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

MARCH | МАРТ VOL. 12 No. 1 2023

BIOTECHNOLOGY

Modern approaches to production of safe and effective genetically modified rabies vaccines for animals p. 6

Biological, cytomorphological and karyological heterogeneity of transformed cell lines derived from domestic pig (*Sus scrofa* L.) organs p. 13

Study of cultural properties of canine enteric coronavirus isolate in different cell cultures p. 23

Spatiotemporal analysis of African swine fever spread in wild boar population in Russian Federation, 2007–2022 p. 57

FEDERAL SERVICE FOR VETERINARY
AND PHYTOSANITARY SURVEILLANCE
(ROSSELKHOZNADZOR)
FGBI "FEDERAL CENTRE FOR ANIMAL HEALTH"
(FGBI "ARRIAH")

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

VETERINARY SCIENCE TODAY

QUARTERLY SCIENTIFIC JOURNAL

MARCH VOLUME 12 No. 1 2023
Published since 2012

ВЕТЕРИНАРИЯ СЕГОДНЯ

ЕЖЕКВАРТАЛЬНЫЙ НАУЧНЫЙ ЖУРНАЛ

МАРТ ТОМ 12 № 1 2023
Основан в 2012 г.

Журнал «Ветеринария сегодня»
включен в Перечень рецензируемых научных изданий (ВАК):
1.5.10 – Вирусология (ветеринарные науки),
4.2.3 – Инфекционные болезни и иммунология животных (ветеринарные науки)

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

Design and composition: Maria Bondar
Technical editor: Elena Guseva
Coordinating Editor: Julia Migulina
Content editor of FGBI "ARRIAH": Julia Nurmukhambetova-Mikhailova
Proof-reader: Irina Zvereva
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.

Scientific Journal "Veterinary Science Today" is included in the information analysis system – Russian Science Citation Index, Directory of Open Access Journals DOAJ, as well as in the Web of Science RSCI database and in the international database of EBSCO. Full-text e-versions of the Journal are published on the website of the Scientific Electronic Library, eLIBRARY.RU, DOAJ, and http://veterinary.arriah.ru/jour/index.

Circulation: 1150. 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

Establisher: 600901, Vladimir, Yur'evets, FGBI "ARRIAH"
Publisher: OOO "Veinard", 129626, Moscow, 102 Prospect Mira, bld. 31, office 12
Editorial Board Office: 600901, Vladimir, Yur'evets, FGBI "ARRIAH"
Printing Office: OOO "Grand Prix", 152900, Yaroslavl Oblast, Rybinsk, Lugovaya str., 7
Approved for print: March 17, 2023
Issued: March 31, 2023

16+

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

Дизайн и верстка: Бондарь Мария
Технический редактор: Гусева Елена
Редактор-координатор: Мигулина Юлия
Редактор-корректор ФГБУ "ВНИИЗЖ": Нурмухамбетова-Михайлова Юлия
Корректор: Зверева Ирина
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.

Научный журнал «Ветеринария сегодня» включен в информационно-аналитическую систему РИНЦ, каталог журналов открытого доступа DOAJ, а также в список журналов, входящих в базу данных RSCI на платформе Web of Science, и международную базу данных EBSCO. Электронные версии журнала размещаются в полнотекстовом формате на сайте Научной электронной библиотеки (НЭБ) eLIBRARY. RU, в каталоге DOAJ и по адресу http://veterinary.arriah.ru/jour/index.

Тираж 1150 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: 17 марта 2023 года
Дата выхода в свет: 31 марта 2023 года

16+

Contents

Содержание

DOI: 10.29326/2304-196X-2023-12-1-6-12

Modern approaches to production of safe and effective genetically modified rabies vaccines for animals

M. I. Doronin, A. Mazloum, D. V. Mikhalishin, M. N. Mitrofanova, A. Yu. Sukharkov, V. V. Kiseleva, A. V. Sprygin
FGBI "Federal Centre for Animal Health" (FGBI "ARRIAH"), Vladimir, Russia

