conditions, while current regulations prohibit diagnostic slaughter as follow-up to skin test results. Studies revealed that the intravenous diagnostic method proved most effective under these conditions, showing particular advantages when testing small herds in private ownership settings

In animals displaying positive reactions to both intradermal and intravenous tests, post-mortem and laboratory analyses confirmed tuberculosis in 95.8% of cases, as demonstrated by comparative evaluation. In addition, in herds with long-term tuberculosis history, an intravenous test additionally reveals tuberculin-anergic animals, with their number ranging from 0.2 to 0.7%, according to numerous reports.

Detection rates of intradermal test reactors were assessed among stable / pasture-kept animals in backyard farming systems of two foothill settlements (Karabudakhkentsky and Buinaksky Raions, 167 animals) and on different farms in the lowland (SPK "Lvovskoye" Babayurtsky Raion, 123 animals, farm "Telmana" Kizlyarsky Raion, 274 animals). The results are shown in Figure 3.

It was found that in all farms, except for "Telmana" farm in the Kizlyarsky Raion, more than 10% of cattle reacted to PPD-tuberculin.

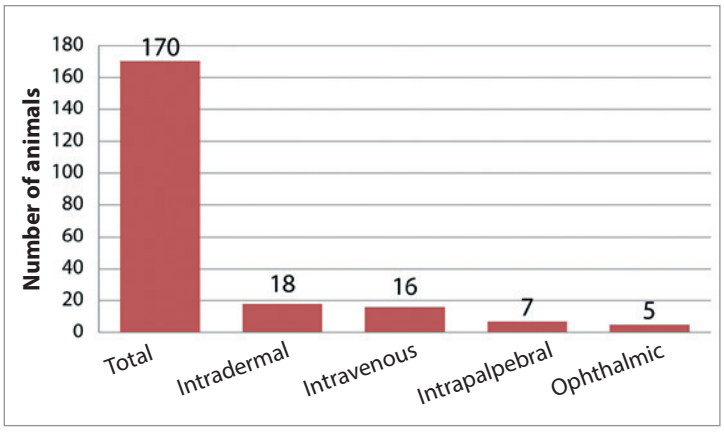

Fig. 1. Assessment of diagnostic value of tuberculin tests in tuberculosis-infected farms

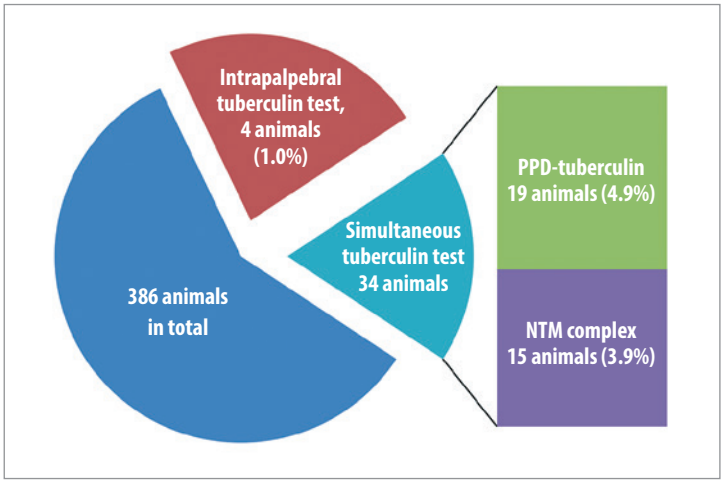

Fig. 2. Results of comparative analysis of simultaneous and intrapalpebral tests

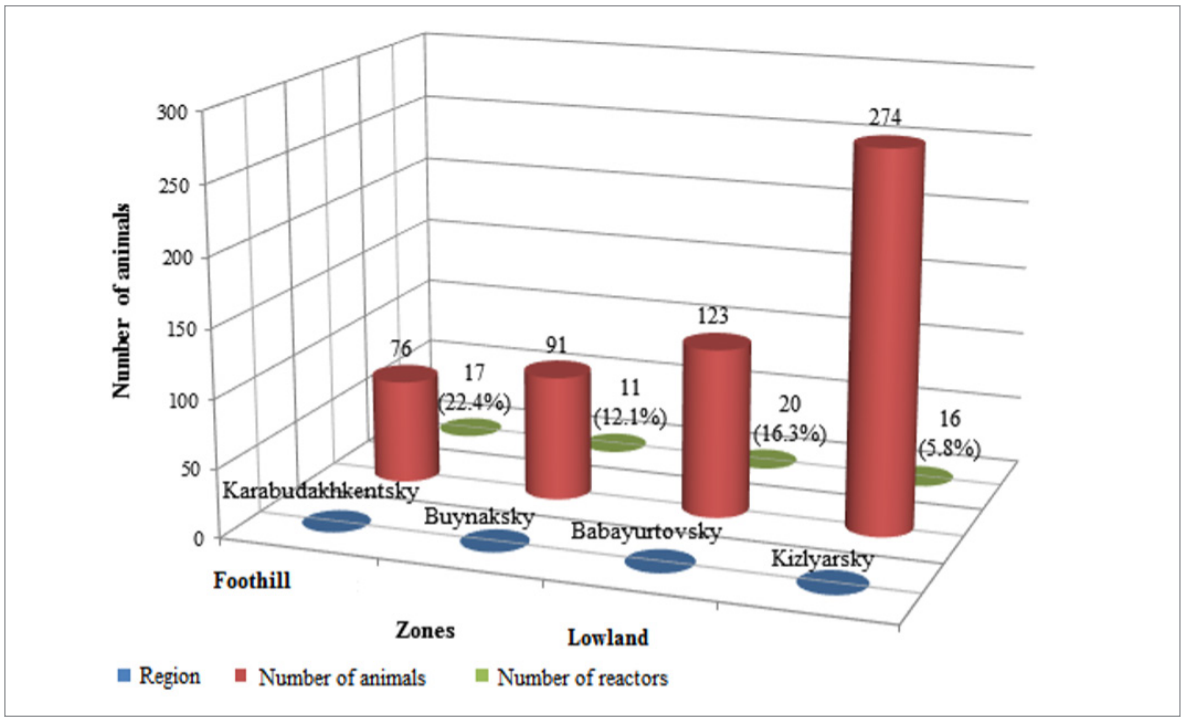

Fig. 3. Number of tuberculin reactors raised in stall / pasture conditions

The analysis of serological test results was based on the parallel use of tuberculin tests: 1,411 serum samples using CFT with MAC and 2,091 samples using IHA test with three RBC diagnostic reagents (*M. bovis*, *M. avium*, *M. fortuitum*). No reliable correlation was revealed between the number of intradermal test reactors and the number of animals demonstrating diagnostic antibody titers in their sera by serological tests.

The CFT demonstrates high specificity (85–100%) but low sensitivity, making it valuable for confirmatory diagnosis CFT shows promise in identification of anergic animals in chronically infected herds. Our observations show that the number of such animals in some farms can reach up to 10%.

The results of the highly sensitive IHA test using RBC diagnostic reagents in most cases are not confirmed by conventional methods (post-mortem and laboratory tests), which is indicative of its low specificity for tuberculosis. This test using several diagnostic reagents can serve as an additional test for tuberculin non-specific reaction differentiation.

Cellular immunity tests (rosette test, LTT, LSLT) exhibit appropriate sensitivity and specificity characteristics, but their complexity confines them primarily to research applications rather than mass screening programs.

The results of bacteriological test revealed that *M. bovis* can be isolated in pure culture in almost all biological samples from animals demonstrating bTB-consistent changes and from about 6% of asymptomatic animals.

Thirty nine mycobacterium cultures were isolated and identified from 57 biological samples: 8 (20.5%) were identified as *M. bovis*; 31 (79.5%) were non-tuberculous acid-resistant species, among them 29 (93.5%) were identified as Runyon II organisms and 2 (6.5%) as Runyon III organisms.

Table 2
Number of isolates from environmental and biological samples

Samples	Total	Isolated	Identified					
			M. bovis		NTM			
					Runyon II organisms		Runyon III organisms	
			No.	%	No.	%	No.	%
Biological samples	57	39	8	20.5	29	93.5	2	6.5
Environmental samples	76	43	2	4.6	23	53.5	18	41.9

Seventy six environmental samples were collected; 43 isolates were recovered of which 2 (4.6%) were identified as *M. bovis*, 23 (53.5%) as Runyon II organisms, and 18 (41.9%) as Runyon III organisms (Table 2).

Isolation rates of certain *Mycobacterium* species differed significantly among sample types and disease progression stages. *M. bovis* isolation frequency decreased significantly from 40% during active disease progression to 14% in the fading stage. Similar changes were observed for non-tuberculous mycobacteria.

The correlation between the frequency and NTM isolation rate from biological and environmental samples can be traced in all natural and climatic zones.

The growth performance of various nutrient media was tested by inoculation of 34 mycobacterial cultures: 7 (20.6%) were identified as *M. bovis*, 27 (79.4%) as non-tuberculosis acid-resistant species, among them 11 (40.7%) were identified as Runyon II organisms and 16 (59.3%) as Runyon III organisms. The isolation rate was estimated by the number of colonies and the growth rate (Table 3).

A noticeable growth of both *M. bovis* (15 colonies without growth of foreign microflora in 17–19 days) and non-tuberculous mycobacteria (19 *M. avium* colonies in 8 days and 16 colonies of *M. scrofulaceum* in 7 days) was recorded on Löwenstein – Jensen's medium. Finn II medium performance was slightly poorer in both growth rates and colony counts (9 small *M. bovis* colonies in 17 days; 6, 17, and 13 *M. bovis* BCG, *M. avium*, and *M. scrofulaceum* colonies, respectively, in 6–11 days). Other media supported slow bacterial growth, producing only small colonies.

CONCLUSIONS

1. The effectiveness of a set of differential diagnostic tests, including intrapalpebral, intravenous and intradermal tuberculin tests was demonstrated. The combined use of these tests identify diseased animals in bTB-infected herds and differentiate nonspecific reactions to PPD-tuberculin for mammals.

2. Studies conducted over the past 3 years demonstrate high prevalence of tuberculin reactors, reaching 18% on some farms. In most cases, the nature of these reactions remains unclear.

3. The analysis of serological results revealed CFT high specificity for tuberculosis diagnosis. We consider it reasonable to use this test as an additional method, in particular to identify animals anergic to tuberculin. Although serological (IHA) and immunological (rosette test, LTT, LSLT) tests are not yet widely adopted for diagnosing animal tuberculosis, they remain scientifically valuable.

4. A comparative study of growth media most commonly used in the laboratory revealed that Löwenstein –

Table 3
Isolation rates for different nutrient media

Growth medium	*M. bovis*		*M. bovis* BCG		*M. avium*		*M. scrofulaceum*	
	Number of colonies	Growth rate, days	Number of colonies	Growth rate, days	Number of colonies	Growth rate, days	Number of colonies	Growth rate, days
Löwenstein –Jensen's	15	17–19	10	8	19	8	16	7
Finn II	9	17	6	7	17	11	13	6
Petraniani	5	20	7	6	10	12	8	10
Gelberg	6	24	3	8	6	10	4	8

Jensen's egg-based medium provides the best growth performance and isolation rates for both typical and nontuberculous mycobacteria. Despite its overall poorer performance, Finn II medium sometimes exhibits faster growth rates compared to Löwenstein – Jensen's medium.

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Received 03.02.2025
Revised 19.03.2025
Accepted 29.04.2025

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Вклад автора: Баратов М. О. – формулировка ключевых целей и задач исследования, проведение исследований, сбор, анализ и интерпретация полученных данных, создание рисунков и таблиц, подготовка рукописи.

https://doi.org/10.29326/2304-196X-2025-14-2-171-178

Development and testing of a set of chromogenic media for rapid diagnosis of bovine mastitis

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ABSTRACT

Introduction. Bovine mastitis remains one of the most prevalent and economically significant diseases in dairy cattle production. Three chromogenic media have been proposed for the diagnosis, each specifically designed for isolation and differentiation of certain mastitis pathogen groups: Medium I is intended for *Enterobacteriaceae* family bacteria, Medium II – for *Staphylococcus* genus microorganisms, Medium III – for *Streptococcus* genus bacteria.

Objective. The objective is to evaluate the sensitivity, specificity, differentiation capacities and inhibitory properties of these chromogenic media, and to test the media using milk samples from mastitic cows.

Materials and methods. For sensitivity testing, the control strains (*Streptococcus agalactiae*, *Staphylococcus aureus* and *Escherichia coli*) at concentrations of 1×10^0, 1×10^1, and 1×10^2 CFU/mL were used. Microbial growth was assessed following 24-hour incubation at 37 °C. Specificity and differentiation capacities were studied using 22 microbial strains, their growth patterns and colony coloration in chromogenic and control media were compared. Inhibitory properties were determined based on presence/absence of culture growth. The media were evaluated using milk samples from mastitic cows and standardized culturing methods.

Results. The chromogenic media demonstrated sensitivity comparable to the control media (Columbia agar supplemented with 5% defibrinated sheep blood), $p > 0.05$. Medium I enabled reliable color-based differentiation but showed limited inhibitory effects. Medium II ensured selective isolation of staphylococci while effectively suppressing growth of other bacteria. Medium III supported growth of both enterococci and streptococci, including *Streptococcus agalactiae*. The tests conducted in milk samples confirmed genus level differentiation capability.

Conclusion. The developed chromogenic media ensure high-accuracy mastitis diagnosis due to their sensitivity, specificity and differentiation properties. Their implementation makes it possible to cover an extensive range of microorganisms and to selectively isolate the targeted bacterial groups. Further work will be aimed at improving the media for fungal growth suppression and increasing the diagnostic accuracy.

Keywords: mastitis, rapid diagnosis, cattle, milk, chromogenic media

Acknowledgements: The study was conducted as part of the state assignment of the Ministry of Science and Higher Education of the Russian Federation, project FGUG-2025-0003.

For citation: Kapustin A. V., Laishevtsev A. I., Savinov V. A., Shastin P. N., Gilmanov Kh. Kh., Khabarova A. V. Development and testing of a set of chromogenic media for rapid diagnosis of bovine mastitis. *Veterinary Science Today*. 2025; 14 (2): 171–178. https://doi.org/10.29326/2304-196X-2025-14-2-171-178

Conflict of interests: The authors declare no conflict of interests.

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УДК 619:618.19-002:636.22/.28:637.075

Разработка и апробация набора хромогенных сред для экспресс-диагностики мастита крупного рогатого скота

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РЕЗЮМЕ

Введение. Мастит крупного рогатого скота является одним из наиболее распространенных и экономически значимых заболеваний в молочном животноводстве. Для его диагностики предложены три хромогенные среды, каждая из которых предназначена для выделения и дифференциации определенных групп возбудителей мастита: среда I – для бактерий семейства *Enterobacteriaceae*, среда II – для микроорганизмов рода *Staphylococcus*, среда III – для бактерий рода *Streptococcus*.

Цель исследования. Оценка чувствительности, специфичности, дифференцирующих и ингибирующих свойств хромогенных сред, а также их апробация на образцах молока от коров с маститом.

Материалы и методы. Для оценки чувствительности использовали контрольные штаммы *Streptococcus agalactiae*, *Staphylococcus aureus* и *Escherichia coli* в различных концентрациях (1×10^0, 1×10^1, 1×10^2 КОЕ/мл). Рост микроорганизмов оценивали через 24 ч инкубации при 37 °C. Специфичность и дифференцирующие свойства изучали на 22 штаммах микроорганизмов, сравнивая их рост и цвет колоний на хромогенных и контрольной средах. Ингибирующие свойства оценивали по наличию или отсутствию роста культур. Апробацию сред проводили с использованием образцов молока от коров с маститом, используя стандартизированные методы посева и культивирования.

Результаты. Хромогенные среды показали сопоставимую с контрольной средой (колумбийский агар с добавлением 5% дефибринированной крови барана) чувствительность ($p > 0,05$). Среда I обеспечила дифференциацию микроорганизмов по цвету колоний, но имела низкие ингибирующие свойства. Среда II избирательно выделяла стафилококки, подавляя рост других бактерий. Среда III поддерживала рост энтерококков и стрептококков, в том числе *Streptococcus agalactiae*. Апробация на образцах молока подтвердила возможность дифференциации культур до вида.

Заключение. Разработанные хромогенные среды обеспечивают высокую точность диагностики мастита, сочетая чувствительность, специфичность и дифференцирующие свойства. Их комплексное использование позволяет охватить широкий спектр микроорганизмов и избирательно выделить целевые группы бактерий. Дальнейшая работа будет направлена на улучшение сред для подавления роста грибов и повышения точности диагностики.

Ключевые слова: мастит, экспресс-диагностика, крупный рогатый скот, молоко, хромогенные среды

Благодарности: Исследование проведено в рамках государственного задания Министерства науки и высшего образования Российской Федерации, проект FGUG-2025-0003.

Для цитирования: Капустин А. В., Лаишевцев А. И., Савинов В. А., Шастин П. Н., Гильманов Х. Х., Хабарова А. В. Разработка и апробация набора хромогенных сред для экспресс-диагностики мастита крупного рогатого скота. *Ветеринария сегодня*. 2025; 14 (2): 171–178. https://doi.org/10.29326/2304-196X-2025-14-2-171-178

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

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INTRODUCTION

Bovine mastitis, an inflammatory condition of the mammary gland, ranks among the most widespread and economically impactful diseases in dairy production [1, 2, 3]. Transmission occurs due to multiple factors, including poor milking hygiene, suboptimal housing conditions, improper milking techniques, weakened animal immunity and inadequate preventive measures [4, 5, 6]. The disease presents in both clinical form – characterized by visible symptoms such as udder swelling, redness, and pain – and subclinical form, which lacks overt inflammation but results in reduced milk quality [7].

The etiology of mastitis comprises two primary causative groups: mechanical and infectious. Mechanical causes involve udder injuries resulting from inappropriate milking techniques, defective milking equipment or traumas during grazing. These injuries establish favorable conditions for microbial invasion, potentially leading to inflammatory development [8]. Nevertheless, pathogenic microorganisms constitute the principal factor in mastitis occurrence [9].

The most common pathogens of mastitis are *Staphylococcus aureus*, *Streptococcus agalactiae*, *Escherichia coli* and others [10, 11]. *S. aureus* is one of the most dangerous pathogens, as it can induce chronic forms of mastitis that are resistant to treatment [12, 13, 14, 15]. *S. agalactiae* is transmitted mainly through milking equipment and can persist in cows for a long time [16]. *E. coli* often causes acute forms of mastitis, accompanied by severe symptoms [17].

To diagnose infectious bovine mastitis, veterinarians have multiple diagnostic tools at their disposal, each tool having its distinct advantages and limitations [18, 19, 20, 21, 22, 23, 24]. Among these, bacteriological milk testing remains one of the most widely used methods [25, 26]. This procedure involves aseptic milk sample collection followed by inoculation onto culture media. After thermostat incubation, microorganisms are identified based on their morphological, biochemical and cultural properties. While this method enables precise pathogen identification and facilitates targeted treatment selection, it requires specialized equipment and has a relatively long turnaround time (2–3 days) [27]. Chromogenic media can be used to speed up the diagnosis of infectious mastitis. These specialized media contain substrates that undergo color changes when acted upon by pathogen-specific enzymes, allowing for etiological agent identification within 24 hours post-inoculation. Current rapid test options include Compact Dry (R-Biopharm AG, Germany) and RIDA® COUNT (Chisso Corporation, Japan) test plates [28, 29]. These test systems feature various specialized assays for determining *S. aureus*, *Enterobacteriaceae*, *Salmonell*a, total microbial count, *E. coli*, as well as yeast and mold contamination.

The Laboratory for Diagnostics and Control of Antibiotic Resistance of Pathogens of the Most Clinically Significant Infectious Animal Diseases, Federal Scientific Centre VIEV has developed its own formulation of chromogenic media for differentiating mastitis pathogens without requiring lengthy laboratory studies. The set consists of three distinct chromogenic media that, when used together, enable identification of the mastitis pathogen spectrum in each specific case. This approach facilitates determination of the pathogenic spectrum, thereby influencing subsequent therapeutic decisions.