SUMMARY

Rabies is a dangerous zoonotic disease that affects the central nervous system, causes encephalomyelitis and paralyses and is almost invariably fatal. The disease causes significant economic losses associated with the death of animals, outbreak consequences, strict restrictions on domestic and international trade in livestock products, preventive and quarantine measures, laboratory tests. The World Organization for Animal Health recommends vaccination to control rabies. Taking into account that there is a lack of affordable high-quality vaccines to globally prevent and control the disease, stable, attenuated production strains of rabies virus with broad cross-activity against various variants of the pathogen shall be considered as ideal candidates to produce high-quality, safe and effective vaccines. Currently, some approaches are applied to reduce the virus virulence and improve safety of rabies vaccines. Reverse genetics is very popular now. It provides new approaches to study functions of a specific gene by analyzing phenotypic effects after direct manipulations with nucleotide sequences. The methods of reverse genetics have revolutionized molecular biology and have become a powerful tool to study genetics of RNA viruses. These methods are widely used to study rabies virus. The use of reverse genetics has made it possible to modify rabies virus production strains for manufacture of modern genetically modified rabies vaccines that induce a persistent and long-term immunity. The review briefly covers general approaches to development of viral vectors with the purpose to create genetically modified rabies vaccines.

Keywords: review, rabies virus, genes, genetically modified rabies vaccines, methods of reverse genetics

Acknowledgements: The work has been done as part of the research program "Creating protection tools for economically and socially significant animal diseases based on production strains of microorganisms selected by genomic sequencing", implemented within some measures of the Federal Program for Genetic Technologies Development for 2019–2027, taken under the Federal Project "Development of large-scale scientific and scientific-technological projects in priority research areas" of the national project "Science and Universities".

For citation: Doronin M. I., Mazloum A., Mikhalishin D. V., Mitrofanova M. N., Sukharkov A. Yu., Kiseleva V. V., Sprygin A. V. Modern approaches to production of safe and effective genetically modified rabies vaccines for animals. *Veterinary Science Today*. 2023; 12 (1): 6–12. DOI: 10.29326/2304-196X-2023-12-1-6-12.

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

For correspondence: Maksim I. Doronin, Candidate of Science (Biology), Head of Sector, Laboratory for FMD Prevention, FGBI "ARRIAH", 600901, Russia, Vladimir, Yur'evets, *e-mail: doronin@arriah.ru*.

УДК 619:578.824.11:615.371:573.6

Современные подходы к созданию безопасных и эффективных генно-инженерных антирабических вакцин для животных

М. И. Доронин, А. Мазлум, Д. В. Михалишин, М. Н. Митрофанова, А. Ю. Сухарьков, В. В. Киселева, А. В. Спрыгин
ФГБУ «Федеральный центр охраны здоровья животных» (ФГБУ «ВНИИЗЖ»), г. Владимир, Россия

РЕЗЮМЕ

Бешенство является одним из опасных зоонозов, который вызывает поражение центральной нервной системы, приводит к энцефаломиелитам, параличам с неизбежным летальным исходом. Заболевание наносит значительный экономический ущерб, который связан с гибелью животных, ликвидацией последствий вспышек болезни, введением строгих ограничений, налагаемых на внутреннюю и международную торговлю продукцией животноводства, проведением профилактических и карантинных мероприятий, осуществлением лабораторных исследований. Для борьбы с бешенством Всемирная организация здравоохранения животных рекомендует вакцинопрофилактику. Для глобальной профилактики и борьбы с этим заболеванием производимого количества доступных высококачественных вакцин недостаточно. Стабильные аттенуированные производственные штаммы вируса бешенства с широкой перекрестной активностью против различных вариантов возбудителя являются идеальными кандидатами для создания надежных, безопасных и эффективных препаратов. На сегодняшний день применен ряд подходов для снижения вирулентности вируса и повышения безопасности антирабических вакцин. Большую популярность имеют методы обратной генетики, которые представляют собой новые подходы к исследованию функции конкретного гена путем анализа фенотипических эффектов за счет непосредственного манипулирования последовательностями нуклеотидов. Данная группа методов произвела революцию в молекулярной биологии, стала мощным инструментом для изучения генетики РНК-содержащих вирусов

и широко используется в исследованиях возбудителя бешенства. Применение методов обратной генетики позволило проводить модификации производственных штаммов вируса бешенства для использования при изготовлении современных генно-инженерных антирабических препаратов, вызывающих стойкий и длительный иммунитет. В представленном обзоре кратко изложены общие подходы к разработке вирусных векторов с целью создания генно-инженерных вакцин против бешенства.

Ключевые слова: обзор, вирус бешенства, гены, генно-инженерные антирабические вакцины, методы обратной генетики

Благодарности: Работа выполнена в рамках исследовательской программы «Создание комплекса средств защиты против экономически и социально значимых болезней животных на основе отобранных методами геномного секвенирования производственных штаммов микроорганизмов», осуществляемой при выполнении отдельных мероприятий Федеральной научно-технической программы развития генетических технологий на 2019–2027 годы, проводимых при реализации федерального проекта «Развитие масштабных научных и научно-технологических проектов по приоритетным исследовательским направлениям» национального проекта «Наука и университеты».

Для цитирования: Доронин М. И., Мазлум А., Михалишин Д. В., Митрофанова М. Н., Сухарьков А. Ю., Киселева В. В., Спрыгин А. В. Современные подходы к созданию безопасных и эффективных генно-инженерных антирабических вакцин для животных. *Ветеринария сегодня*. 2023; 12 (1): 6–12. DOI: 10.29326/2304-196X-2023-12-1-6-12.

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

Для корреспонденции: Доронин Максим Игоревич, кандидат биологических наук, заведующий сектором лаборатории профилактики ящура ФГБУ «ВНИИЗЖ», 600901, Россия, г. Владимир, мкр. Юрьевец, *e-mail: doronin@arriah.ru.*

INTRODUCTION

Rabies is an acute viral disease affecting almost all mammalian species, including humans [1]. Once clinical symptoms appear, rabies is virtually 100% fatal. The World Health Organization estimates that the rabies virus transmitted via bites from infected animals causes more than 59,000 deaths worldwide.

To eradicate the disease successfully, it is necessary to vaccinate wild carnivorous animals (oral vaccination program); to vaccinate domestic animals; to ensure post-exposure and preventive immunization of people who seek medical help and to ensure preventive vaccination of people at risk, primarily, professional risk; to monitor ongoing anti-rabies measures, comprising a number of tasks and techniques. Taking into account that there is a lack of affordable, high-quality vaccines to globally prevent and control the disease; stable, attenuated production strains of rabies virus with broad cross-activity against various variants of the pathogen shall be considered as ideal candidates to make affordable, high quality, safe and effective vaccines [2], however, significant efforts shall be made.

Since the first rabies vaccine was developed at the end of the XIX century by Pasteur, the product has significantly improved, and vaccination is now used for both domestic and farm animals, as well as for reservoir species [3]. Brain tissues affected by the virus were used for a long time to make vaccines. Severe adverse reactions were observed when using rabies vaccines produced from nerve tissues or from tissues of developing avian embryos. The modern technologies of industrial cell cultivation and fermentation have significantly expanded the possibilities to produce high-quality vaccines with a specified amount of immunogenic components [2].

Inactivated and attenuated vaccines are widely used to control rabies, however, they have some disadvantages, most importantly, and some of them are related to safety and strict requirements for biosafety level in the laboratory. Modern rabies vaccines, which are chemically inactivated whole viral products combined with an adjuvant, or live vaccines (most often used in enzootic areas), have been very successful in reducing the number of outbreaks worldwide [4, 5]. However, when using live rabies vaccines, there is a risk that the used strain may easily revert to a virulent phenotype, which is extremely hazardous. In addition, there is an important problem related to commercial production. For this purpose the followings things are required: expensive production lines installed in BSL-3 facilities and a great amount of infectious agent for the vaccine production. An opportunity to bypass these restrictions has appeared, thanks to genetically modified vaccines, because their production is safe. The modified constructions are non-infectious and can be used to produce vaccines in BSL-2 laboratories. However, at the same time, it is necessary to prove that the virus obtained by reverse genetics is avirulent or it belongs to Pathogenicity Groups 3–4 [6–8].

In 1994, M. J. Schnell et al. cloned cDNA of each rabies virus gene and obtained a modified virus. Recently, due to a large amount of data on gene sequences, methods of reverse genetics have been used to determine their functions. The essence of this approach is to move "from genotype to phenotype", i.e. with the help of various genetic manipulations to change or to knock out a particular gene, and then analyze what these changes will result in [9]. At the same time, in contrast to classical approaches, reverse genetics allows to use nucleotide sequences to analyze a specific role of a gene in the phenotype.

Currently, there are four main methods: gene replacement/knockout based on homologous recombination; RNA interference/gene silencing; T-DNA insertion mutagenesis (T-DNA tagging); TILLING (Targeting Induced Local Lesions in Genomes). It should be noted that the reverse genetics greatly facilitates molecular and biological analysis of RNA-containing viruses due to a possibility to do tests directly with ribonucleic acid molecules [10].