The study aims to evaluate the efficacy and diagnostic quality of these chromogenic media for bovine mastitis diagnosis.

MATERIALS AND METHODS

Chromogenic media. Three chromogenic nutrient media were prepared.

Medium I is intended for the determination and differentiation of the most frequently encountered microorganisms of the *Enterobacteriaceae* family.

Medium II is intended for the determination and differentiation of microorganisms of the genus *Staphylococcus*.

Medium III is intended for the determination and differentiation of microorganisms of the genus *Streptococcus* (in particular, *S. agalactiae*).

For ease of use, the three media were placed in one Petri dish with sectors.

The efficacy of chromogenic nutrient media was determined according to the following criteria: sensitivity, specificity, cultural properties of microorganism control strains, differentiating and inhibitory properties. A commercially available medium, Columbia blood agar (HiMedia Laboratories Pvt Ltd., India) supplemented with 5% defibrinated ram blood served as control.

Control strains. The control strains comprised 22 microbial cultures from the collection of pathogenic and vaccine strains maintained at the Federal Scientific Center VIEV, including: *E. coli* ATCC 25922, *S. agalactiae* ATCC 8057, *S. aureus* ATCC 12600, *Klebsiella pneumoniae* B-1392, *Proteus mirabilis* B-1382, *Pseudomonas aeruginosa* B-1366, *Salmonella typhimurium* B-1025, *Enterococcus faecalis* B-1399, *Enterococcus faecium* 1921, *Acinetobacter baumannii* 2516, *Enterobacter cloacae* 1322, *Staphylococcus hominis* 1377, *Staphylococcus equorum* 2511, *Staphylococcus haemolyticus* 2505, *Staphylococcus pseudintermedius* B-1849, *Morganella morganii* 1418, *Streptococcus uberis* 2114, *Streptococcus dysgalactiae* 2432, *Streptococcus pyogenes* 1972, *Aerococcus viridans* 2320, *Streptococcus canis* 2326, *Streptococcus suis* 2383.

Preparation of bacterial suspension dilutions. Initial bacterial suspensions were prepared at concentrations of 1×10^8 to 1×10^9 CFU/mL using the pharmacopoeial reference standard (PhRS 3.1.00085). To achieve the required seeding densities, serial ten-fold dilutions of the initial suspensions were performed.

Determination of sensitivity. S. agalactiae, *S. aureus* and *E. coli* strains were inoculated onto the studied chromogenic and control media in 1 mL at different concentrations: 1×10^0, 1×10^1, 1×10^2 CFU/mL. After (24 ± 2) hours of incubation at 37 °C, the number of grown colonies in all the inoculations was compared. The experiment was performed in triplicate. To compare the mean values of the groups and determine statistically significant differences between them, the Student's test (*t*-criterion) was used: the differences are considered statistically significant if p-value < 0.05.

Specificity assessment. Specificity was determined for each chromogenic medium separately. The growth and nature of changes in colonies of different bacterial strains on the same experimental medium were compared and the presence of similarities or differences was noted.

Evaluation of differentiating properties. To determine the differentiating properties, changes in control strains inoculated in chromogenic and control media (structure, color of colonies, color of the medium around the colonies) were compared.

Evaluation of inhibitory properties. Inhibitory properties were determined by the presence or absence of growth of cultures on chromogenic media in comparison with the presence of growth on a control medium.

Testing of media with mastitis milk samples. Eight milk samples (10 mL each) were collected from cows with mastitis confirmed by the Kenotest somatic cell test (CID Lines, Belgium). Samples were collected in sterile biological specimen containers and processed within 2 hours of collection, with storage maintained at +4 to +8 °C. For inoculation, a sterile cotton swab was immersed in each milk sample, excess moisture was removed by touching the container walls, and then streaked in a lawn pattern onto three chromogenic media. The cultures were incubated at 37 °C for 24 hours before result interpretation.

RESULTS AND DISCUSSION

To determine the sensitivity of chromogenic media, three target strains (*S. agalactiae*, *S. aureus*, and *E. coli*) inoculated in 1 mL at three different concentrations (1×10^0, 1×10^1, 1×10^2 CFU/mL) were used. After culturing for 24 hours at 37 °C, the number of colony-forming units for all media was counted. The results are presented in Table 1.

To identify statistically significant differences or similarities, the Student's *t*-test was used, the results of which are presented in Table 2.

Table 1
Mean colony-forming unit values for each tested microorganism species in experimental and control media

CFU/mL		Medium I	Medium II	Medium III	Control
S. agalactiae	1×10^2	116.0 ± 11.4	Inhibited	117.7 ± 12.7	105.3 ± 21.4
	1×10^1	20.3 ± 1.2		21.7 ± 3.8	23.0 ± 6.1
	1×10^0	3.7 ± 1.2		5.7 ± 1.5	3.3 ± 3.5
S. aureus	1×10^2	112.0 ± 14.0	111.3 ± 8.3	Inhibited	118.3 ± 10.0
	1×10^1	21.7 ± 3.2	17.3 ± 2.1		23.0 ± 6.1
	1×10^0	3.7 ± 2.5	4.0 ± 2.6		3.0 ± 2.6
E. coli	1×10^2	100.3 ± 4.9	Inhibited	Inhibited	118.3 ± 9.1
	1×10^1	27.0 ± 2.0			21.7 ± 1.5
	1×10^0	4.3 ± 2.1			2.3 ± 2.3

Table 2
Statistical significance assessment (Student's *t*-test) between compared groups

Compared groups	CFU/mL	*S. agalactiae*	*S. aureus*	*E. coli*
Medium I *vs* control	1×10^2	0.63	0.18	0.06
	1×10^1	0.45	0.81	0.12
	1×10^0	0.89	0.42	0.18
Medium II *vs* control	1×10^2	Inhibited	0.46	Inhibited
	1×10^1		0.30	
	1×10^0		0.76	
Medium III *vs* control	1×10^2	0.39	Inhibited	Inhibited
	1×10^1	0.84		
	1×10^0	0.48		

Based on the obtained data, chromogenic media demonstrate sensitivity comparable to the control medium, as confirmed by statistical analysis (Student's *t*-test, $p > 0.05$). The observed differences between chromogenic media and the control medium showed no statistical significance across all tested strains and concentrations. Thus, these results indicate that chromogenic media effectively support growth of target microorganisms even at minimal inoculum levels.

The specificity, differentiating properties and inhibitory characteristics of the chromogenic media were evaluated concurrently using 22 microbial strains representing diverse species. The results are presented in Table 3.

Medium I was found to be highly specific: most of the tested bacteria formed colonies with unique colors. For example, *E. coli* formed burgundy colonies, *S. aureus* – golden, *P. aeruginosa* – gray-green, and *S. equorum* – violet-pink. However, some microorganisms, such as *E. cloacae* and *K. pneumoniae*, had similar colony colors (violet-blue), which may make it difficult to distinguish them visually. Inhibitory properties were weak: all studied strains

Table 3
Results of tests for specificity, differentiation capacities and inhibitory properties of chromogenic media as compared with control medium

Microorganisms	Medium I		Medium II		Medium III		Control	
	Growth	Colony color	Growth	Colony color	Growth	Colony color	Growth	Colony color
Escherichia coli	Good	Burgundy	Inhibited		Inhibited		Good	Grayish-white
Klebsiella pneumoniae	Good	Violet-blue	Inhibited		Inhibited		Good	Grayish-white
Proteus mirabilis	Good	Transparent	Good	Transparent	Moderate	Transparent	Good	Grayish-white
Pseudomonas aeruginosa	Good	Grey-green	Inhibited		Good	Blue-green	Good	Blue-green
Salmonella typhimurium	Good	Transparent	Inhibited		Inhibited		Good	Grayish-white
Enterococcus faecalis	Good	Blue-light blue	Inhibited		Good	Blue-green	Good	Grayish-white
Enterococcus faecium	Good	Blue-green	Inhibited		Good	Blue-green	Good	Grayish-white
Acinetobacter baumannii	Good	Pale-yellow	Inhibited		Inhibited		Good	Grayish-white
Enterobacter cloacae	Good	Violet-blue	Inhibited		Inhibited		Good	Grayish-white
Morganella morganii	Good	Amber	Inhibited		Moderate	White	Good	Grayish-white
Staphylococcus aureus	Good	Golden	Moderate	Violet	Inhibited		Good	Golden
Staphylococcus hominis	Good	White	Good	Blue-green	Inhibited		Good	White
Staphylococcus equorum	Good	Violet-pink	Good	Blue-green	Inhibited		Good	White
Staphylococcus haemolyticus	Good	White	Good	Green	Inhibited		Good	Grayish-white
Staphylococcus pseudintermedius	Good	Beige-pink	Good	Blue-green	Inhibited		Good	Grayish-white
Streptococcus agalactiae	Moderate	Pale-pink	Inhibited		Good	Blue	Good	Grayish-white
Streptococcus uberis	Moderate	White	Inhibited		Moderate	White	Good	Grayish-white
Streptococcus dysgalactiae	Moderate	Pale-pink	Inhibited		Moderate	White	Good	Grayish-white
Streptococcus pyogenes	Moderate	White	Inhibited		Moderate	White	Good	Grayish-white
Aerococcus viridans	Moderate	White	Inhibited		Inhibited		Good	Greenish
Streptococcus canis	Moderate	White	Inhibited		Moderate	White	Good	Grayish-white
Streptococcus suis	Moderate	Pale-pink	Inhibited		Moderate	White	Good	Grayish-white

of microorganisms demonstrated growth within 24 hours. Nevertheless, medium I ensured effective differentiation of control strains by colony color, which allows visually distinguishing microorganisms already at early stages.

The inhibitory properties of Medium II are pronounced: the growth of most bacteria was absent, with the exception of the target microorganisms – *Staphylococcus* spp. It is worth noting that the specificity of the medium is low – most staphylococci were stained blue-green. However, the same color was predominantly saprophytic microorganisms, while potentially pathogenic staphylococci (*S. aureus* and *S. haemolyticus*) differed in color. For example, *S. aureus* formed purple colonies, and *S. hominis* and *S. equorum* formed blue-green ones, which made it possible to visually distinguish them. Medium II as compared with the control one, provided a differentiation of staphylococci by color.

Medium III demonstrated good inhibitory properties, effectively suppressing the growth of most microorganisms, with the exception of gram-positive cocci and some representatives of the *Enterobacteriaceae* family. The differentiating and specific properties of the medium were weakly expressed and manifested mainly for enterococci, which were stained blue-green, and for *S. agalactiae*, which formed blue colonies.

The use of all three chromogenic media in combination provides a comprehensive approach to mastitis diagnosis, demonstrating high sensitivity, specificity and differentiating properties. This method allows for a wide range of microorganisms to be covered, selectively isolating target bacterial groups such as staphylococci, streptococci and enterococci.

For testing in the field, milk samples were collected and then inoculated onto three chromogenic media. The results are shown in the Figure.

The simultaneous use of three media for milk sample inoculation enables nearly species-level differentiation of cultures. In Figure (a) it is evident that *Enterococcus* sp. grew on Media I and III (supposedly *E. faecalis*, as *E. faecium* typically exhibits a darker green coloration). Single colonies on Medium II consist of *Staphylococcus* sp., while the presence of *S. aureus* can be ruled out, as it would appear purple on Medium II. Additionally, burgundy colonies on Medium I indicate the presence of *E. coli* in the sample.

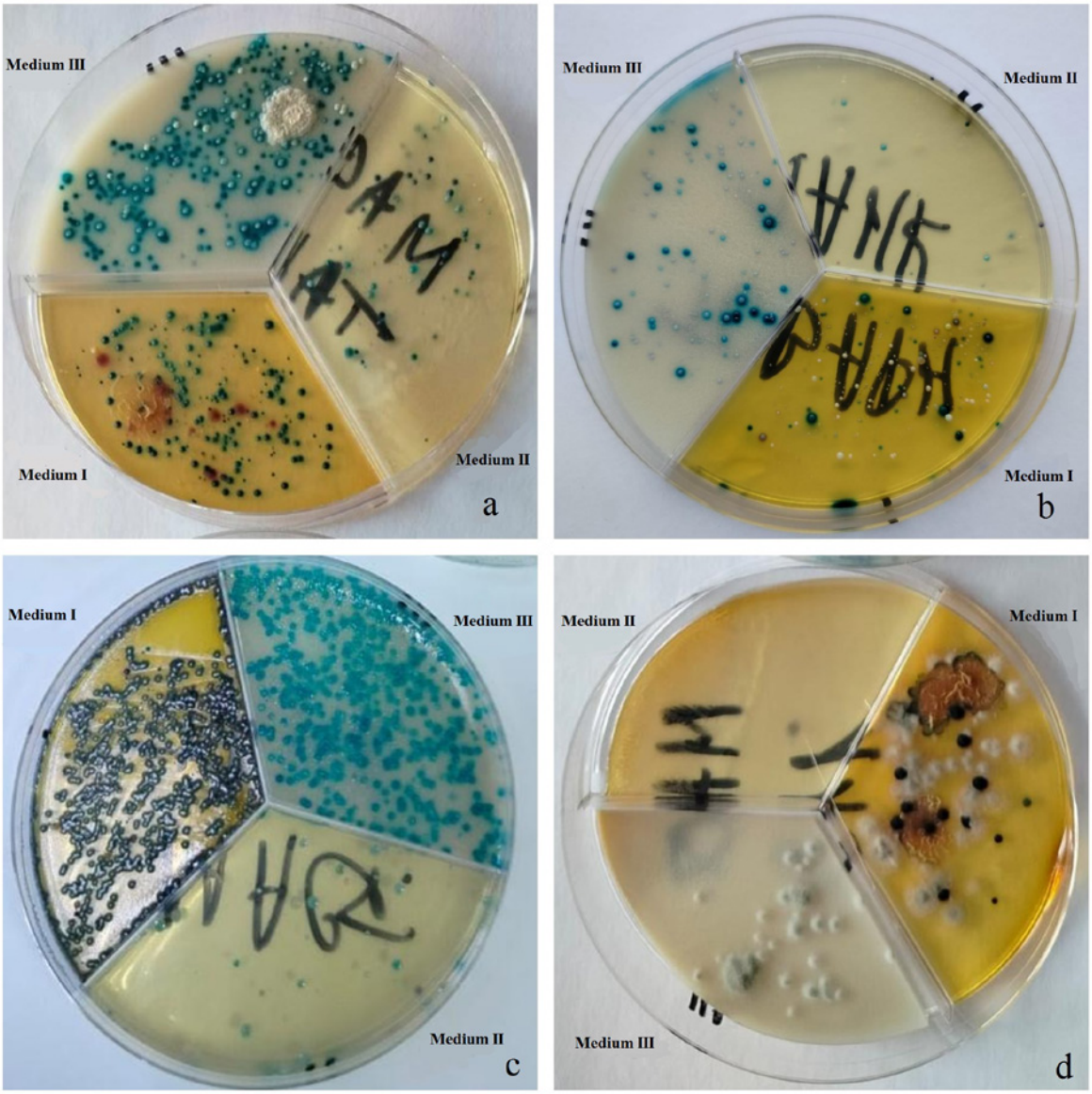

Fig. Testing of culture media using milk samples (cultivated at 37 °C for 24 hours)

In Figure (b), the sample microbiome consists almost exclusively of *Enterococcus* sp. White and green colonies on Media I and II, respectively, are formed by *Staphylococcus* sp. microorganisms, excluding *S. aureus*. Figure (c) reveals a monoculture of *Enterococcus* sp., most likely *E. faecium*. The fourth sample shown in Figure (d), contained only filamentous fungi. Another milk sample yielded results similar to (b), while three other cultures showed no growth.

CONCLUSION

The developed chromogenic media demonstrate high efficiency in bovine mastitis diagnosis. Medium I, with its high sensitivity and differentiating properties, enables primary screening and detection of a broad spectrum of microorganisms, including members of the *Enterobacteriaceae* family. Medium II, due to its strong inhibitory properties, selectively isolates staphylococci, which is a critical feature for identifying pathogenic species such as *S. aureus*. Medium III, while having more limited differentiating capabilities, effectively supports the growth of enterococci and streptococci, including *S. agalactiae*, making it essential for mastitis diagnosis.

The integrated use of all three media ensures high diagnostic accuracy, enabling not only broad microbial coverage but also selective identification of target bacterial groups. This significantly accelerates pathogen detection and facilitates timely administration of effective therapy. Testing of the media on milk samples from mastitic cows confirmed the media's practical applicability and effectiveness when used in the field.

During the testing, occasional development of filamentous fungi was observed, which may complicate result interpretation. To address this, further work will focus on optimizing the media composition by evaluating various antifungal preparations at different concentrations. These improvements aim to enhance media specificity by suppressing non-target fungal growth, thereby reducing the risk of false-positive results.

It is worth noting that standardized disposable loops for milk culture make it possible to roughly estimate the number of colony-forming units. While this method lacks high precision, it provides a practical approximation of milk contamination levels, offering valuable preliminary insights into infection severity.

Thus, the developed chromogenic media represent a promising tool for rapid mastitis diagnosis, combining high sensitivity, specificity and differentiating capabilities. Their implementation in veterinary practice could significantly accelerate diagnostic procedures and enhance mastitis treatment efficacy, ultimately improving animal health and dairy herd productivity.

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Received 21.03.2025
Revised 18.04.2025
Accepted 28.04.2025

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https://doi.org/10.29326/2304-196X-2025-14-2-179-185

Testing of vaccine against canine distemper, parvovirus and coronavirus enteritis, adenovirus infection and dog rabies for its antigenic properties

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ABSTRACT

Introduction. Recently "Carnican-5R" vaccine against canine distemper, parvovirus and coronavirus enteritis, adenovirus infection and dog rabies has been developed at the Rosselkhoznadzor-subordinated Federal Center for Animal Health (FGBI "ARRIAH", Vladimir) in accordance with the Russian Federation legislative requirements. The virus strains currently circulating and significant in the country were used for the vaccine development.

Objective. Testing of "Carnican-5R" vaccine for its antigenic properties in target animals including determination of humoral immunity development time and duration during the observation period.

Materials and methods. "Carnican-5R" combined vaccine containing two components: freeze-dried component and liquid component were used for the test. Dogs at the age of 10–12 weeks served as animal models for testing the vaccine for its antigenic properties. The antibody levels were determined with virus neutralization test, hemagglutination inhibition test and fluorescent antibody virus neutralization test.

Results. Vaccination of dogs was found to induce antibodies to the pathogens of the specified infections. Double "Carnican-5R" vaccine administration at 21-day interval induced strong humoral response by day 35 after its first administration and an increase in the antibody titers to canine distemper – by 8.6 times, to canine parvovirus type 2 – by 2.1 times, to canine coronavirus – by 5.0 times, to canine adenovirus serotype 2 – by 5.36 times, to the rabies virus – by 5.72 times. The specific immunity lasted for at least 12 months and virus-specific antibodies titers to the pathogens remained at the protective levels.

Conclusion. "Carnican-5R" vaccine is safe and non-reactogenic for target animals and induces strong immunity in dogs that lasts for at least 12 months from the date of booster vaccination.

Keywords: viral diseases of dogs, canine distemper, parvovirus enteritis, coronavirus enteritis, adenovirus infection, rabies, specific prevention, "Carnican-5R" vaccine

Acknowledgements: The work was funded by the Federal Centre for Animal Health within the framework of the research topic "Development of a comprehensive control system for infectious animal diseases and improvement of the test methods for residues of banned and harmful substances in animals, feedstuffs and animal products".

For citation: Klimova A. A., Komarova A. A., Kiselev A. M., Galkina T. S. Testing of vaccine against canine distemper, parvovirus and coronavirus enteritis, adenovirus infection and dog rabies for its antigenic properties. *Veterinary Science Today.* 2025; 14 (2): 179–185. https://doi.org/10.29326/2304-196X-2025-14-2-179-185

Conflict of interests: The authors declare no conflict of interests.

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УДК 619:616.98:578:636.7:615.371

Изучение антигенных свойств вакцины против чумы плотоядных, парвовирусного и коронавирусного энтеритов, аденовирусной инфекции и бешенства собак

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РЕЗЮМЕ

Введение. В настоящий момент на базе подведомственного Россельхознадзору Федерального центра охраны здоровья животных (ФГБУ «ВНИИЗЖ», г. Владимир) разработана в соответствии с требованиями законодательства Российской Федерации вакцина против чумы плотоядных, парвовирусного и коронавирусного энтеритов, аденовирусной инфекции и бешенства собак «Карникан-5R». Для ее создания были использованы штаммы вирусов, циркулирующие на территории страны и актуальные в настоящее время.

Цель исследования. Изучение антигенных свойств вакцины «Карникан-5R» на целевых животных: определение срока формирования гуморального иммунитета и продолжительности иммунитета на протяжении периода наблюдения.

Материалы и методы. В исследовании использовали ассоциированную вакцину «Карникан-5R», состоящую из двух компонентов: лиофилизированного и жидкого. В качестве животных моделей для изучения антигенных свойств препарата служили собаки 10–12-недельного возраста. Уровень антител оценивали в реакции нейтрализации, реакции торможения гемагглютинации и реакции нейтрализации методом FAVN (Fluorescent Antibody Virus Neutralization).

Результаты. Установлено, что вакцинация собак индуцировала выработку антител к возбудителям указанных инфекций. Двукратное введение вакцины «Карникан-5R» с интервалом 21 сут стимулировало формирование напряженного гуморального ответа к 35-м сут после первого введения и прирост титра антител к вирусу чумы плотоядных в 8,6 раза, к парвовирусу собак типа 2 – в 2,1 раза, к коронавирусу собак – в 5,0 раза, к аденовирусу собак серотипа 2 – в 5,36 раза, к вирусу бешенства – в 5,72 раза. Продолжительность специфического иммунитета составила не менее 12 мес. с сохранением протективного уровня титра вирусспецифических антител к указанным возбудителям.

Заключение. Вакцина «Карникан-5R» безвредна и ареактогенна для целевых животных, способствует формированию у собак напряженного иммунитета продолжительностью не менее 12 мес. с момента бустерной вакцинации.

Ключевые слова: вирусные болезни собак, чума плотоядных, парвовирусный энтерит, коронавирусный энтерит, аденовирусная инфекция, бешенство, специфическая профилактика, вакцина «Карникан-5R»

Благодарности: Работа выполнена за счет средств ФГБУ «ВНИИЗЖ» в рамках тематики научно-исследовательских работ «Разработка комплексной системы контроля инфекционных болезней животных и совершенствование методов исследования остатков запрещенных и вредных веществ в организме животных, кормах и продуктах животного происхождения».

Для цитирования: Климова А. А., Комарова А. А., Киселев А. М., Галкина Т. С. Изучение антигенных свойств вакцины против чумы плотоядных, парвовирусного и коронавирусного энтеритов, аденовирусной инфекции и бешенства собак. *Ветеринария сегодня*. 2025; 14 (2): 179–185. https://doi.org/10.29326/2304-196X-2025-14-2-179-185

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

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INTRODUCTION

Canine distemper virus (CDV) affects some animal species of carnivorous order including canids, raccoons, felines, etc., as well as pandas. The pathogen is polytrophic and can affect almost all body systems. According to the international classification, the virus belongs to the order *Mononegavirales*, family *Paramyxoviridae*, subfamily *Orthoparamyxovirinae*, genus *Morbillivirus*, and species *Morbillivirus canis* [1, 2].

Canine parvovirus type 2 (CPV-2) is the parvovirus enteritis agent and the main cause of dog fatalities associated with viral diseases; it is extremely contagious. Clinical signs are as follows: acute gastroenteritis, large intestine mucosal lining sloughing and hemorrhagic inflammation, hemorrhagic diarrhea, dehydration, leukopenia and neutropenia [2]. The virus belongs to the order *Piccovirales*, family *Parvoviridae*, subfamily *Parvovirinae*, genus *Protoparvovirus* and species *Protoparvovirus carnivoran 1* [1].

Canine coronavirus (CCoV) causes enteritis with characteristic symptoms including anorexia, vomiting, diarrhea, lymphopenia, and lethargy [3, 4, 5, 6]. The disease varies from asymptomatic to fatal [7]. Coronavirus ranks second position among viral enteropathogens in the world [8, 9, 10]. The pathogen belongs to the order *Nidovirales*, suborder *Cornidovirineae*, family *Coronaviridae*, subfamily *Orthocoronavirinae*, genus *Alphacoronavirus* [1].

Canine adenovirus serotype 2 (CAV-2) causes infectious laryngotracheitis in transient, asymptomatic or mild forms; it can cause severe necrotizing bronchitis, interstitial pneumonia [11], diarrhea [12] and central nervous system disorders [2]. The virus belongs to the order *Rowavirales*, family *Adenoviridae*, genus *Mastadenovirus*, species *Mastadenovirus canidae*, serotype 2 [1].

Rabies virus (RABV) affects the central nervous system of warm-blooded animals and humans and causes fatal disease [13, 14]. There is no treatment for rabies. The virus belongs to the order *Mononegavirales*, family *Rhabdoviridae*, subfamily *Alpharhabdovirinae* and genus *Lyssavirus* [1, 15, 16].

The strains formulated in "Carnican-5R" combined vaccine against canine distemper, parvovirus and coronavirus enteritis, adenovirus infection and dog rabies were selected according to the guidelines published by the Vaccination Guidelines Group (VGG) of the World Small Animal Veterinary Association (WSAVA). According to the guidelines, canine distemper, canine parvovirus enteritis and canine adenovirus infection (CAV-2) are the major viral diseases that should be prevented regardless of the geographical location of the animal. The leptospirosis prevention with vaccines is classified as additional in the guidelines [17]. Rabies virus is included in the vaccine as anti-rabies immunization is envisaged by the Russian Federation legislation [18, 19]. However, these guidelines are not mandatory, and vaccination schedules are developed by veterinarians taking into account occurrence of viral diseases in the particular region. In view of increasing number of deaths in the dog population due to coronavirus enteritis [5, 6, 10], the canine coronavirus strain responsible for this disease is also included in the vaccine composition.

Dog immunization schedule was developed based on scientific data on humoral immunity and the WSAVA recommendations. Generally, veterinarians around the world recommend to start vaccination of dogs at the age of 12 weeks, to perform booster vaccination at the age of 16 weeks, and then vaccinate the animal once

a year or once every three years, depending on the viral animal disease situation in the region, during the whole animal life [17].

During "Carnican-5R" vaccine development, tests of the viruses formulated in the vaccine for their non-reactogenicity and safety were performed in laboratory animals in addition to the tests of the said viruses for their properties and determination of the proportions of the components formulated in the vaccine. Also, tests for selection of optimal vaccine immunizing dose and administration route were carried out. The tests have shown that the vaccine is nonreactogenic and safe, the immunizing dose is 1.0 cm^3 (the liquid vaccine component serves as diluent for the freeze dried vaccine component), the vaccine is administrated by subcutaneous or intramuscular routes. The storage period after combining the components was 2 hours at a temperature of 18–25 °C.

For testing the vaccine for its antigenic properties, experiments were designed and carried out in target animals to study the humoral immunity development and the virus-specific antibody persistence time after the vaccine administration during the observation period (12 months).

The study was aimed at testing the vaccine against canine distemper, parvovirus and coronavirus enteritis, adenovirus infection and dog rabies for its antigenic properties in target animals. As a result, high-quality immunobiological product compliant with specified parameters was prepared, test program was designed, tests including serological tests for determination of antibody levels before and after vaccination were carried out, obtained data were processed and structured.

MATERIALS AND METHODS

"Carnican-5R" vaccine against canine distemper, parvovirus and coronavirus enteritis, adenovirus infection and dog rabies was tested for its antigenic properties in accordance with the requirements of Order No. 101 of the Ministry of Agriculture of the Russian Federation of 6 March 2018 on approval of the rules for veterinary medicinal product preclinical studies, clinical trials and bioequivalence studies.

Vaccine. "Carnican-5R" vaccine, developed by the Federal Centre for Animal Health consists of 2 components: freeze-dried vaccine component contains attenuated canine distemper virus and liquid vaccine component contains inactivated canine parvovirus, canine coronavirus, canine adenovirus and dog rabies virus. The vaccine has been registered in the Russian Federation, and the patent for the invention has been obtained [20].

The active substance of the freeze dried vaccine component is attenuated CDV Rockborn strain; the active substance of the liquid vaccine component is aminoethylethylenimine-inactivated CPV-2 Grey strain, CCoV Rich strain, CAV-2 Unity strain, RABV ARRIAH strain. The freeze dried vaccine component is supplemented with stabilizers: lactalbumin hydrolysate, sucrose and gelatose, the liquid vaccine components is supplemented with aluminum hydroxide as an adjuvant. All source materials used for the vaccine production have passed comprehensive incoming quality control. One immunizing dose of the vaccine contains at least 3.0 lg $TCID_{50}/cm^3$ of attenuated CDV and inactivated CPV-2 (virus titre before inactivation – at least 7.0 log_2, HAU 1:128), CCoV (virus titre before inactivation – at least 3.0 lg $TCID_{50}/cm^3$), CAV-2 (virus titre before inactivation – at least 3.0 lg $TCID_{50}/cm^3$), RABV (virus titre before inactivation – at least 1.0 IU/cm^3).

Animals. Clinical trials were carried out in 10–12 week-old dogs ($n = 35$).

The animals were kept in shelters, veterinary clinics and individually in private households. The health status of the dogs was assessed before the trial and during the whole observation period.

To test "Carnican-5R" vaccine for its effectiveness, puppies were immunized twice with a 21-day interval; the vaccine was injected at one immunizing dose intramuscularly into the caudal proximal area of hind leg.

All tests were carried out in accordance with the requirements of the following Federal Centre for Animal Health standards: STO 00495527-0002 "Laboratory animals used for tests and experiments" and STO 00495527-0230 "Preclinical studies of veterinary medicinal products".

Serological tests. Sera from dogs were tested for antibodies to CDV, CCoV, CAV-2 with virus neutralization test (VNT) in microplates [21, 22, 23], for antibodies against RABV with fluorescent antibody virus neutralization test (FAVN test) and for antibodies against CPV-2 with hemagglutination inhibition test (HI test) according to the approved methodical guidelines [24].

Statistical analysis of the test results. The test results were processed using statistical methods in the Microsoft Excel program. Specific antibody titres were calculated using the Karber formula and expressed as log_2.

RESULTS AND DISCUSSION

No body temperature changes, general physiological state deterioration, anorexia, and local reactions at the site of the vaccine injection were observed after vaccination. No signs of canine distemper, canine parvovirus and coronavirus enteritis, canine adenovirus infection and dog rabies were observed.

Tests of puppy sera showed that mean group specific antibody titres in sera collected before vaccination were as follows: mean group specific antibody titre against CDV, CCoV, CAV-2 was < 1.0 log_2 (when sera were tested with VNT); against CPV-2 – 4.23 ± 0.63 log_2 (when sera were tested with HI test); against RABV – < 0.5 log_2 (when sera were tested with FAVN test).

Figure 1 shows the humoral immunity dynamics in dogs after "Carnican-5R" vaccine administration. Vaccination was found to induce virus-specific antibodies against CDV, CPV-2, CCoV, CAV-2 and RABV. The antibody levels 21 days after the first immunization were as follows: against CDV – 4.00 ± 0.25 log_2; against CPV-2 – 5.67 ± 0.58 log_2; against CCoV – 2.67 ± 0.14 log_2; against CAV-2 – 2.83 ± 0.14 log_2; against RABV – 0.82 ± 0.03 log_2, and were significantly higher than threshold values.

Mean group virus-specific antibodies titres on day 7, 14, 21, and 35 were higher than the titres in dogs before their immunization. On day 35, virus-specific antibody level was significantly higher than that ones determined at previous test points ($p \geq 0.1$) and was as follows: antibody titre against CDV – 8.60 ± 0.14 log_2; against CPV-2 – 8.93 ± 0.58 log_2; against CCoV – 5.0 ± 0.25 log_2; against CAV-2 – 5.36 ± 0.14 log_2; against RABV – 2.86 ± 0.07 log_2. Antibody levels demonstrated a "plateau effect" on day 42 and day 51. Based on the data obtained, it was concluded that double administration of "Carnican-5R" vaccine at a 21-day interval induced strong humoral response by

day 35 after the first vaccine administration. Thus, the following schedule for vaccination of dogs against canine distemper, parvovirus and coronavirus enteritis, adenovirus infection and dog rabies was recommended: first vaccination – at the age of 10–12 weeks, booster vaccination – after 21 days and then annual vaccination. The vaccination schedule for adult animals is similar and does not depend on the age of the dog.

At the next stage, the immunity duration after double vaccination against canine distemper, parvovirus and coronavirus enteritis, adenovirus infection and dog rabies was studied. For this purpose, blood samples were taken from the animals every 30 days for 12 months (observation period).

As shown in Figure 2, the duration of immunity to the pathogens of these diseases was at least 12 months. Slight decrease in the specific antibody levels was recorded at the time of booster vaccination, one year after the first vaccination of dogs. However, according to the published studies, the protective level of antibodies to CDV is 2–4 $\log_2$ [25, 26]. Double vaccine administration induced an increase in antibodies up to 9.15 ± 0.14 $\log_2$, the minimum value was 7.75 ± 0.14 $\log_2$ during the observation period. The level of antibodies should be at least 4 $\log_2$ to protect dogs from parvovirus enteritis [25, 26, 27, 28, 29, 30, 31]. After double vaccination, the maximum level of antibodies to CPV-2 was 10.40 ± 0.58 $\log_2$, the minimum anti-CPV-2 antibody level was 9.0 ± 0.28 $\log_2$. Double immunization induced an increase in antibodies to CAV-2 up to 6.35 ± 0.25 $\log_2$. The minimum anti-CAV-2 antibody level was 5.90 ± 0.14 $\log_2$ during the observation period of 12 months. There are

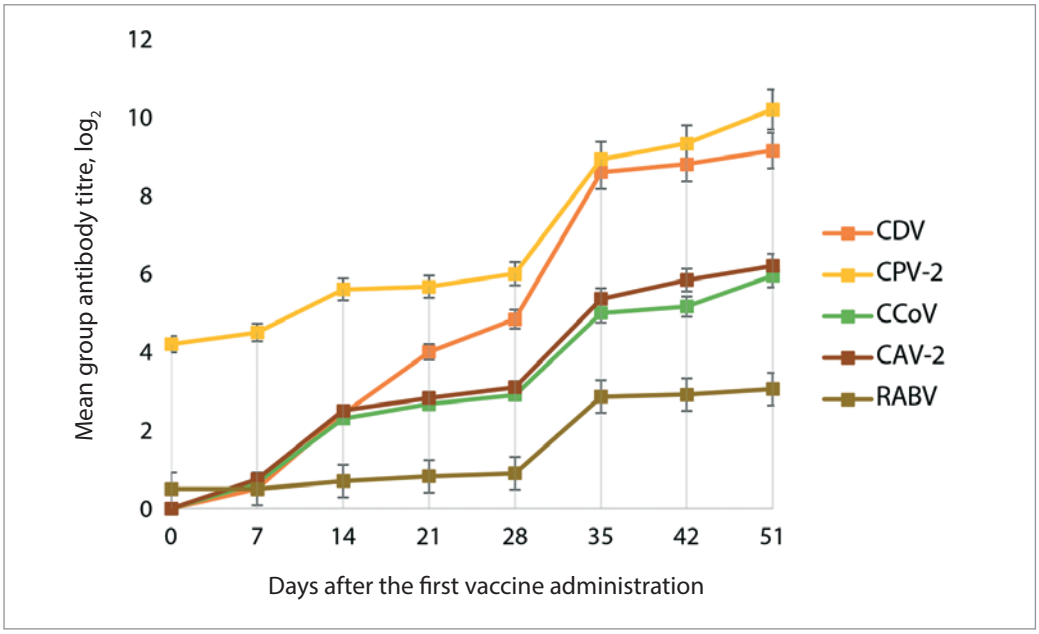

Fig. 1. Development of humoral immunity in dogs after "Carnican-5R" vaccine administration

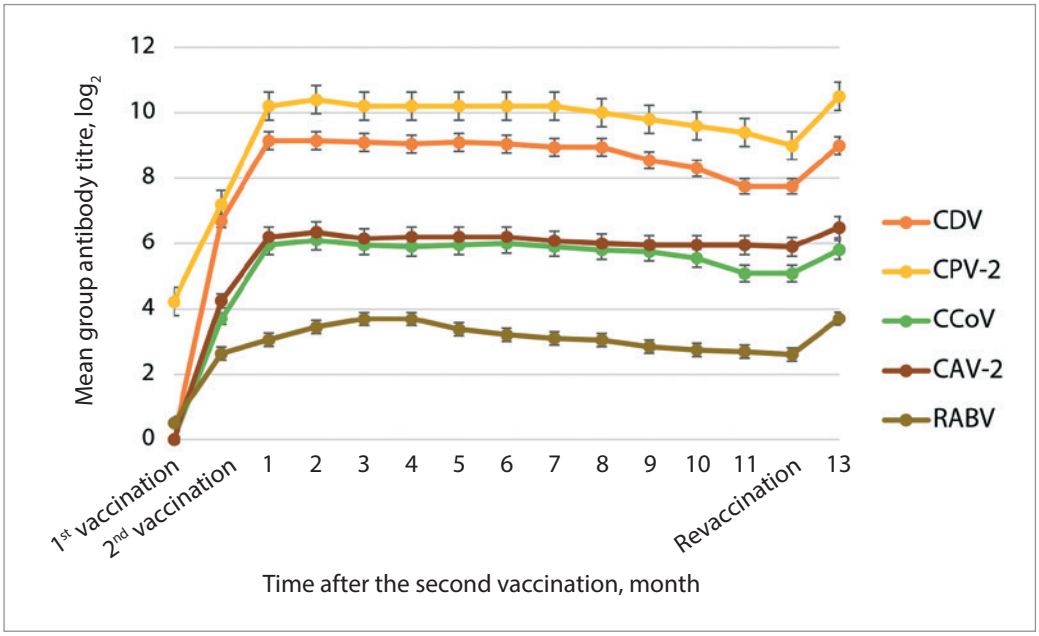

Fig. 2. Duration of the immunity in dogs after double "Carnican-5R" vaccine administration

no reliable data on protective level of antibodies against CCoV, however, the anti-CCoV antibody titre was higher than 5.95 ± 0.14 $\log_2$ after the second vaccine administration, and the minimum anti-CCoV antibody titre was 5.08 ± 0.28 $\log_2$ suggesting that animals were protected from the disease [32]. According to scientific data and the requirements of the World Organization for Animal Health, the anti-rabies vaccine should induce anti-rabies virus antibodies at a titre of ≥ 0.5 IU/cm^3 [14, 33]. In our study, the maximum titre of antibodies against RABV was 3.69 IU/cm^3, the minimum titre of antibodies against RABV was 2.6 IU/cm^3 after immunization.

During the year, mean titre of antibodies against CDV was 8.74 ± 0.53 $\log_2$, against CPV-2 was 9.95 ± 0.42 $\log_2$, against CCoV was 5.75 ± 0.34 $\log_2$, against CAV-2 was 6.09 ± 0.14 $\log_2$, against RABV was 3.12 ± 0.37 IU/cm^3. Thus, "Carnican-5R" vaccine induces humoral antibodies to canine distemper virus, canine parvovirus and coronavirus, canine adenovirus serotype 2 and rabies virus.

CONCLUSION

During the study, the vaccine against canine distemper, parvovirus and coronavirus enteritis, adenovirus infection and dog rabies was tested for its antigenic properties. Based on the data obtained, "Carnican-5R" vaccine was found to induce seroconversion in the target animals. The immunity lasts at least 12 months after double vaccine administration at a 21-day interval. The immune response develops 21 days after double vaccine administration. The mean antibody titres one month after booster vaccination were as follows: against CDV – 9.15 $\log_2$ (when tested with VNT), against CPV-2 – 10.2 $\log_2$ (when tested with HI test), against CCoV – 5.95 $\log_2$ (when sera were tested with VNT), against CAV-2 – 6.2 $\log_2$ (when sera were tested with VNT), against RABV – 3.05 IU/cm^3 (when sera were tested with FAVN test). The level of antibodies to these viruses in dogs is higher than the protective level and protects the animal from these infections.