Due to *in vitro* reversion, the viral RNA is converted into its corresponding cDNA. Further manipulations with the sequence of the resulting nucleic acid, such as site-specific mutation, deletion, insertion, replacement of a gene site, or the entire gene, can be performed at the cDNA level to study the structure and function of a particular gene [11]. Thus, M. J. Schnell et al. in 1994 used reverse genetics to study attenuated SAD-B19 strain of fixed rabies virus [9]. Later, many researchers began to use this powerful tool to study the molecular biology of rabies viruses belonging to genetic group RABV. Reverse genetics offer great opportunities for development of modern genetically engineered rabies vaccines, which are much safer than the conventional ones [10, 12, 13].

The review briefly covers general approaches to development of viral vectors with the purpose to create genetically engineered rabies vaccines. We have analyzed many publications on reverse genetics of negative-strand RNA viruses, as well as the history and development of rabies vaccines [2, 3, 5, 7, 14–16]. Methods of reverse genetics currently used to reduce virulence and improve safety of rabies vaccines are summarized in the paper. The paper also covers general points related to the use of these methods for negative-sense RNA viruses.

BRIEF DESCRIPTION OF RABIES VIRUS STRUCTURE

Rabies virus (RABV) is a neurotropic virus, a member of the *Lyssavirus* genus belonging to the *Rhabdoviridae* family. The virus of genetic group RABV causes most rabies cases [8].

The virion is a bullet-shaped particle, approximately 250 nm long and 70 nm in diameter. The rabies virus genome is a single-tranded RNA of negative polarity (11,000–12,000 bp in size), encoding the following five structural proteins in a conservative order of 3′→5′: nucleoprotein (N-protein), phosphoprotein (P-protein), matrix protein (M-protein), glycoprotein (G-protein) and RNA-dependent RNA polymerase (L-protein) [4].

Transcription starts from short-leader RNA synthesis, at the 3′ end of the genomic RNA. Viral nucleic acid serves as a matrix for transcription with an RNA-dependent RNA polymerase consisting of L- and P-proteins, which results in synthesis of mRNA for expression of N-, P-, M-, G- and L-proteins. Replication of negative-chain genomes leads to formation of antigenomes that serve as templates for synthesis of negative-strand genomes. Genomic and its complementary antigenomic RNA are tightly encapsulated by a nucleoprotein to form a helical ribonucleoprotein. Only ribonucleoprotein, but not a free RNA, is suitable as a template for replication and transcription. The viral capsid is surrounded by a membrane derived from the host cell, which interacts with the matrix protein and rabies virus glycoprotein [17–19].

Each link between the genes in the rabies virus genome contains a sequence that determines the end of the upstream gene, the intergenic region and the beginning for the downstream gene. These sequences function as a signal for polyadenylation, as well as for initiation, capping, and methylation of the downstream RNA. Intergenic regions of the rabies virus N-P, P-M, M-G and G-L (untranslated pseudogenic ψ-region) include 2, 5, 5 and 24 nucleotides, respectively [14, 20].

ANALYSIS OF RABIES VIRUS GENES FOR DEVELOPMENT OF GENETICALLY MODIFIED RABIES VACCINES

G-gene of rabies virus and its expression. Glycoprotein is a protein of rabies virus, located on the surface of the bullet-shaped virion. It is the main structural protein and the rabies virus antigen, which triggers a strong immune response. The G-protein has two major functions: it determines pathogenicity of the viral particle and induces humoral and cellular immunity against this pathogen [19, 21]. In addition, the glycoprotein ensures interaction between the virion and corresponding receptors on the cell surface resulting in its penetration, and determines the neurotropic nature of the infection [22]. It is important to note that, unlike field isolates, attenuated vaccine strains of rabies virus can synthesize higher levels of glycoprotein in infected neurons [23]. Rupprecht C. E. et al. focus on the fact that the attenuated strains of rabies virus cause mass apoptosis in neurons; however, in case of infection with pathogenic street isolates, these phenomena are much less frequent [8]. Artificially induced mutations of the G-gene make it possible to obtain materials for creation of a new-generation rabies vaccine based on genetically modified structures.

Rabies virus gene containing two and three copies of the G-gene. According to some researchers, modification of the rabies virus containing two copies of G-gene allowed to increase the expression of glycoprotein, which significantly improved the vaccine effectiveness due to an increase in their immunogenicity. At the same time, pathogenicity of the strains decreased sharply. The researchers also showed that the level of G-protein expression is inversely related to the rabies virus pathogenicity [21, 24, 25]. Increased glycoprotein synthesis is associated with enhanced apoptosis, which contributes to regulation of genes associated with host immune responses observed in neurons infected with attenuated virus strains [21].