The vaccine is nonreactogenic and safe, does not cause any pronounced local reaction when administered intramuscularly or subcutaneously, the vaccine has no adverse effect on the physiological state of animals. The vaccine induces a pronounced immune response owing to production of virus-specific antibodies at protective titres.

"Carnican-5R" combined vaccine against canine distemper, parvovirus and coronavirus enteritis, adenovirus infection and dog rabies developed at the Federal Centre for Animal Health can be used for specific prevention of the viral diseases in dogs. The vaccine has passed testing at the Russian State Center for Animal Feed and Drug Standardization and Quality and is registered in the Russian Federation.

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Received 14.01.2025
Revised 04.03.2025
Accepted 06.03.2025

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https://doi.org/10.29326/2304-196X-2025-14-2-186-193

Identification of *Escherichia coli, Escherichia albertii, Proteus vulgaris* biofilms detected in poultry with respiratory and gastrointestinal diseases

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ABSTRACT

Introduction. When the body resistance-associated compensatory mechanisms are impaired or evolutionarily developed microbiocenoses are changed the quorum sensing signaling molecules facilitates excessive growth of pathogenic microorganisms. Antibacterial potential of inhibitors of intercellular communication molecule synthesis is achieved through reducing the microorganism adhesion and, consequently, *in vivo* and *in vitro* contamination.

Objective. Study of the dynamics of morphometric and densitometric parameters of biofilms formed by *Escherichia coli, Escherichia albertii, Proteus vulgaris* isolates identified in poultry with respiratory and gastrointestinal diseases.

Materials and methods. Dynamics of the biofilms formed by reference strains and isolates recovered from pathological samples from ROSS-308 chickens at the age of 40–42 weeks ($n = 20$) were studied. The sample optical densities were determined using Immunochem-2100 photometric analyzer (HTI, USA), wavelength 580 nm (OD_{580}). Morphometric parameters were recorded at ≥ 90.0% reliable frequency in the field of view of H604 Trinocular Unico optical microscope (United Products & Instruments Inc., USA) and Hitachi TM3030 Plus scanning electron microscope (Hitachi, Japan).

Results. *Escherichia coli, Escherichia albertii,* and *Proteus vulgaris* were isolated from pathological samples from the poultry with catarrhal hemorrhagic aerosacculitis, hemorrhagic enteritis, fibrinous polyserositis and splenomegaly signs and then identified. Direct correlations ($r = 0.91$) between morphometric and densitometric parameters depending on the cultivation time were established. Cells with defective cell walls, spheroplasts, needle-like and giant structures as well as revertant cells dominated during heterogeneous population dispersion.

Conclusion. General patterns of the heterogeneous microorganism population development are mediated by adhesion, synthesis of exocellular molecules, intensive cell proliferation and differentiation depending on the cell cycle stage.

Keywords: biofilms, bacteria, heteromorphism, densitometry, optical microscopy, scanning electron microscopy

Acknowledgements: The authors thank the Russian Biotechnological University, Belgorod Branch of the Federal Centre for Animal Health, and Peoples' Friendship University of Russia named after Patrice Lumumba for the study support.

For citation: Lenchenko E. M., Ponomarev V. V., Sachivkina N. P. Identification of *Escherichia coli, Escherichia albertii, Proteus vulgaris* biofilms detected in poultry with respiratory and gastrointestinal diseases. *Veterinary Science Today*. 2025; 14 (2): 186–193. https://doi.org/10.29326/2304-196X-2025-14-2-186-193

Conflict of interests: The authors declare no conflict of interests.

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УДК 619:616-076:579.84:636.5

Индикация биопленок изолятов *Escherichia coli, Escherichia albertii, Proteus vulgaris*, идентифицированных при болезнях органов дыхания и пищеварения птиц

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РЕЗЮМЕ

Введение. При снижении компенсаторных механизмов резистентности организма, изменении состава эволюционно сложившихся микробиоценозов избыточному росту патогенных микроорганизмов способствует репрезентация сигнальных молекул quorum sensing. Антибактериальный потенциал

ингибиторов синтеза молекул межклеточных коммуникаций достигается за счет снижения адгезии микроорганизмов, а соответственно, и степени контаминации *in vivo* и *in vitro.*

Цель исследования. Изучение динамики изменений морфометрических и денситометрических показателей биопленок изолятов *Escherichia coli, Escherichia albertii, Proteus vulgaris,* идентифицированных при болезнях органов дыхания и пищеварения птиц.

Материалы и методы. Исследовали динамику развития биопленок референтных штаммов и изолятов, выделенных из патматериала птицы: куры кросса ROSS-308 40–42-недельного возраста ($n = 20$). Оптическую плотность исследуемых образцов определяли с применением фотометрического анализатора Immunochem-2100 (HTI, США), длина волны 580 нм (OD_{580}). Морфометрические показатели учитывали при достоверной частоте встречаемости ≥ 90,0% поля зрения оптического микроскопа H604 Trinocular Unico (United Products & Instruments Inc., США) и сканирующего электронного микроскопа Hitachi TM3030 Plus (Hitachi, Япония).

Результаты. Из патматериала птиц с признаками катарально-геморрагического аэросаккулита, геморрагического энтерита, фибринозного полисерозита и спленомегалии были выделены и идентифицированы *Escherichia coli, Escherichia albertii, Proteus vulgaris.* В зависимости от времени культивирования установлены прямые коррелятивные зависимости ($r = 0,91$) между морфометрическими и денситометрическими показателями. При дисперсии гетерогенной популяции доминируют клетки с дефектной клеточной стенкой, сферопласты, игольчатые и гигантские структуры, а также клетки-ревертанты.

Заключение. Общие закономерности динамики развития гетерогенной популяции микроорганизмов опосредованы адгезией, синтезом экзоцеллюлярных молекул, интенсивной пролиферацией и дифференциацией клеток в зависимости от стадии клеточного цикла.

Ключевые слова: биопленки, бактерии, гетероморфизм, денситометрия, оптическая микроскопия, сканирующая электронная микроскопия

Благодарности: Авторы благодарят РОСБИОТЕХ, Белгородский филиал ФГБУ «ВНИИЗЖ», РУДН за предоставленные возможности для проведения исследовательской работы.

Для цитирования: Ленченко Е. М., Пономарев В. В., Сачивкина Н. П. Индикация биопленок изолятов *Escherichia coli, Escherichia albertii, Proteus vulgaris*, идентифицированных при болезнях органов дыхания и пищеварения птиц. *Ветеринария сегодня*. 2025; 14 (2): 186–193. https://doi.org/10.29326/2304-196X-2025-14-2-186-193

Конфликт интересов. Авторы заявляют об отсутствии конфликта интересов.

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INTRODUCTION

In view of globalization of the spread of new and variants of known nosological forms characterized by high epidemiological potential, there is a statistically significant trend to increase in incidence of the infections caused by antibiotic-resistant bacteria of the order *Enterobacterales* [1, 2, 3, 4]. Due to their multidrug resistance, these bacteria are classified to the first category of critical priority level for research according to the WHO Bacterial Priority Pathogens List (2024) [5].

Clinical *Escherichia coli* isolates identified in humans with septicemia, neonatal meningitis, and urologic disorders are genetically similar and share common virulence genes with avian pathogenic *E. coli* (APEC) [6, 7].

High population density in limited areas, keeping animals of the same species and age in the holding, use of antibiotics as well as frequent changes in the vaccination schedule including use of vaccines based on "hot" and variant strains contribute to the wide spread of infectious diseases [8]. According to the veterinary reports, colibacillosis is registered everywhere and responsible for significant economic losses [9, 10]. In poultry with systemic infection, the dominance of *E. coli* as an etiological agent ranges from 50.7 to 100% depending on the disease situation on commercial poultry farms of various types as well as family-operated and backyard farms [11, 12]. Increasing resistance of APEC to different classes of antibiotics, including socially important antibiotics (*β*-lactams, colistin, and carbapenems) is a marker of multiple APEC resistance [13, 14, 15, 16].

E. coli pathogenic properties are accounted for virulence factors encoded by chromosomal, plasmid genes and chromosome-integrated bacteriophages [17, 18]. When intestinal compensatory mechanisms of mucociliary clearance and colonization resistance are impaired and microbiocenosis quantitative and species composition are changed, representation of the quorum sensing (QS) signalling molecules contributes to the excessive growth of pathogenic microorganisms [19]. The therapeutic and disinfecting effect of QS inhibitors owing to blocking the intercellular communication molecules synthesis enables reducing the adhesion of microorganisms and, consequently, level of contamination *in vivo* and *in vitro* [20, 21].

Studies of the etiological factors of respiratory and gastrointestinal diseases in poultry are of priority for identification of pathogenetical factors of initiation, development and outcome of the avian infectious pathology characterized by pathogenic enterobacteria excessive growth and dissemination. Study of the general patterns of multilevel algorithms for differentiation of heterogeneous population including viable uncultivated cells will facilitate optimization of the long-term retrospective identification of ubiquitous bacteria as well as development of methods for biofilm eradication in the future.

The aim of the work is to study the dynamics of morphometric and densitometric parameters of biofilms of *E. coli*, *Escherichia albertii*, *Proteus vulgaris* isolates identified in poultry with respiratory and gastrointestinal diseases.

MATERIALS AND METHODS

Strains. Isolates recovered in pathological samples collected from ROSS-308 cross chickens at the age of 40–42 weeks ($n = 20$) were used for tests. Reference

Escherichia coli strain (ATCC 25922) from the Collection of the Tarasevich State Research Institute for Standardization and Control of Biological Products (Moscow) was used as a control [22].

Nutrient media. The following nutrient media were used: Endo medium, bismuth-sulfite agar (BSA; HiMedia, India), meat-peptone broth (MPB), meat-peptone agar (MPA), Hiss medium, Olkenitsky's medium, Simmons' citrate agar (State Research Center for Applied Microbiology, Russia), Tryptone Bile X-glucuronide agar, Chromocult® coliform agar (Merck, Germany).

Test system. The following test systems were used: Paper indicator systems for the microorganism identification; kit No. 2 for *Enterobacteriaceae* genus and species differentiation (Microgen, Russia); Boichemical plate for enterobacteria identification (Diagnostic Systems, Russia); ENTERO-Rapid 24, NEFERMtest 24 (Erba Lachema s.r.o., Czech Republic).

Postmortem examination. Dead chickens (n = 20) submitted to the Belgorod Branch of the Federal Centre for Animal Health for bacteriological examination from poultry farms located in the Central Black Earth region of the Russian Federation were subjected to postmortem examination (necropsy). The tests were performed in accordance with the Methodical guidelines for pathomorphological diagnosis of animal, avian, and fish diseases in veterinary laboratories: approved by the Veterinary Department of the Ministry of Agriculture of the Russian Federation on 11 September 2000, No. 13-7-2/2137 [23]. Postmortem examination was carried using common methods and taking into account the chicken anatomical and topographic features [24, 25, 26].

Microbiological tests were carried out in accordance with the Methodical guidelines for bacteriological diagnosis of mixed intestinal infection in young animals caused by pathogenic enterobacteria, approved by the Veterinary Department of the Ministry of Agriculture of the Russian Federation on 11 October 1999, No. 13-7-2/1759; Methodical guidelines for bacteriological diagnosis of animal colibacillosis (escherichiosis), approved by the Veterinary Department of the Ministry of Agriculture of the Russian Federation on 27 July 2000, No. 13-7-2/2117; Methodological guidelines for Isolation of bacteria from the animal gastrointestinal tract and identification thereof, approved by the Veterinary Department of the Ministry of Agriculture of the Russian Federation on 11 May 2004, No. 13-5-02/1043 [27, 28, 29].

The authors confirm compliance with institutional and national standards in accordance with the Consensus Author Guidelines for Animal Use (IAVES, July 23, 2010). The test protocol was approved by the Ethics Committee of the RUDN University, Moscow, Russian Federation (Protocol No. 9a/3 of 8 October 2024).

Small intestine and caecum contents were examined for microorganism quantification. Test samples weighing 1.0 g were placed in test tubes and of 0.85% sodium chloride solution was added to the tubes, 9.0 cm^3 per tube. Diagnostically significant dilutions were made, then 0.1 mL of the test sample was inoculated onto differential media.

Test pathological sample (heart with ligated vessels, lungs, tubular bone, liver with gall bladder, spleen) was put with a Pasteur pipette to the middle part of a Petri dish and evenly distributed with a glass spatula. For small intestine examination, its contents were removed, mucous membrane was carefully scraped off using a scarifying cone of a Pasteur pipette and the material was inoculated onto the medium. In order to avoid the swarming bacteria growth, Endo medium surface was irrigated with 96% ethanol (1–2 cm^3) before material inoculation. Microorganisms were cultured at (37 ± 1) °C for (24 ± 1) hours and (48 ± 1) hours. To isolate pure *Proteus* spp. cultures, the materials were inoculated according to Shukevich method in condensed fluid of freshly slanted MPA and cultivated at (37 ± 1) °C for (24 ± 1) hours. When the growth was observed, the microorganisms were transferred to the BSA medium and cultured at (37 ± 1) °C for (24 ± 1) and (48 ± 1) hours [24, 27, 28].

For species identification, three species-characteristic colonies of microorganisms were transferred into tubes with slanted MPA and cultured at (37 ± 1) °C for (24 ± 1) hours. The microorganisms were tested for their morphological, cultural, and biochemical properties using common methods [1, 27, 28, 29].

Biofilm tests. For densitometric tests, the test samples were added to wells of a 96-well plate (Medpolymer OJSC, Russia) and cultured at (37 ± 1) °C under static aerobic conditions for 6, 18, 24, 48 hours. After the specified time, the fluid was removed from the plate wells, and the sediment was washed with 200 µL of phosphate buffer solution (pH 7.2) three times. At each washing stage, the plate contents were stirred at 2,000 rpm for 10 minutes using MixMate vortex shaker (Eppendorf, Germany). The samples were fixed with 96% ethanol for 15 minutes and dried at (37 ± 1) °C for 20 minutes. Then, 0.5% crystalline violet solution (HiMedia, India) was added to the wells and the plates were cultured at (37 ± 1) °C for 5 minutes. The well contents were removed, the wells were washed with 200 µL of phosphate buffer solution (pH 7.3) three times, and dried. The dye was eluted with 200 µL of 96% ethanol for 30 minutes [30, 31]. The optical densities of the test samples were determined using an ImmunoChem-2100 photometric analyzer (HTI, USA) at a wavelength of 580 nm (OD_{580}).

For morphometric tests, the preparations were fixed with ethanol-ether mixture (1:1) for 10 minutes and stained with an aqueous gentian violet solution (1:2,000) and Gram stained (BioVitrum, Russia). For scanning electron microscopy, the preparations were fixed with 25% glutaraldehyde solution vapours for 8 hours, and then with 1% osmium tetroxide solution vapours for 4 hours. The test samples were thickened with ethanol at increasing concentration: 30, 50, 96, 100%. Then, test samples were exposed to gold ions using a Q150T ES device (Quorum Technologies Ltd., Great Britain). Morphometric parameters were recorded at significant ≥ 90.0% frequency in the field of view of the H604 Trinocular Unico optical microscope (United Products & Instruments Inc., USA) and Hitachi TM3030 Plus scanning electron microscope (Hitachi, Japan).

Statistical analysis using the Student's criterion was used for the test result processing; the results were considered reliable at $p \leq 0.05$ [19].

RESULTS AND DISCUSSION

Postmortem examination. Postmortem examination of dead ROSS-308 chickens at the age of 40–42 weeks (n = 20) showed the following: the chicken feathers

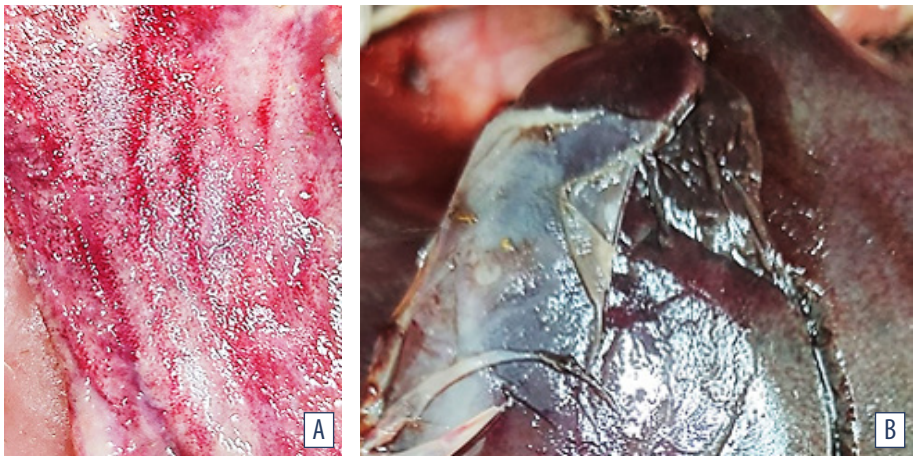

Fig. 1. Postmortem gastrointestinal lesions in poultry:
A – multiple hemorrhages in intestinal mucosa; B – perihepatitis

were dull and ruffled; the dead chickens were emaciated. Cyanosis of mucous membranes, uneven and extreme swelling of stomach, small intestine and caecum were detected. Multiple petechial and striated haemorrhages were found in the muscles and tracheal, stomach and intestinal mucosa. Acute congestive hyperemia of cardiovascular organs was characterized by blood vessel congestion, edematous fluid accumulation in loose connective tissue, red blood cell hemolysis. Catarrhal hemorrhagic aerosacculitis, splenomegaly, hemorrhagic enteritis and fibrinous polyserositis manifestations were detected (Fig. 1).

Detection and identification of microorganisms. The bacteria formed round glossy convex colonies with even edges, 1.5–2.5 mm in diameter when the test samples were inoculated onto differential nutrient media intended for primary identification.

On Endo medium, lactose fermenting microorganisms formed red colonies, some of which had a characteristic metallic glitter. The number of colonies grown onto media inoculated with small intestine and cecum contents was $(1.43 \pm 0.25) \times 10^6$ CFU/g; and $(4.6 \pm 0.32) \times 10^7$ CFU/g, respectively. Lactose-non-fermenting microorganisms were isolated from the chicken small intestine contents together with the specified bacteria; number of the pinkish colonies colourless in the centre was $(0.85 \pm 0.34) \times 10^4$ CFU/g (Fig. 2A).

When test samples were inoculated with Shukevich method in the condensed fluid of freshly slanted MPA, microorganism growth was observed. The cultures transferred from MPA to BSA medium formed dark-green colonies surrounded by zone of inhibition, the number of colonies was $(0.77 \pm 0.87) \times 10^3$ CFU/g (Fig. 2B).

Gram-negative, facultative anaerobic, oxidase-negative, and catalase-positive *E. coli* isolates were identified when pure cultures of the microorganisms isolated from the pathological samples of all tested chickens (100%) were tested for their morphological, tinctorial, and biochemical properties. *E. coli* monocultures were detected in small intestine content samples from 16 chickens (80%). *E. albertii* and *P. vulgaris* bacteria were detected together with *E. coli* in tested small intestine samples from 4 chickens (20%).

Morphological and densitometric parameters of biofilms. *E. coli*, *E. albertii*, and *P. vulgaris* isolates cultivated at (37 ± 1) °C for 6, 18, 24, 48 hours under static aerobic conditions showed common patterns for biofilm development regardless of the isolation origin. The changes in absolute values of tested sample optical density and the biofilm formation intensity are given in the Table.

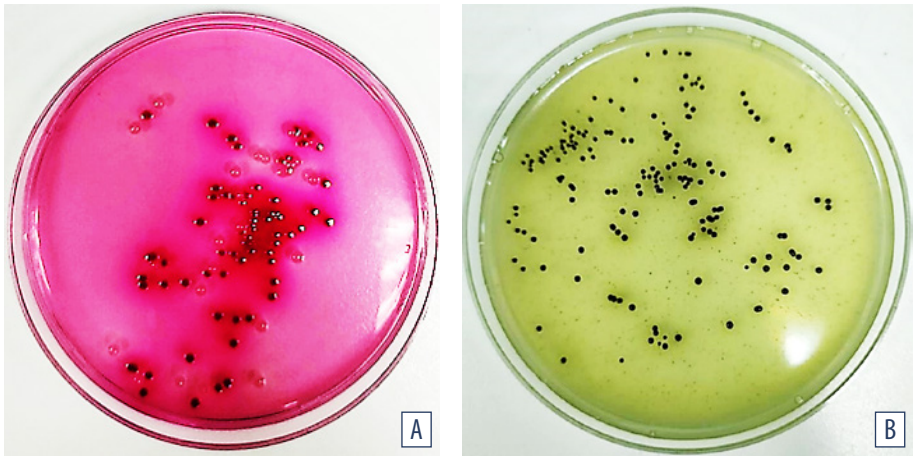

Fig. 2. Microorganism cultures isolated from chicken small intestine contents:
A – Endo medium, cultivation at (37 ± 1) °C for 24 hours;
B – BSA cultivation at (37 ± 1) °C for (24± 1) hours

Table
Densitometric parameters of biofilms

Sample cultivation time, hours	Absolute value of optical density	Biofilm formation intensity
6	(0.102 ± 0.04) – (0.111 ± 0.06)	≥ 0.1–0.2
18	(0.172 ± 0.07) – (0.191 ± 0.05)	≥ 0.1–0.2
24	(0.246 ± 0.03) – (0.284 ± 0.08)	≥ 0.2–0.3
48	(0.348 ± 0.07) – (0.526 ± 0.18)	≥ 0.3–0.4

Depending on cultivation duration, direct correlations ($r = 0.91$) were observed between densitometric parameter intensities and increased frequency of visualized bacterial coaggregation within the intercellular matrix.

The following stages of biofilm development were identified at representative ≥ 90.0% field of view of the microscope: adhesion, fixation, microcolony, growth, and dispersion. Adsorption and nonspecific adhesion of microorganisms to the tested substrate surface – glass owing to conditioning were detected at the initial stages of development. Moreover, at this stage, cells can either attach to the substrate surface or detach from it and return to planktonic phase of growth. Intermolecular interactions between specific microbial cell wall structures mediate irreversible adhesion and surface attachment. Once microorganisms were firmly attached to the substrate surface they promoted adhesion of subsequent cells. Cells exhibiting distinct morphologies and sizes but interconnected within an extracellular matrix were differentiated depending on the cell cycle stage (Fig. 3).

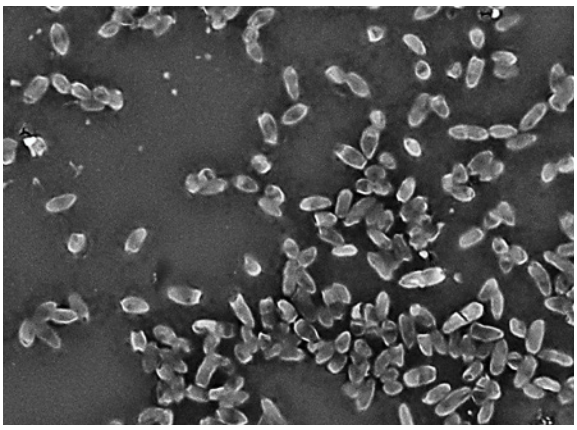

Fig. 3. E. coli biofilm morphology (MPB medium; cultivation at (37 ± 1) °C for 18 hours; Hitachi TM3030 Plus, Japan)

Clusters (aggregates, conglomerates) formed and grew owing to the binary division of bacteria during intensive proliferation of the cells synthesizing exocellular molecules. Rounded fluid-filled structures – canals serving for the population hydration were detected between clusters of orderly and at the same time multidirectionally arranged cells. Extracellular matrix exhibited progressive thickening correlating with both increased numbers of attached dividing cells and enhanced synthesis of exopolymeric components. The matrix components were differentiated based on the chemical composition when the cells were stained with metachromatic aniline dyes with properties: protein structures stained blue, polysaccharides stained pink (Fig. 4).

Mature three-dimensional heteromorphic biofilm becomes immobilized through QS-mediated intercellular communication driven by population expansion and extracellular matrix development. Dispersion of the heteromorphic population increased with prolongation of the cultivation time. Bacteria characteristic for L-transformation were detected together with the cells typical for the bacteria species. The following cells dominated: cells with defective cell walls, spheroplasts, needle-like and giant structures, as well as cells capable of reverting to their original phenotypic and metabolic state. The destruction, partial or complete autolysis of cells losing typical morphofunctional features (uncultivable cells) were accompanied by enhanced light refraction combined with decrease in the optical density of the biofilm (Fig. 5).

In case of microorganism overgrowth, their pathogenicity is regulated by transcriptional control of polymer molecule adhesion, invasion, and synthesis [32, 33]. QS molecules are considered as promising targets in the development of the medicinal products that significantly reduce APEC adhesion and inhibit anti-inflammatory cytokine expression [34, 35].

The results of biofilm dynamics studies will be useful for optimization of methods for microbiological monitoring of critical control points in poultry production, and can also be used for development of medicinal products and disinfectants blocking synthesis of intercellular communication molecules.

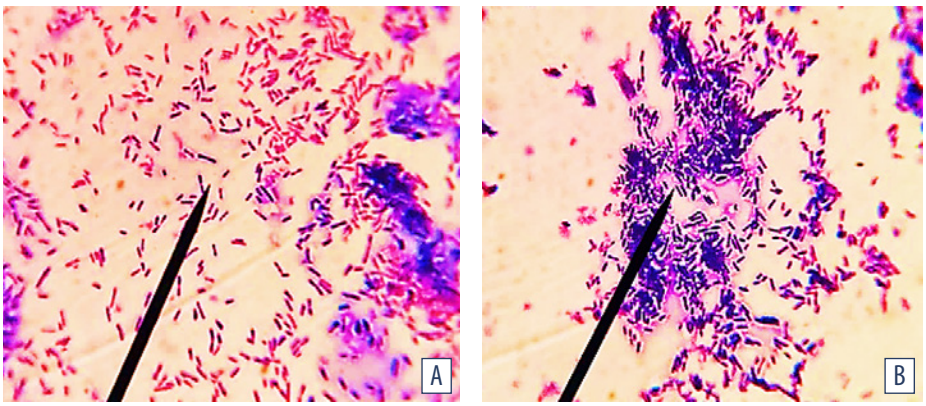

Fig. 4. E. coli biofilm morphology (MPB medium, temperature (37 ± 1) °C, cultivation period: A –18 hours, B – 24 hours; Gram staining; oc. 10×, obj. 100×, immersion, H604 Trinocular Unico, USA)

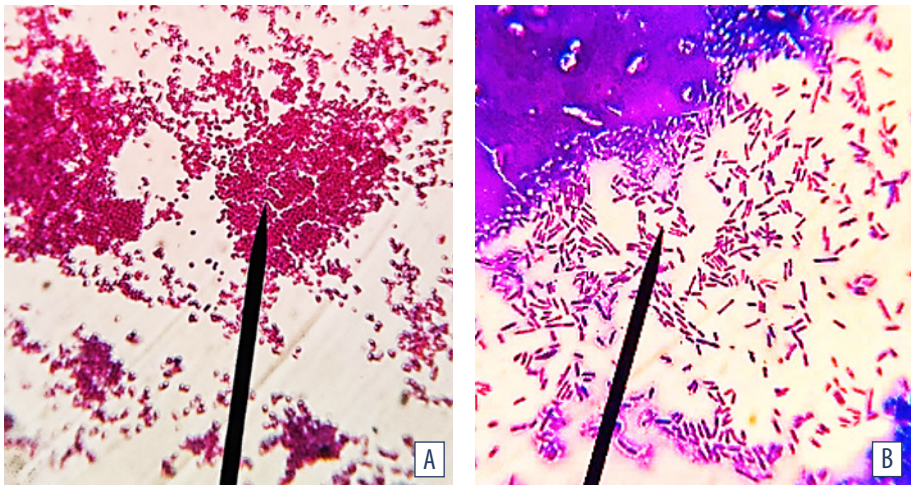

Fig. 5. Biofilm morphology: A – E. albertii; B – E. coli (MPB medium; cultivation at (37 ± 1) °C for 48 hours; Gram staining; oc. 10×, obj. 100×, immersion, H604 Trinocular Unico, USA)

CONCLUSION

E. coli, *E. albertii*, and *P. vulgaris* isolates cultivated at (37 ± 1) °C for 6, 18, 24, 48 hours under static aerobic conditions were shown to have common patterns for biofilm formation and growth. Biofilm initiation and growth is multistage process where motile planktonic microorganisms differentiate into an attached, structured form, with QS playing a crucial role in intercellular communication. Coaggregation of heteromorphic cells of different sizes and shapes depending on the cell cycle stage is the general pattern of heterogeneous microorganism population dynamics mediated by adhesion, intensive cell proliferation, and exocellular molecule synthesis. Bacteria characteristic for L-transformation dominated during heteromorphic population dispersion. Spheroplasts, needle-like and giant structures as well as cells capable of reverting to their original phenotypic and metabolic state were differentiated together with the cells typical of the species.

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Received 14.01.2025
Revised 07.03.2025
Accepted 14.04.2025

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Contribution of the authors: The authors contributed to the study equally: data collection and analysis; determination of the study goals, tasks and methods; conclusion formulation and scientific justification; presentation of the key study results as the paper.

Вклад авторов: Авторы внесли равный вклад в проведение исследования: сбор и анализ материала; определение целей и задач, методов исследования; формулирование и научное обоснование выводов; оформление ключевых результатов исследования в виде статьи.

https://doi.org/10.29326/2304-196X-2025-14-2-194-200

Clostridium species diversity in cattle

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ABSTRACT

Introduction. Clostridial infections, though relatively sporadic, are globally ubiquitous and specified by high mortality rates, resulting in substantial economic losses to agriculture. In cattle, pathogenic *Clostridia* cause diseases such as enterotoxemia, malignant edema, tetanus, and botulism. The most clinically significant species include *Clostridium septicum*, *Clostridium perfringens*, *Clostridium chauvoei*, *Clostridium novyi*, and *Clostridium sordellii*.

Objective. Study of *Clostridium* spp. diversity by examination of autopsy samples and sections of cattle from different regions of Russia; determination of their anatomical localization as well as antibiotic resistance of *Clostridium perfringens* to the most common groups of antibiotics.

Materials and methods. Throughout the study, we adhered to internationally recognized regulatory frameworks and methodological guidelines, employing standardized microbiological and mass-spectrometric methods. Antibiotic resistance was tested against multiple drug groups, such as macrolides, monobactams, penicillins, polypeptides, glycopeptides, aminoglycosides, carbapenems, lincosamides, tetracyclines, ansamycins, diaminopyrimidines, fusidic acid derivatives, etc. *Clostridium* isolates were recovered and identified using routine bacteriological methods coupled with MALDI-ToF mass spectrometry.

Results. Analysis of 359 biological samples resulted in isolation and identification of 137 *Clostridium* isolates (*Paraclostridium bifermentans*, *Clostridium perfringens*, *Clostridium tertium*, *Clostridium butyricum*, *Clostridium septicum*, *Clostridium sporogenes*, *Clostridium cadaveris*, *Clostridium sphenoides*, *Clostridium cochlearium*, *Clostridium sartagoforme*, *Clostridium chauvoei*, *Clostridium novyi*, *Clostridium sordellii*, *Clostridium paraputrificum*, *Clostridium* spp.), of which 25 exhibited pathogenic potential and 17 demonstrated toxigenic properties. *Clostridia* were most frequently isolated from the liver, small and large intestinal segments, and muscular tissues. Herewith, *Clostridium perfringens* prevailed (17.5%). This bacterium isolates demonstrated multiple drug resistance to cefixime, fusidic acid, cefotaxime, cefaclor, spectinomycin, piperacillin, clarithromycin, doripenem and doxycycline.

Conclusion. The obtained results can be used for modification of current clostridial infection treatment protocols, reformulation of immunobiological products, development of evidence-based guidelines for use of antibiotics in livestock production to mitigate antimicrobial resistance risks.

Keywords: *Clostridium*, *Clostridiaceae*, cattle, antibiotic resistance, toxigenicity, biosafety, pathogenicity, anaerobes

Acknowledgements: The study was conducted under the state assignment of the Ministry of Science and Higher Education of the Russian Federation (Project FGUG-2025-0003).

For citation: Shastin P. N., Savinov V. A., Laishevtsev A. I., Mandryka E. D., Fabrikantova E. A., Supova A. V. *Clostridium* species diversity in cattle. *Veterinary Science Today*. 2025; 14 (2): 194–200. https://doi.org/10.29326/2304-196X-2025-14-2-194-200

Conflict of interests: The authors declare no conflict of interests.

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УДК 619:579.852.13:636.22/.28

Видовое разнообразие клостридий у крупного рогатого скота

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РЕЗЮМЕ

Введение. Клостридиозы, несмотря на относительно спорадические случаи их возникновения, имеют повсеместное распространение и характеризуются высокой летальностью, что наносит экономический ущерб сельскому хозяйству. У крупного рогатого скота патогенные клостридии вызывают такие заболевания, как энтеротоксемия, злокачественный отек, столбняк, ботулизм. Этиологически значимыми видами клостридий являются *Clostridium septicum*, *Clostridium perfringens*, *Clostridium chauvoei*, *Clostridium novyi*, *Clostridium sordellii*.

Цель работы. Изучение видового разнообразия клостридий на основании исследований патолого-анатомического и секционного материала крупного рогатого скота из различных регионов России, определение мест их локализации в организме животных, а также антибактериальной устойчивости *Clostridium perfringens* к наиболее распространенным группам антибиотиков.

Материалы и методы. В период проведения исследования руководствовались общепринятыми нормативно-правовыми документами, методическими указаниями, рекомендациями, инструкциями; применяли микробиологические, масс-спектрометрические методы. Для определения антибактериальной устойчивости использовались различные группы препаратов: макролиды, монобактамы, пенициллины, полипептиды, гликопептиды, аминогликозиды, карбапенемы, линкозамиды, тетрациклины, ансамицины, диаминопиримидины, фузидины и др. Изоляты клостридий выделяли, используя рутинные бактериологические методы, видовую идентификацию выполняли с помощью времяпролетной масс-спектрометрии MALDI-ToF.

Результаты. При исследовании 359 образцов биоматериала было выделено и идентифицировано 137 изолятов клостридий (*Paraclostridium bifermentans*, *Clostridium perfringens*, *Clostridium tertium*, *Clostridium butyricum*, *Clostridium septicum*, *Clostridium sporogenes*, *Clostridium cadaveris*, *Clostridium sphenoides*, *Clostridium cochlearium*, *Clostridium sartagoforme*, *Clostridium chauvoei*, *Clostridium novyi*, *Clostridium sordellii*, *Clostridium paraputrificum*, *Clostridium* spp.), из которых 25 обладали патогенными и 17 – токсигенными свойствами. Чаще всего клостридии обнаруживали в печени, тонком и толстом отделах кишечника, мышцах. При этом выявлено превалирование *Clostridium perfringens* (17,5%). Установлена полирезистентность изолятов данного вида бактерии к цефиксиму, фузидиевой кислоте, цефотаксиму, цефаклору, спектиномицину, пиперациллину, кларитромицину, дорипенему, доксициклину.
Заключение. Полученные результаты могут быть использованы для модификации существующих протоколов лечения клостридиозов, корректировки состава иммунобиологических препаратов, разработки рекомендаций по использованию антибиотиков в животноводстве для снижения рисков развития антимикробной резистентности.

Ключевые слова: клостридии, *Clostridiaceae*, крупный рогатый скот, антибиотикорезистентность, токсигенность, биобезопасность, патогенность, анаэробы

Благодарности: Исследование проведено в рамках государственного задания Министерства науки и высшего образования Российской Федерации, проект FGUG-2025-0003.

Для цитирования: Шастин П. Н., Савинов В. А., Лаишевцев А. И., Мандрыка Е. Д., Фабрикантова Е. А., Супова А. В. Видовое разнообразие клостридий у крупного рогатого скота. *Ветеринария сегодня*. 2025; 14 (2): 194–200. https://doi.org/10.29326/2304-196X-2025-14-2-194-200

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

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INTRODUCTION

The genus *Clostridium* was first described by A. Prażmowski in 1880. Over 225 species of *Clostridia* have been currently identified in various regions of the planet. *Clostridia* are gram-positive rods that form spores. They are widespread in the environment, and are also part of the human and animal microbiome. However, only some of them are capable of causing diseases in animals [1, 2, 3]. Clostridial infections are characterized by high mortality. Due to the spore-forming ability of *Clostridia*, they can persist in the soil for a long time, thus posing a potential threat of the disease emergence [4, 5, 6]. The pathogen entry into the body of animals occurs mainly by ingestion of contaminated feed (alimentary route), through wounds or by inhalation. The main factors of *Clostridium* pathogenicity are exotoxins and enzymes [7, 8, 9, 10], which have hemolytic, necrotizing and lethal effects. The most potent toxins of clostridial origin are botulinum and tetanus neurotoxins, as well as epsilon toxin produced by *Clostridium perfringens* types B and D [11, 12, 13, 14].

The emergence of polyresistant *Clostridium* strains results in wider spread of clostridial infections. A number of scientists have noted low therapeutic efficacy of antibacterial drugs against the clinical manifestation of anaerobic enterotoxemia in young cattle, high mortality and the need for specific prevention [7, 15, 16, 17, 18, 19].

According to "Galen" component of the FGIS "VetIS", the list of registered vaccines against bovine clostridial infections in the Russian Federation is currently includes the following products: Clostrivax (Tecnovax S. A., Argentina); Coglavax (Ceva Sante Animale, France; Ceva-Phylaxia Veterinary Biologicals Company, Hungary); Clostbovac-8 (Vetbiochem LLC, Russia); Clostarm-9 (Armavir Biofactory, Russia); Cubolac (CZ Vaccines S. A. U., Spain); Antox 9 (Stavropol Biofactory, Russia); One Shot Ultra 8 (Zoetis Inc., USA); Scourguard 4KS (Zoetis Inc., USA).

The relevance and novelty of the work lies in obtaining data on the antibiotic resistance of the etiologically relevant *Clostridium* isolates, on the structure of the strains isolated from cattle, and on their toxigenic and pathogenic properties. The resulted data will contribute to the improvement of the clostridial infection control system in cattle, which in turn will reduce the economic losses in the livestock production.

The aim of the work was to conduct the monitoring studies to identify *Clostridia*, as well as to assess the level of antimicrobial resistance of *Clostridium perfringens* isolates recovered from cattle in various regions of Russia, and to study their toxigenic and pathogenic properties.

MATERIALS AND METHODS

The work was performed in 2022–2024 at the Laboratory for Diagnostics and Control of Antibiotic Resistance of Pathogens of the Most Clinically Significant Infectious Animal Diseases of the Federal Scientific Centre VIEV, as part of the state project (FGUG-2025-0003) supported by the Ministry of Science and Higher Education of the Russian Federation. As a result of our own research, monitoring data was obtained and the practical part was completed. Sectional and autopsy materials collected from cattle were delivered from various regions of Russia: Nizhny Novgorod, Moscow, Leningrad, Ryazan, Novosibirsk, Penza Oblasts and Republic of Mordovia.

Biological material. A total of 359 samples were examined (liver, heart, spleen, lung, kidney, muscle, small and large intestines, stomach, hoof sections, amniotic fluid, etc.).

Recovery of isolates, determination of their pathogenic and toxigenic properties. The study aimed at the recovery of the isolates of the microorganisms that are etiologically most relevant for commercial animal husbandry, namely the *Clostridiaceae* family, was implemented in accordance

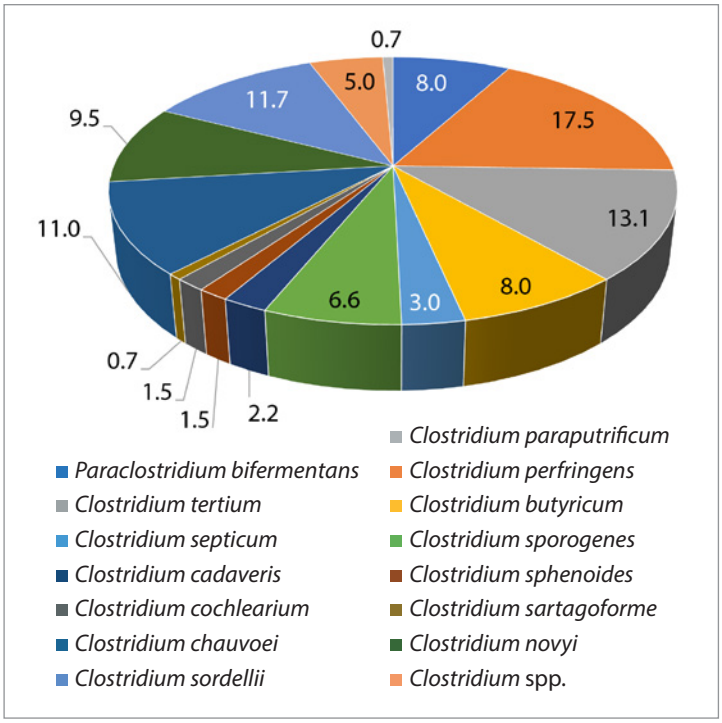

Fig. 1. Species diversity of Clostridium isolates circulating in the Russian Federation (n = 137), %

with GOST 26503-85 "Agricultural animals. Methods for laboratory diagnostics of clostridium"[1].

Identification of Clostridia. Species identification of the microorganisms was performed by mass spectrometry using MALDI Biotyper system (Bruker Daltonik GmbH, Germany) according to the "Guidelines for the identification of microorganisms using MALDI Biotyper mass spectrometer for the examination of food raw materials and food products" (approved by the Rosselkhoznadzor RTC on 3 April 2014).

Antibiotic resistance of the microbial cultures was determined by disc diffusion method in accordance with Methodological Guidelines MUK 4.2.1890-04 "Guidelines for susceptibility testing of microorganisms to antibacterial agents"[2]. Within the research activities, antibacterial drugs of various groups were used (HiMedia Laboratories Pvt Ltd., India): macrolides (azithromycin 15 µg, clarithromycin 15 µg, pristinamycin 15 µg, spiramycin 30 µg, tylosin 15 µg, erythromycin 15 µg), monobactams (aztreonam 30 µg), penicillins (amoxiclav 30 µg, amoxicillin 25 µg, ampicillin 25 µg, benzylpenicillin 10 µg, carbenicillin 100 µg, piperacillin 100 µg), polypeptides (bacitracin 10 µg, polymyxin B 50 µg), chloramphenicol 30 µg, glycopeptides (vancomycin 30 µg), aminoglycosides (gentamicin 30 µg, kanamycin 30 µg, spectinomycin 100 µg, streptomycin 25 µg), carbapenems (doripenem 10 µg), lincosamides (clindamycin 2 µg, lincomycin 10 µg), fluoroquinolones (levofloxacin 5 µg, norfloxacin 10 µg, ofloxacin 5 µg, pefloxacin 5 µg, ciprofloxacin 30 µg, enrofloxacin 10 µg), tetracyclines (oxytetracycline 30 µg, tetracycline 30 µg, chlortetracycline 30 µg, doxycycline 30 µg), ansamycins (rifampicin 15 µg), sulfonamides (sulfadiazine 100 µg, sulfafurazole 300 µg), diaminopyrimidines (trimethoprim 25 µg), cephalosporins (cefixime 5 µg, cefazolin 30 µg, cefaclor 30 µg, cefalexin 30 µg, cefotaxime 30 µg, cefepime 30 µg, cefoperazone 75 µg, cefpirome 30 µg, ceftriaxone 30 µg), phosphonic acid derivatives (fosphomycin 50 µg), fusidines (fusidic acid 30 µg).

The results were interpreted in accordance with CLSI (Clinical and Laboratory Standards Institute) and EUCAST (European Committee on Antimicrobial Susceptibility Testing) recommendations [20, 21].

The results were statistically processed using Microsoft Excel.

RESULTS AND DISCUSSION

As a result of the studies, 137 *Clostridium* isolates were recovered and identified: *Paraclostridium bifermentans*, *Clostridium perfringens*, *Clostridium tertium*, *Clostridium butyricum*, *Clostridium septicum*, *Clostridium sporogenes*, *Clostridium cadaveris*, *Clostridium sphenoides*, *Clostridium cochlearium*, *Clostridium sartagoforme*, *Clostridium chauvoei*, *Clostridium novyi*, *Clostridium sordellii*, *Clostridium paraputrificum*, *Clostridium* spp.

Diversity of *Clostridium* spp. circulating in the Russian Federation has been established, which is shown in Figure 1.

Prevalence of *C. perfringens* was established – 17.5%, followed by *C. tertium* – 13.1%, *C. sordellii* – 11.7%, *C. chauvoei* – 11.0%, *C. novyi* – 9.5%, *P. bifermentans* and *C. butyricum* – 8.0%, *C. sporogenes* – 6.6%, *Clostridium* spp. – 5.0%, *C. septicum* – 3.0%, *C. cadaveris* – 2.2%, *C. sphenoides* and *C. cochlearium* – 1.5% each, the smallest proportion is made up of *C. sartagoforme* and *C. paraputrificum* isolates – 0.7%.

Results of determination of antibiotic resistance of *C. perfringens* isolates ($n = 24$) recovered from cattle in various regions of the Russian Federation are demonstrated in Figure 2.

According to the obtained data, it can be concluded that *C. perfringens* isolates ($n = 24$) demonstrated resistance to cefixime, fusidic acid, cefotaxime, cefaclor, spectinomycin, piperacillin, clarithromycin, doripenem, and doxycycline. Antibiotic resistance to ampicillin demonstrated 85% of the isolates, to amoxicillin, chlortetracycline, vancomycin, rifampicin and ciprofloxacin – 80%, to tylosin and amoxiclav – 75%, to sulfadiazine, cefalexin, ofloxacin and polymyxin B – 60%, to pefloxacin and cefoperazone – 55%, to benzylpenicillin, clindamycin, ceftriaxone and chloramphenicol – 50%, to enrofloxacin, cefazolin, tetracycline and streptomycin – 45% of the isolates; 40% of *C. perfringens* isolates were resistant to bacitracin, norfloxacin, fosfomycin; 35% of the isolates demonstrated resistance to levofloxacin, lincomycin, oxytetracycline; 25% of isolates were resistant to erythromycin, spiramycin and gentamicin and 20% – to azithromycin, cefepime and cefpirome. All studied *C. perfringens* isolates were susceptible to sulfafurazole and carbenicillin (100%), trimethoprim – 90%, azithromycin – 70%, levofloxacin – 65%, and kanamycin – 45% of the isolates. All the tested strains were intermediately susceptible to aztreonam and pristinamycin, 75% of the isolates – to spiramycin, 60% – to fosfomycin, and 55% – to cefazolin, 45% – to kanamycin.

Among 137 recovered *Clostridium* isolates, 25 demonstrated pathogenic properties and 17 had toxigenic properties. The obtained data is presented graphically as a percentage in Figures 3 and 4.

[1] https://base.garant.ru/5916932 (in Russ.)

[2] https://docs.cntd.ru/document/1200038583 (in Russ.)

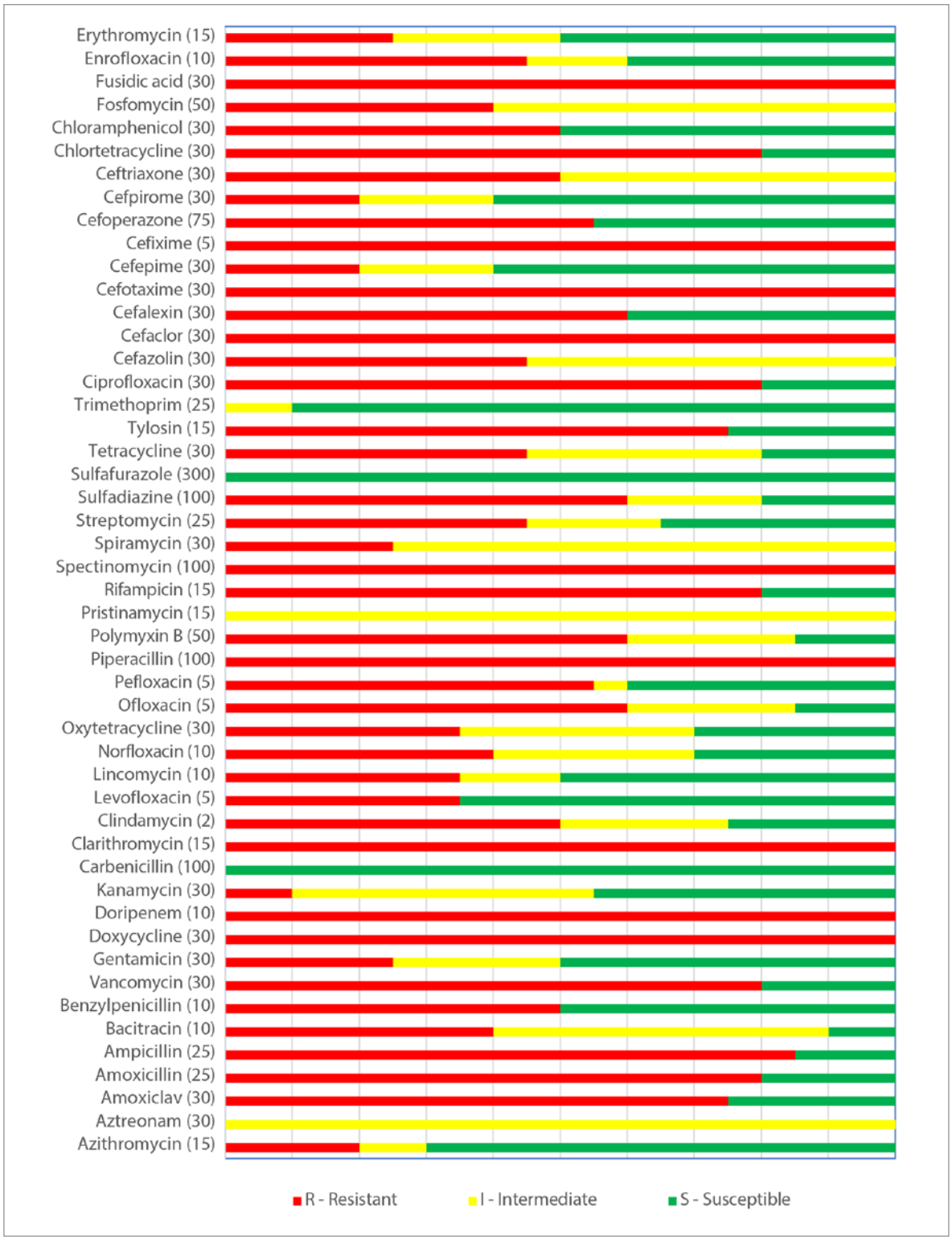

Fig. 2. Antibiotic resistance of C. perfringens isolates (n = 24) recovered from cattle

In most cases, *C. perfringens* isolates possessed pathogenic properties (6.6%). Pathogenicity factors were detected in 5.1% of *C. novyi* strains, 4.4% of *C. chauvoei* isolates, in 1.5% of *C. septicum* strains and in 0.7% of *Clostridium* spp. isolates. Toxigenic properties were determined for *C. sordellii* (3.7%), *C. perfringens* (3.7%), *C. novyi* (3.0%), *C. septicum* (1.5%) and *Clostridium* spp. (0.7%).

The localization sites of *Clostridia* in cattle are presented in the Table.

According to the demonstrated data, *Clostridia* were most often isolated from the liver, small and large intestine and from muscles.

Clostridia are widespread bacteria that cause diseases in animals, birds and humans. Antibiotic resistance is a serious challenge for the veterinary medicine due to the fact that 80% of all antibiotics in the world are used in agriculture, *inter alia* as feed additives and growth promoters. The results obtained during the present study

Table
Localization of *Clostridia* in cattle

Clostridium species	Biological material										
	Heart	Liver	Spleen	Lung	Kidney	Muscle	Small intestine	Large intestine	Stomach	Hoof sections	Amniotic fluid
Paraclostridium bifermentans	–	+	–	–	–	+	+	+	+	+	–
Clostridium tertium	–	+	+	–	–	+	+	+	–	–	–
Clostridium perfringens	–	+	+	–	+	+	+	+	+	–	+
Clostridium butyricum	–	+	–	–	–	–	+	+	+	–	–
Clostridium cochlearium	–	–	–	–	–	–	–	+	–	–	–
Clostridium sartagoforme	–	–	–	–	–	–	–	+	–	–	–
Clostridium septicum	–	+	–	–	+	+	–	+	–	–	–
Clostridium sporogenes	–	+	–	–	–	+	+	+	–	–	–
Clostridium sphenoides	–	–	–	–	–	–	–	+	–	–	–
Clostridium chauvoei	–	+	–	–	–	–	+	+	+	–	–
Clostridium novyi	–	+	–	–	–	–	–	–	–	–	–
Clostridium sordellii	–	+	–	–	–	–	–	–	–	–	–
Clostridium paraputrificum	–	+	–	–	–	–	–	–	–	–	–
Clostridium spp.	–	+	–	–	–	+	+	+	–	–	–
Clostridium cadaveris	–	+	–	–	+	–	–	+	–	–	–

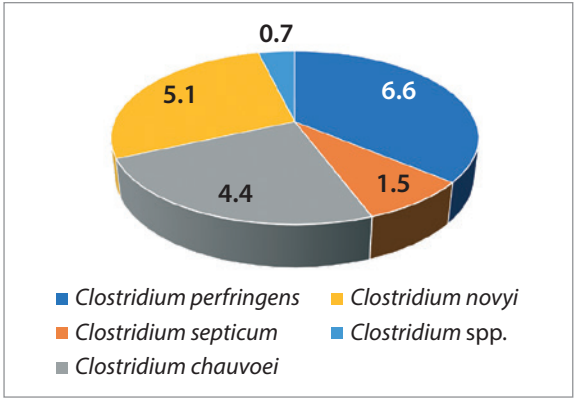

Fig. 3. Species composition of Clostridium isolates with pathogenic properties, %

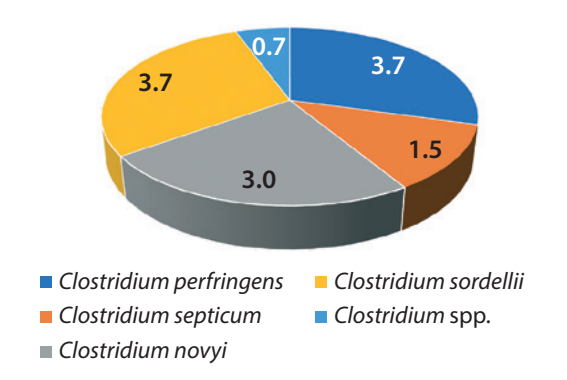

Fig. 4. Species composition of Clostridium isolates with toxic properties, %

on antibiotic resistance to cefotaxime are consistent with the data reported by N. A. Bezborodova et al. [7], H. A. Ahmed et al. [22]. In the studies carried out by the Iranian researchers F. Khademi et al., resistance of *C. perfringens* to ampicillin (25.8%), erythromycin (32.9%), gentamicin (45.4%), tetracycline (19.5%), amoxicillin (19.3%), bacitracin (89.1%) was reported [23]. A group of scientists from China and Pakistan studied eleven of the most commonly used antibiotics, two of them had no inhibitory effect, five were effective, and four had moderate effect against *C. perfringens*. Lincomycin and amikacin did not inhibit the isolates, tetracycline, penicillin, erythromycin and oxytetracycline inhibited *Clostridium* growth to a lesser extent. The scientists have concluded that it was advisable to use several types of antibiotics, which was a more effective approach to inhibit the bacterial infection [24]. Researchers from Ivory Coast determined in their studies that the level of antibiotic resistance of *C. perfringens* to tetracycline, doxycycline, chloramphenicol, and erythromycin ranged from 20 to 50% [25]. A group of scientists from South Korea, when studying the prevalence and resistance of *C. perfringens* to antibiotics, found that resistance to tetracycline was 100%, to ampicillin – 31.6%, to chloramphenicol – 68.4%, to metronidazole – 34.2% and to imipenem – 71%. The researchers also noted an important point of the combined resistance of 78.9% of the isolates to several antimicrobial drugs [26].

CONCLUSION

As a result of the examination of the sections and pathological materials from cattle in 2022–2024, 137 *Clostridium* isolates were recovered, of which 25 demonstrated pathogenic properties, and 17 – toxigenic ones. The most

common *Clostridium* localization sites included liver, large and small intestine, muscles, and stomach. The bacteria were also detected in kidneys, spleen, amniotic fluid, and hoof swabs.

Monitoring studies aimed at the determination of the antimicrobial resistance of *C. perfringens* isolates revealed their resistance to cefixime, fusidic acid, cefotaxime, cefaclor, spectinomycin, piperacillin, clarithromycin, doripenem, and doxycycline.

The results of this study can be used to modify existing treatment protocols for clostridial infections, adjust the composition of immunobiological products, and develop recommendations for the use of antibiotics in animal husbandry to reduce the risk of antimicrobial resistance developing.

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Received 10.01.2025
Revised 20.02.2025
Accepted 24.04.2025

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https://doi.org/10.29326/2304-196X-2025-14-2-201-209

Analysis of RASFF notifications for mycotoxins in 2020–2022

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ABSTRACT

Introduction. Mycotoxins are secondary metabolites of various fungi. The contamination with mycotoxins is subject to control. Pursuant to the accepted classification in accordance with Council Directive 96/23/EC they belong to group B3: "Other substances and environmental contaminants". Information on detected exceedances of maximum permitted levels in feed and food is notified to the RASFF and ACN information systems, which operate across the European Union.

Objective. Analysis of RASFF and ACN notifications for mycotoxins in food and feed in 2020–2022.

Materials and methods. 1,335 publications on exceedances of maximum permitted levels of mycotoxins (aflatoxins, ochratoxin A, deoxynivalenol, zearalenone and patulin) in food and feed have been analysed.

Results. Breakdown of mycotoxin notifications during the analyzed period was as follows: aflatoxins – 87.1%, ochratoxin A – 11.6%, patulin – 0.6%, deoxynivalenol – 0.5%, zearalenone – 0.2%. Aflatoxin contaminations were most often reported in groundnuts (764 notifications), ochratoxin A in dried figs (43 notifications), patulin in apple juice (6 notifications), zearalenone and deoxynivalenol in cereals and bakery products. Feedstuffs and feed ingredients were found to be contaminated only with aflatoxins (33 notifications), and 66.7% of notifications accounted for groundnuts intended for feeding. An analysis of mycotoxin contamination dynamics demonstrated that there was an increase in the number of notifications in 2021 and 2022.

Conclusion. According to RASFF and ACN notifications, mycotoxins were the third most notified hazard category in 2020–2022. Elevated mycotoxin concentrations were detected exclusively in plant products.

Keywords: mycotoxins, aflatoxins, ochratoxin, patulin, deoxynivalenol, zearalenone, RASFF system, European Union, agricultural products, animal products, feed, contamination, exceeding maximum levels

Acknowledgements: The study was funded by the Federal Centre for Animal Health within the research topic "Veterinary Welfare".

For citation: Ibragimova S. S., Pruntova O. V., Shadrova N. B., Zhbanova T. V. Analysis of RASFF notifications for mycotoxins in 2020–2022. *Veterinary Science Today*. 2025; 14 (2): 201–209. https://doi.org/10.29326/2304-196X-2025-14-2-201-209

Conflict of interests: Pruntova O. V. is a member of the editorial board of the "Veterinary Science Today" journal since 2012, but was not involved into the decision making process related to this article publication. The manuscript has passed the review procedure accepted in the journal. The authors did not declare any other conflicts of interests.

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УДК 619:615.9:63-021.66

Анализ выявлений микотоксинов по данным информационной системы RASFF за период с 2020 по 2022 г.

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РЕЗЮМЕ

Введение. Микотоксины – вторичные метаболиты плесневых грибов, являются контаминантами, подлежат контролю. Согласно принятой классификации, по требованиям Директивы Совета Европейского союза 96/23ЕС, относятся к группе В3: «Прочие вещества и загрязнители окружающей среды». Информация о выявлении превышения предельно допустимых концентраций в кормах и пищевых продуктах вносится в информационную систему RASFF и ACN, функционирующую на территории стран Европейского союза.

Цель исследования. Анализ сведений о контаминации микотоксинами пищевой продукции и кормов за период с 2020 по 2022 г., зарегистрированных в информационной системе RASFF и ACN.

Материалы и методы. Объектом анализа были 1335 сообщений о превышении предельно допустимых концентраций микотоксинов (афлатоксинов, охратоксина А, дезоксиниваленола, зеараленона и патулина) в пищевых продуктах и кормах.

Результаты. Распределение случаев выявления микотоксинов в анализируемый период: афлатоксины – 87,1%, охратоксин А – 11,6%, патулин – 0,6%, дезоксиниваленол – 0,5%, зеараленон – 0,2%. Превышение предельно допустимой концентрации афлатоксинов чаще всего обнаруживали в арахисе (764 сообщения), охратоксина А – в сушеном инжире (43 сообщения), патулина – в яблочном соке (6 сообщений), зеараленона и дезоксиниваленола – в продукции из категории «крупы и хлебобулочные изделия». В кормах и кормовом сырье были выявлены несоответствия по содержанию исключительно афлатоксинов (33 сообщения), которые в 66,7% случаев обнаруживали в арахисе, предназначенном для кормовых целей. Анализ динамики контаминации продукции микотоксинами показал, что в 2021 и 2022 гг. наблюдали рост количества регистрируемых сообщений об их детекции.

Заключение. Согласно отчетам RASFF и ACN за 2020–2022 гг., микотоксины представляли третью по распространенности категорию опасности. Нарушение законодательства в части превышения предельно допустимых концентраций микотоксинов выявлено исключительно в продукции растительного происхождения.

Ключевые слова: микотоксины, афлатоксины, охратоксин, патулин, дезоксиниваленол, зеараленон, система RASFF, Европейский союз, сельскохозяйственные продукты, продукты животного происхождения, корма, контаминация, превышение предельно допустимых концентраций

Благодарности: Работа выполнена за счет средств ФГБУ «ВНИИЗЖ» в рамках тематики научно-исследовательских работ «Ветеринарное благополучие».

Для цитирования: Ибрагимова С. С., Прунтова О. В., Шадрова Н. Б., Жбанова Т. Б. Анализ выявлений микотоксинов по данным информационной системы RASFF за период с 2020 по 2022 г. *Ветеринария сегодня*. 2025; 14 (2): 201–209. https://doi.org/10.29326/2304-196X-2025-14-2-201-209

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

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INTRODUCTION

Currently, more than 400 mycotoxin types are known which are produced by fungi belonging to *Aspergillus*, *Penicillium*, *Fusarium*, *Claviceps*, *Neotyphodium*, *Myrothecium*, *Stachybotrys*, *Trichoderma*, *Trichothecium* genera, etc. [1, 2, 3, 4, 5].

According to domestic and foreign publications, feed and plant products are highly contaminated with micromycetes (up to 80–100%), including toxin-producing micromycetes (up to 40–60%), and in 21% of cases mycotoxins are produced in concentrations dangerous to animal and human health. The problem of feed and food contamination by micromycetes of mold fungi and their metabolites is prevalent and has no geographical boundaries. The contamination level depends on environmental conditions (temperature and humidity), compliance with the rules of agricultural technology, plant resistance to phytopathogens, etc. [6, 7, 8, 9, 10, 11].

Feeding animals with mycotoxin-contaminated feeds causes numerous non-infectious diseases, known as food-borne mycotoxicosis. Clinical signs and symptoms depend on a variety of factors: the mycotoxin type, the amount and duration of exposure, general health condition and immune status of the animal. Mycotoxicoses are divided by the type of a toxin or fungus they are caused by: fusariotoxicosis, aflatoxicosis, ochratoxicosis, patulinotoxicosis, stachybotriotoxicosis, etc. Depending on livestock and poultry species, age, and physical condition, different sensitivity to the action of various mycotoxins is reported. For example, piglets under 3 months of age, pregnant sows, calves, fattening pigs, adult cattle and sheep are the most susceptible to aflatoxins. Turkeys, ducklings, and goslings are highly sensitive among poultry. Pigs and poultry are susceptible to ochratoxins. Horses and cattle are sensitive to stachyobothriotoxin, horses, pigs; poultry are susceptible to fusariotoxins and fumonisins, pigs and cattle to patulin [1, 12, 13, 14, 15, 16].

In the Russian Federation, the ten-year monitoring data obtained from annual mycotoxicological tests of complete feeds for pigs and poultry, provided by farms and processing plants located in the Northwestern, Central, Southern, Volga and Ural Federal districts, reveal the following toxins: T-2 mycotoxin, diacetoxyscirpenol, deoxynivalenol, zearalenone, fumonisins B, alternariol, ochratoxin A, citrinin, aflatoxin B_1, sterigmatocystin, cyclopiazonic acid, mycophenolic acid, ergot alkaloids and emodin. The results obtained confirmed the relevance of mycotoxin contamination systematic control [17].

According to BIOMIN GmbH (Austria) scientific data obtained by testing of 6,844 samples of agricultural products, the most frequent mycotoxins in the world are deoxynivalenol (66%), fumonisins (56%) and zearalenone (53%) [18].

According to literature sources, among several hundred known mycotoxins, aflatoxins, T-2 mycotoxin, ochratoxin A, patulin, fumonisins, zearalenone and deoxynivalenol are the most common and dangerous to livestock health and performance [19, 20, 21, 22].

Aflatoxins, when ingested, inhaled or adsorbed through the skin, have hepatotoxic, teratogenic and cytotoxic effects. The toxic effect is enhanced by the presence of T-2 toxin or ochratoxin in feed and relatively low levels of crude protein, methionine, and vitamin D_3. Out of the ochratoxins, ochratoxin A is the most dangerous one, which inhibits protein synthesis and disrupts carbohydrate metabolism by inhibiting the activity of a specific enzyme that initiates protein synthesis [23, 24, 25, 26, 27, 28].

Fusariotoxins commonly encountered in the world are deoxynivalenol (DON, vomitoxin) and zearalenone. DON is most often detected in wheat, less often in corn, barley, rye, oats and grain products. Zearalenone differs from other mycotoxins as it has hormone-like effect and is less toxic, not leading to death. It is an uterotrophic and estrogenic substance that induces hyperestrogenism in pigs, infertility and stunted growth in cattle and poultry [29, 30, 31, 32, 33].

Patulin is usually found in rotten fruits, berries and vegetables; it has mutagenic, neurotoxic, nephrotoxic and immunotoxic effects, and can cause gastrointestinal injuries [1, 34].

Geographically, aflatoxins are most widespread in regions with a tropical climate (Africa and Southeast Asia); ochratoxins are found in regions with a cool, humid climate (Northern Europe); fusariotoxins and zearalenone are widespread everywhere, including in the Russian Federation [1, 13].

According to the Food and Agriculture Organization of the United Nations (FAO), up to 30% of food and fodder crops are contaminated with mycotoxins. Reports from domestic and foreign information sources confirm that mycotoxicosis significantly impairs the livestock performance and reproduction, bringing significant economic losses for livestock farming. In addition, toxic substances produced by mold fungi pose a serious danger to the health of consumers of agricultural products. In this regard, the issues related to the detection of mycotoxins in agricultural and food products are relevant [1, 6, 33, 34, 35, 36].

In the Russian Federation, the maximum levels of mycotoxins in agricultural and food products are regulated by the Technical Regulations of the Customs Union, namely: TR CU 015/2011 "On grain safety", TR CU 021/2011 "On food safety", TR CU 033/2013 "On the safety of milk and dairy products". In addition, mycotoxins have been included in the list of parameters to be tested for monitoring purposes since 2007. The monitoring is organized and conducted annually by the Federal Service for Veterinary and Phytosanitary Surveillance (Rosselkhoznadzor) [37].

The novelty of this work consists in the analysis and interpretation of information on mycotoxin contamination of food and feed in European countries.

The aim of the study is to analyze information on mycotoxin contamination of food and feed in the European Union (EU) countries based on reports from the RASFF (Rapid Alert System for Food and Feed) for 2020–2022.

MATERIALS AND METHODS

The RASFF notifications on the detection of mycotoxins (aflatoxins, ochratoxin A, deoxynivalenol, zearalenone and patulin) in food and feed for 2020–2022 became the object of analysis.

RESULTS AND DISCUSSION

Rapid Alert System for Food and Feed (RASFF). The common legal framework regarding safety of agricultural products, food raw materials and feed based on Regulations No. 178/2002 and No. 882/2004 is valid in the EU [38, 39].

Regulation No. 178/2002 lays down general principles and requirements for the quality and safety of agricultural products and food raw materials, covering all stages of production and processing. In addition, this act establishes and defines the powers of the European Food Safety Agency (EFSA) and provides the legal basis for the RASFF in the EU. Regulation No. 882/2004 establishes the general principles of official control performed to ensure compliance with feed and food law [40, 41, 42, 43].

The RASFF is a key tool for the rapid exchange of information on detections of contaminants in food or feed posing risks for human and animal health. This system was created in 1979 under Food Safety Directive. The feeds were not officially covered by the system. Since January 28, 2002 Article 50 of Regulation No. 178/2002 of the European Parliament and the Council has been the legal basis for the RASFF. The Article establishes the general principles and requirements of the EU food legislation, covering all stages of food production and processing within the food chain "from stable to table", including feed and feed raw materials [39, 40, 44].

In 2020, hazards were established to be included into notifications categorized by feed products, origin countries, and notifying countries. From March 2021 RASFF, together with the Administrative Assistance and Cooperation Network (AAC) and the Agri-Food Fraud Network (FFN) have been merged into the Alert and Cooperation Network (ACN). The Network was established by Commission Implementing Regulation (EU) 2019/1715 which sets up and manages a computerized information management system for official controls (IMSOC). The ACN notification system includes three networks (RASFF, AAC and FFN), ensuring an unhindered exchange of information between the competent authorities of the Member States and facilitating cooperation between them [45].

The RASFF notifications concern product controls at the EU's external borders, at entry points or border inspection posts, and inspections by competent authorities or food poisoning incidents. Contact points have been set up in all RASFF Member States and the European Commission, between which information is exchanged [38, 45, 46, 47].

The RASFF member notifies of the existence of a serious, direct or indirect, risk to public health linked to food or feed. After receiving the notification, other members can trace whether these products are available on their market. Next, these members report back on what they have found and what measures they have taken. The notifications also concern controls at European Economic Area borders, at points of entry or border inspection posts when a consignment was not accepted for import [45, 46, 47].

When a problem is detected in the internal market, it is the task of the national food and feed authorities to take action. This includes any action necessary to immediately address the risk but also to prevent a similar risk reoccurring. A whole range of actions are carried out and reported back through RASFF: withdrawal or recall of the products and their possible destruction, information to the public, re-dispatch to origin etc. [45, 46].

Mycotoxin hazards (aflatoxins, ochratoxin A, patulin, DON, zearalenone) notified by the EU countries in 2020–2022. According to the RASFF and ACN annual reports, mycotoxins rank third behind pesticide residues and *Salmonella* among hazards [45, 46, 47].

An analysis of mycotoxin contamination dynamics, focusing on concentrations exceeding maximum levels (MLs) in RASFF notifications, revealed a clear upward trend in 2021–2022. In 2021, reported mycotoxin cases increased by 6% compared to 2020, followed by a further 10.5% rise in 2022. The 2020 RASFF Annual Report documented a 23% decline in hazard detections compared to 2019, which authorities attributed primarily to COVID-19 pandemic disruptions [45, 46, 47].

According to RASFF reports for 2020 and ACN reports for 2021 and 2022, 1,335 notifications on exceeding MLs of mycotoxins in food and feed were reported. In 2020, 400 notifications were registered, 450 notifications were

Table
Mycotoxin detection dynamics in 2020–2022 according to RASFF and ACN

Mycotoxin	2020		2021		2022		Total number of notifications
	No.	%*	No.	%*	No.	%*	
Aflatoxins	343	87.0	387	88.8	413	85.7	1,143
Ochratoxin A	41	10.4	46	10.5	65	13.5	152
DON	6	1.5	1	0.2	0	0	7
Zearalenone	1	0.3	0	0	1	0.2	2
Patulin	3	0.8	2	0.5	3	0.6	8
Total	394	100	436	100	482	100	1,312

* % of the total number of detections for the year.

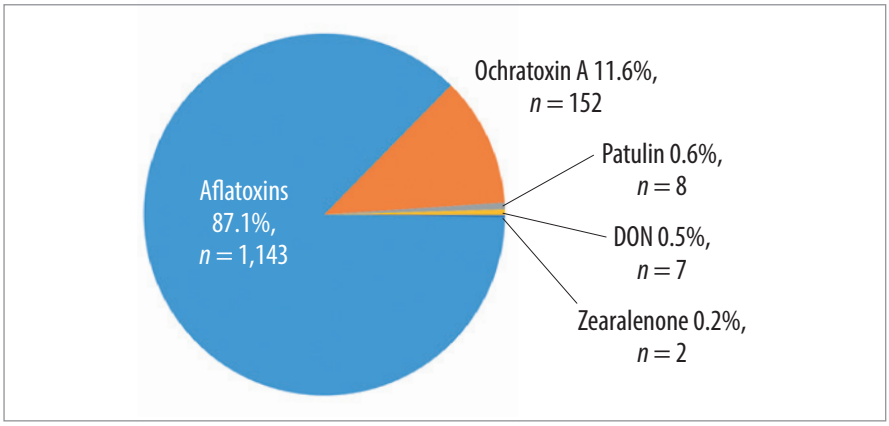

Fig. 1. Types of mycotoxins and rates of their detection in plant product samples in 2020–2022

made in 2021, and 485 in 2022. Of 1,335 reports, 1,312 contain information on mycotoxin findings covered by this paper: aflatoxins, ochratoxin A, patulin, DON and zearalenone. Mycotoxin detection dynamics in 2020–2022 according to RASFF and ACN is given in the table below.

Of the 1,312 notifications on mycotoxin findings, 1,007 were reported during border control, 162 during official market control, 142 as a result of internal inspections, and one notification was generated in the RASFF system following a consumer poisoning incident due to ochratoxin A exceeding levels [48].

It was found that during this period, aflatoxins (87.1%), ochratoxin A (11.6%), patulin (0.6%), DON (0.5%), zearalenone (0.2%) were most frequently notified mycotoxins in the RASFF system. Data are shown in Figure 1. It should be noted that only plant-based commodities contained mycotoxin concentrations above maximum limits.

Aflatoxin findings (according to RASFF data for 2020–2022). Of 1,143 RASFF notifications, 97.1% were for aflatoxins detected in food products and 2.9% in feed and feed materials.

The exceeding MLs of aflatoxins in food are evidenced by 1,110 notifications in the following product categories: "Nuts, nut products and seeds", "Fruits and vegetables", "Cereals and bakery products", "Herbs and spices", "Other food product / mixed", "Confectionery", "Cocoa and cocoa preparations, coffee and tea" and "Ice cream and desserts".

Notifications in the category "Nuts, nut products and seeds" category were made in 67.9% of cases (776 notifications); 52.0% (403) of them were made for peanuts; 27.6% (214) for pistachios; 9.1% (71) for hazelnuts; 3.6% (28) for almonds; 2.1% (16) for peanut butter; 1.2% (9) for watermelon seeds; 1.0% (8) for melon seeds; 0.5% (4 for each) notifications were reported for Brazil nuts and sesame seeds; 0.4% (3) for almond flour; 0.3% (2 for each) notifications for ogbono seeds, apricot kernels, hazelnut paste, cashews; 0.1% (1 for each) for sunflower, lotus seeds, nut mix, chia seeds, pistachio flour, almond nougat, peanut paste and nut crackers.

163 notifications (14.3%) were reported in "Fruits and vegetables" category. Most findings were reported for dried figs – 94.5% (154), as well as for dried dates – 3.1% (5), mulberry –1.8% (3), date syrup – 0.6% (1).

7.4% of notifications (85) were made in "Cereals and bakery products" category, of which 75.0% (64) were findings in rice; 4.7% (4) in corn, 3.5% (3) in wheat flour, 2.4% (2 for each) in buckwheat, millet seeds, dry soy product, a mixture of millet, corn and baobab juice, 1.2% (1 for each) in wheat, rice flour, corn flour, spelt flour, almond flour and buckwheat husk flour.

74 (6.5%) notifications were made to the RASFF about aflatoxins in "Herbs and spices" category products within the mentioned period. Among them 21 notifications (28.4%) were on hazards in spice mixture; 16 (21.6%) in nutmeg, 13 (17.6%) in whole dried chili peppers; 10 (13.5%) in crushed chili peppers; 5 (6.8%) in turmeric, 4 (5.4%) in ground ginger, 3 (4.0%) in curry powder; 2 (2.7%) in black pepper.

In "Other food product / mixed" category, which included hazelnut paste, date syrup, shelled peanuts, rice flour, paste for filling and ice cream sprinkles, only 5 notifications (0.4%) were made; one for each type of product. Only 5 notifications (0.4%) were reported in the "Confectionery" category, of which 3 (60.0%) were for peanut candies; 1 (20.0% each) for peanut halva and pistachio halva. In the "Cocoa, cocoa preparations, coffee and tea" (in cocoa powder) and "Ice cream and desserts" (in peanut paste for ice cream) categories, 1 notification was registered (0.1% for each).

33 notifications were made on exceeded MLs of aflatoxins in feed and feed materials in the following categories: "Source material / feed" – 25 notifications (75.8%); "Nuts, nut products and seeds" (peanuts) – 6 (18.2%) notifications; one notification was made in "Feed materials" (non-compliances found in corn gluten) and "Pet food" categories (3.0% each). In the "Source material / feed" category: 16 notifications (64.0%) were made for peanuts, 2 (8.0% each) for millet and sunflower seeds, 1 notification for each of (4.0% each) in rice flour, rice bran and protein, corn gluten and cottonseed flour.

Ochratoxin A findings (according to RASFF data for 2020–2022). During the study period, 152 notifications of ochratoxin A non-compliant concentration were made.

In products from "Fruits and vegetables" category, 73 non-compliances (48.0%) were detected, of which 43 (58.9%) were in dried figs; 20 (27.4%) in raisins, 3 (4.1%) in mulberries and 3 in dates, 1 (1.4%) in date syrup, fig bread with almonds, canned plums and apricot kernels.

In the "Cereals and bakery products" category, 36 notifications were made in 2020–2022, which accounted for 23.7% of the total number of notifications on ochratoxin A. Among them, 44.4% (16 notifications) were on exceeding MLs in rice, 11.1% (4 notifications) in wheat flour, notifications were made for wheat, oats, rye bread, rye flakes, rye flour, bread rolls, muesli, dry soy product, quinoa groats, corn flour, whole-grain rye pasta, oat flakes, baby food, fruit and oat bars, red quinoa and rolls (one for each).

27 notifications (17.7%) were made in the "Herbs and spices" category, 11 (40.7%) on exceeding MLs in nutmeg, 10 (37.1%) in ground pepper, 3 (11.1%) in crushed licorice root, 2 (7.4%) in chili seasoning, 1 (3.7%) in dietary supplements.

5 notifications (3.3%) were made in the "Nuts, nut products and seeds" category: 4 (80%) in pistachios and 1 (20%) in watermelon seeds.

There were 4 notifications (2.6%) on ochratoxin A in date syrup ("Other food product / mixed" category), and 4 notifications (2.6%) on exceeding MLs in instant coffee (3) and in a mixture of roasted and ground coffee (1) in the "Cocoa, cocoa preparations, coffee and tea" category.

During the studied period, the RASFF received notifications about exceeding MLs of ochratoxin A in astragalus extract powder ("Dietetic foods, food supplements and fortified foods"), Rossa wine ("Wine" category) and fruit bars ("Prepared dishes and snacks"), one for each (0.7%).

Deoxynivalenol and zearalenone findings (according to RASFF data for 2020–2022). Violations of European MLs for DON were reported only in "Cereals and bakery products" category: 7 notifications were sent during the period under study. This mycotoxin was found in wheat and corn grains (2 notifications, 28.6% each), wheat flour, instant noodles and breadcrumbs (1 notification, 14.3% each). Most DON detections were made in 2020 (6 RASFF notifications were registered), and 1 notification was received in 2021. In 2020, one wheat sample, in addition to DON levels above MLs, zearalenone concentration was also too high. The second case of zearalenone detection was reported in 2022; this mycotoxin was detected in rice crackers.

Patulin findings (according to RASFF data for 2020–2022). During the study period, 8 notifications were received about non-compliant MLs of patulin in two RASFF categories: "Fruits and vegetables" (37.5%) and "Non-alcoholic beverages" (62.5%). In 75.0% of cases (6 notifications), this mycotoxin was detected in apple juice, 12.5% (1 notification for each) for apple sauce and natural apple-cherry juice.

Analysis of the distribution of identified mycotoxins by categories of plant products and feeds according to the RASFF classification. It should be noted that exceeding MLs of several types of mycotoxins have been reported in various product categories. Thus, in products of "Cereals and bakery products" category, exceeding MLs of four mycotoxins were detected; in "Fruits and vegetables" category, three mycotoxins were identified; in products from the "Nuts, nut products and seeds", "Herbs and spices", "Other food product / mixed" and "Cocoa, cocoa preparations, coffee and tea" categories two mycotoxins were detected. The mycotoxin distribution (aflatoxins, ochratoxin A, DON, zearalenone, patulin) by product category is demonstrated in Figure 2.

The MLs of aflatoxins (85 notifications; 65.4%), ochratoxin A (36 notifications; 27.7%), DON (7 notifications; 5.4%) and zearalenone (2 notifications; 1.5%) were reported in products from the "Cereals and bakery products" category. Moreover, in 2022, there was more than double increase in the number of reports of identified non-compliant aflatoxin levels in this category.

In the "Fruits and vegetables" category, there were notifications about levels of 3 types of mycotoxins above MLs: aflatoxins – 181 notifications (70.4%), ochratoxin A – 173 notifications (28.4%) and patulin – 3 notifications (1.2%).

Non-compliant levels of aflatoxins (758 (99.3%) and 74 (73.3%) notifications, respectively) and ochratoxin A (5 (0.7%) and 27 (26.7%) notifications, respectively) were reported in "Nuts, nut products and seeds" and "Herbs and spices" categories.

During the period under review, there were 5 notifications on aflatoxins and 4 notifications on ochratoxin A in products from "Other food product / mixed" category. In 2021, 4 notifications were reported to the RASFF about exceeding MLs of ochratoxin A and aflatoxins in "Cocoa, cocoa preparations, coffee and tea" products.

In categories such as "Confectionery", "Prepared dishes and snacks", "Dietetic foods, dietary supplements", "Ice cream and desserts", "Wine" and "Soft drinks", only one of the mycotoxins was above the established MLs.

In products intended for feed purposes of "Pet food", "Feed materials" and "Nuts, nut products and seeds", categories exceeding MLs of aflatoxins were found.

During the analytical study, co-contamination with several mycotoxins was noted in 16 cases, co-contamination with aflatoxins and ochratoxin A was reported in 14 notifications, co-contamination with zearalenone and DON, as well as with zearalenone and aflatoxins were also notified (1 notification for each case).

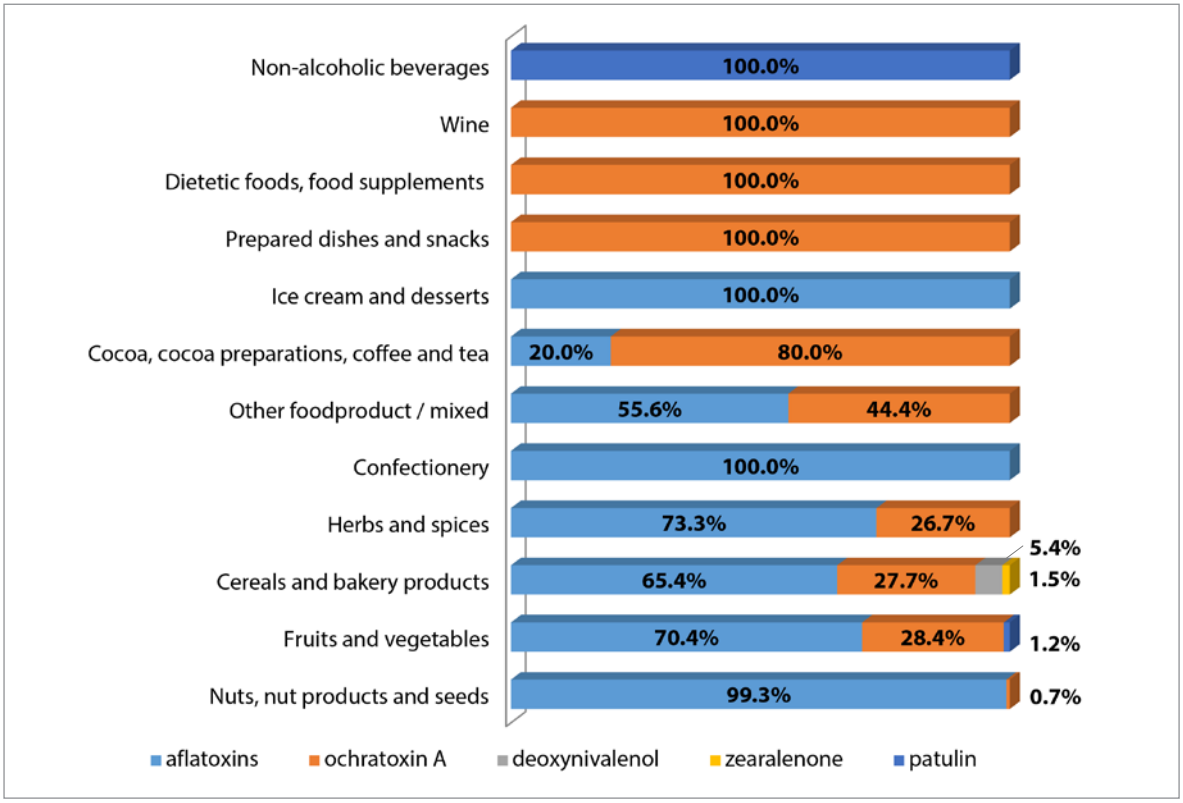

Fig. 2. Percentage ratio of detected mycotoxins in different plant product categories (according to RASFF for 2020–2022)

Based on the information provided in the RASFF notifications for 2020, 2021 and 2022, an increasing trend in the number of contaminations of feed and plant products with aflatoxins and ochratoxin A was noted. There were only a few notifications regarding the exceeding MLs of mycotoxins such as DON, zearalenone and patulin (Table), and they are insufficient to establish reliable trends in contamination of plant products and feed. At the same time, the findings confirm that this issue is prevalent, warranting systematic monitoring and control measures, as well as a thorough risk assessment of mycotoxin contamination in feed and plant-derived commodities.

According to literature data, DON, T-2 toxin, zearalenone and aflatoxins are most often detected in the Russian Federation. An analysis of contamination of food grain harvested in 2020 showed that 10% of the samples are co-contaminated with two or more mycotoxins. The most common contaminants of grain were tentoxin, DON, and cyclopiazonic acid, while those of corn were fumonisins B_1 and B_2. Ochratoxin A, aflatoxins, zearalenone, T-2 and HT-2 toxins, citrinin, sterigmatocystin, ochratoxin B, alternariol and its methyl ester, altenuene, and mycophenolic acid were also detected [49].

Mycotoxins exhibit organism-specific pathological effects, combining high toxicity, bioaccumulation potential, and diverse impacts – including embryotoxicity, teratogenicity, mutagenicity, carcinogenicity, immunosuppression, and cytotoxic, hepatotoxic, neurotoxic, dermatotoxic, and nephrotoxic effects. Mycotoxins disrupt protein synthesis, induce lymphatic tissue hypoplasia and bone marrow alterations, impair protein/lipid/mineral metabolism, exacerbate allergic responses, and cause hepatic, renal, and reproductive system damage [1, 12, 50].

For EU countries, the MLs of mycotoxins in food and agricultural products are established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The Committee consists of independent international experts who, based on the results of scientific research, issue recommendations on MLs, on measures to prevent and reduce contamination, laboratory methods for concentration measurement, etc. JECFA publications are regarded as international reference documents underlying international and regional standard development.

CONCLUSION

According to RASFF annual reports for 2020–2022, there were 1,312 notifications on exceeding MLs of mycotoxins. It was found that aflatoxins were the most frequently reported contaminants during the period under review (87.1%), with ochratoxin A ranking second (11.6%). Levels of patulin (0.6%), DON (0.5%), zearalenone (0.2%) above the established MLs were also notified. Herewith, exceeding MLs of studied mycotoxins were reported only for plant-derived products.

In products of "Cereals and bakery products" category, exceeding MLs of all mycotoxins were detected: aflatoxins (65.4%), ochratoxin A (27.7%), DON (5.4%) and zearalenone (1.5%), in "Fruits and vegetables" category three mycotoxins were prevalent: aflatoxins (70.4%), ochratoxin A (28.4%), and patulin (1.2%).

Of the 1,312 RASFF notifications in 2020–2022, 97.5% mycotoxins were detected in plant-derived foods and 2.5% in feed and feed materials. Moreover, only aflatoxins were found in the feed.

Co-contamination with several mycotoxins was noted in 16 cases, co-contamination with aflatoxins and

ochratoxin A was reported in 14 notifications, co-contamination with zearalenone and DON, as well as with zearalenone and aflatoxins were also notified (1 notification for each case).

During the period under review, 76.8% of notifications on mycotoxins concerned border control; 12.3% were made following official market control; 10.8% resulted from internal inspections; 0.1% reported consumer complaints.

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Received 01.11.2024
Revised 16.12.2024
Accepted 26.02.2025

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Contribution of the authors: Ibragimova S. S. – research conceptualization, data search, analysis and interpretation, compilation of tables and graphical material for data visualization, article drafting and writing; Pruntova O. V. – supervision, scientific consultations, research conceptualization, article drafting and writing; Shadrova N. B. – scientific consultations on RASSF searches and analysis, article drafting and writing; Zhbanova T. V. – article drafting and writing.

Вклад авторов: Ибрагимова С. С. – формирование концепции исследования, проведение поисково-аналитической работы, анализ и интерпретация полученных данных, составление таблицы и графического материала для визуализации данных, подготовка и написание статьи; Прунтова О. В. – курирование, научное консультирование, формирование концепции исследования, подготовка и написание статьи; Шадрова Н. Б. – научное консультирование по проведению поисково-аналитической работы в системе RASFF, подготовка и написание статьи; Жбанова Т. Б. – подготовка и написание статьи.

On the 70th anniversary of Idris G. Idiatulin

Idris G. Idiatulin was born on June 6, 1955. In 1982, he graduated from the Leningrad Veterinary Institute. He pursued postgraduate studies and earned a PhD in 1982–1983.

Here is a clear chronological breakdown of his employment history:

- Deputy Director, Agricultural Dairy Farm (1993–1998);
- Head, Baltiysky Border Crossing Point at the Northwestern Territorial Administration of Gosvetnadzor on the State Border of the Russian Federation and Transport (1998–2005);
- Deputy Head, Organizational and Inspection Department;
- Head, Organizational and Inspection Department;
- Deputy Head, Rosselkhoznadzor Territorial Administration for St. Petersburg & Leningrad Oblast (2005–2011);
- Head, Veterinary Administration, Committee for Agriculture and Fisheries of the Leningrad Oblast (2011–2013);
- Head, Veterinary Administration of the Leningrad Oblast (2013–2019).

With over 26 years of experience in veterinary medicine and animal production, he played a significant role in safeguarding the country's borders against the introduction of infectious animal diseases and ensuring veterinary oversight for the export/import of animals, animal products and raw materials. He contributed to the reorganization of the state veterinary service, particularly in the areas of state veterinary surveillance at the border and transport within the Leningrad Oblast.

As Head of the Veterinary Administration of the Leningrad Oblast, he exercised extensive organizational and leadership responsibilities. He deserves credit for swiftly addressing challenges during the implementation of a new system to record the fulfillment of state assignments in veterinary service institutions: drafting state assignments, calculating necessary funding from the regional budget, ensuring timely budget allocation, and supplying municipal and city veterinary institutions with essential material, technical, and financial resources. He elevated the paid veterinary services offered by state veterinary institutions in the Leningrad Oblast to a modern level by spearheading the development of scientifically grounded pricing, which ensured the cost-effective operation of these facilities. Among his contributions, Idris G. Idiatulin organized the calculation of time norms for veterinary work and the standardization of veterinarians' labor in medical, preventive, diagnostic, and veterinary-sanitary institutions. This work led to the establishment of scientifically grounded standards in state veterinary institutions. Under his leadership, successful efforts were made to prevent and control infectious animal diseases, including African swine fever and highly pathogenic avian influenza.

With decades of expertise, Idris G. Idiatulin serves as the chairman of the Public Council under the Veterinary Administration of the Leningrad Oblast. As a seasoned specialist, he plays a key role in guiding initiatives aimed at supporting and improving the management of stray animals.

Idris G. Idiatulin has been honored with awards at the federal, regional, and district levels, earning well-deserved respect among colleagues and agricultural professionals.

On this special anniversary, please accept our heartfelt wishes for continued success in all your endeavors, along with good health, happiness, and prosperity!

Dedicated to the 100th anniversary of the birth: Dr. Vladislav P. Onufriev (1925–1998)

On April 16, 2025, we commemorate the 100th anniversary of the birth of Vladislav Petrovich Onufriev, an eminent virologist, distinguished coordinator in the field of veterinary medicine, Doctor of Science (Biology), Professor, Corresponding Member of the Lenin All-Union Academy of Agricultural Sciences (VASKhNIL), the Russian Academy of Agricultural Sciences (RAAS), Honored Scientist of Ukraine, veteran of the Great Patriotic War, and Director of the All-Union Foot-and-Mouth Disease Research Institute (AUFMDRI) for 18 years.

Vladislav P. Onufriev was born in the village of Alekseevka, the Kalanchak Raion, the Kherson Oblast. After completing junior high school in 1938–1941, he studied at the Tsyurupynsk Veterinary Technical School (the Kherson Oblast).

As the war broke out, he was mobilized to participate in the defence of the Crimea. From November 1943 to August 1946, he served in the Soviet Army, participated in the Great Patriotic War, fought for a year and a half as a scout and commander of a motorized reconnaissance company in the 33rd Guards Rifle Division, and was wounded. In late 1943, he was awarded the Medal "For Courage" for his bravery, followed by the Order of Glory 3rd Class in 1944 and the Order of Glory 2nd Class in 1945. The battles near Königsberg were particularly fierce, and as a participant of the events he received the Medal "For the Capture of Königsberg". He was also awarded the Order of the Patriotic War 2nd Class (1985) and numerous other medals.

After demobilization, from 1946 to 1951, he was a student at the Kharkiv Veterinary Institute, which he graduated with honors. From 1951 to 1955, he worked as the chief veterinarian of the Mglin District Agricultural Department (the Bryansk Oblast) and later on as a senior veterinarian at the Prosyana MTS (the Dnipropetrovsk Oblast).

From 1955 to 1958, he pursued postgraduate studies at the Leningrad Research Veterinary Institute. In 1960, he defended his dissertation on "The Toxic Effects of Certain Preparations on the Tick Ixodes Ricinus" for the Candidate of Science Degree. From 1959 onward, he engaged in research as a senior scientist at the Foot-and-Mouth Disease Laboratory of the Ukrainian Research Institute of Experimental Veterinary Medicine (Kharkiv), was head of the Epizootology Department at the Institute of Animal Husbandry and Veterinary Medicine of the Tajik SSR Academy of Sciences (1960), and director of the Tajik Research Veterinary Institute (Dushanbe, 1961–1963).

In March 1963, Vladislav Onufriev was appointed as a director of the newly founded AUFMDRI subordinated to the USSR Ministry of Agriculture. In this role, he proved

himself an excellent organizer and authoritative leader. He oversaw the construction of the modern, well-equipped institute, staffing it with scientific and technical personnel, and launching research on foot-and-mouth disease. Combining administrative duties with scientific work, he also headed the institute's Immunology Laboratory. In 1969, he successfully defended his dissertation on "Experimental Studies on the Immunology of Foot-and-Mouth Disease" for Doctor's Degree (Biology) and in March 1972 was awarded the title of Professor in Virology. The research work performed by Dr. Onufriev and his students was focused on foot-and-mouth disease, its prevention and eradication in the USSR. Considerable attention was paid to passive immunity in animals caused by anti-foot-and-mouth sera, immunolactone, and lactoglobulins, as well as to studying active immunity induced by inactivated mono- and polyvalent vaccines. His diagnostic and preventive methods significantly improved the animal health situation and helped eradicate the disease.

With the direct involvement of Vladislav Onufriev, justification was prepared for construction of the Transcaucasian (Yerevan) and Central Asian (Dushanbe) branches of the AUFMDRI. Since 1970 these branches had served as bases for scientific and practical work in the areas where

foot-and-mouth disease and other highly dangerous animal diseases could be introduced into our country.

For successful completion of the planned research and successful implementation of the obtained results into production, the institute headed by Vladislav P. Onufriev was repeatedly awarded with the Transferable Red Banner Prize from the Central Committee of the Communist Party of the Soviet Union (CPSU), the USSR Council of Ministers, the All-Union Central Council of Trade Unions (ACCTU), and the Central Committee of the Komsomol, diplomas from the Exhibition of Achievements of National Economy (VDNKh) and from the Ministry of Agriculture of the USSR. For developing a set of measures that ensured elimination of foot-and-mouth disease outbreaks nationwide and established sustainable control over this infection, a group of veterinary specialists from the institute was awarded the State Prize of the Russian Federation in Science and Technology.

From 1968 to 1981, Vladislav P. Onufriev was a Chairman of the Coordination Council on Foot-and-Mouth Disease for CMEA Member Countries (Council for Mutual Economic Assistance). In this role, he did extensive work to improve scientific research in specialized laboratories across the USSR and in the CMEA Member Countries and to speed up implementation of the latest breakthroughs into veterinary practice. He undertook numerous international assignments and participated in congresses, symposia, and sessions of the OIE (now the WOAH) and CMEA. His scholarly output includes over 300 scientific publications and 18 invention patents. Under his mentorship, 14 researchers earned Doctor of Science Degree and more than 40 obtained Candidate of Science degree. Professor Onufriev chaired the Academic and Dissertation Councils at AUFMDRI and served on the expert council of the USSR Higher Attestation Commission (VAK).

In 1975, Vladislav P. Onufriev was elected as a Corresponding Member of the VASKhNIL. By 1991, he became a Corresponding Member of the RAAS and the Ukrainian Academy of Agrarian Sciences, Honored Worker of Science and Technology of Ukraine. Concurrently, he engaged in extensive public work, i.e. since 1963 he had been annually elected to the institute's Communist Party Bureau and since 1964 he had been a Candidate Member to the Vladimir Regional Committee of the CPSU. From 1966 to 1980 he became its Full Member. His agricultural research breakthroughs earned him Order of Lenin, Two Orders of the Red Banner of Labor, Diplomas and Certificates of Honor from the USSR VDNKh.

In April 1981, he became head of the Epizootology Department and later the Microbiology and Virology Department at the All-Ukrainian Academy of Agricultural Sciences (Kyiv), where he worked until his passing on December 16, 1998.

Vladislav P. Onufriev was renowned for his scholarly erudition, dedication, and kindness. He is remembered as a man of great warmth and vision who advanced the frontiers of veterinary science and earned deep respect both in the USSR and abroad.

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