Hosokawa-Muto J. et al. created R (NPMGGL) strain of recombinant rabies virus carrying double glycoprotein (G) genes. This structure was obtained with the help of reverse genetics using cloned cDNA of RC-HL strain. The biological properties of the created virus were compared with those of the recombinant RC-HL (rRC-HL) strain. The reproduction intensity of strain R(NPMGGL) in cell lines and virulence for adult mice were almost the same as those of strain rRC-HL. At the same time, the G-protein content in the purified virion of strain R(NPMGGL) and the level of glycoprotein expression in infected cells were 1.5 times higher than those in strain rRC-HL. Following serial passages of strain R(NPMGGL) in cell culture, G-protein expression level was the same and the virus infectious titer increased in the process of its adaptation to the cells. It was also demonstrated that strain R(NPMGGL) has higher immunogenicity than strain rRC-HL [6]. Thus, the modified rabies virus strain carrying a double G-gene will make it possible to develop new genetically modified rabies vaccines in future. It should also be noted that in this case we are talking about an inactivated vaccine, since the only modification is the duplication of G-gene, which significantly increases the concentration of immunogenic components and, as a consequence, the vaccine immunogenicity.

Tan Y. et al. conducted a research, where they used self-cleaving sequence of FMDV 2A gene to express

double or triple copies of rabies virus G gene from a one open reading frame obtained from human adenovirus type 5 (AdHu-5). Recombinant adenoviruses produce the virus in similar titers, which suggests that the insertion of double or triple copies of G-gene (rabies virus) associated with the 2A gene sequence (FMDV) does not affect the virus replication. The glycoprotein was effectively expressed by constructs containing 2A gene sequence and retained its antigenic properties. The 2A self-cleaving peptide mediated effective generation of individual rabies virus glycoprotein in the assessment of transient expression. Flow cytometry proved that G-gene expression levels were higher in recombinant adenovirus constructs carrying multiple copies of the rabies virus glycoprotein gene [26].

Thus, the increase in the G-gene expression level has a number of advantages for creation of genetically modified vaccines: 1) it significantly improves production capacity and biosafety; 2) reduces economic losses. These factors are crucial for modern production of safe, effective and affordable rabies vaccines. Therefore, the recombinant rabies virus expressing two or three copies of a glycoprotein is a candidate to develop new-generation genetically modified rabies vaccines.

Significant nucleotide substitutions in the rabies virus G-gene. According to M. Faber et al., the substitution of one amino acid at position 333 on the glycoprotein from a positively charged arginine (Arg) or lysine (Lys) residue for glutamine (Gln) or isoleucine (Ile) makes a virulent strain of rabies virus apathogenic for adult mice when administered intracerebrally [21]. At the same time, there is evidence that the glycoprotein amino acids at position 333 are not fully responsible for the virus pathogenicity, therefore, some rabies virus strains that have a substitution for Gln_{333} retain neuroinvasive abilities and pathogenicity [17].

Ito Y. et al. analyzed rabies virus strain RC-HL using reverse genetics and concluded that amino acids located between positions 164–303 in G-protein, especially amino acids at positions 242, 255 and 268, also play an important role in making the strain apathogenic [27]. M. Faber et al. found that only the amino acid substitution at position 194 of glycoprotein from asparagine (Asn) to lysine (Lys) is responsible for restoring pathogenicity in an apathogenic, attenuated rabies virus strain SPBNGA [21].

Taking into account the experience of many researchers, it can be concluded that when using recombinant strains of rabies virus that carry two or more G-genes encoding glycoprotein with mutations in positions 149, 194 and 333, the risk of returning to the pathogenic phenotype is significantly reduced.

Significant mutations in the M-protein of the rabies virus. The matrix protein of rabies virus is multifunctional, has a small molecular weight of about 20–25 kDa and a length of 202 amino acid residues. This phosphoprotein is represented by two isoforms M_1 and M_2, which differ from each other in phosphorylation degree. The matrix protein is structurally a bridge between the N- and G-protein. M-gene is much more conservative compared to P-protein. The matrix protein is believed to form a layer between the glycoprotein in the virion shell and the helical-shaped nucleocapsid nucleus consisting of RNA and N-, L-, P-proteins. M-protein is the main factor contributing to virion morphogenesis [28].

Finke S. et al. demonstrated that the M-protein establishes a balance between the virus transcription and replication [15, 16]. It interacts with the viral ribonucleoprotein, which condenses into a dense bullet-shaped form, and plays a key role in the assembly and budding of mature virions [29].

Wirblich C. et al. found that the matrix protein structure has an L-domain with four motifs, one of which is PPxY (PPEY) close to the amino terminus of M-protein. The authors created constructs with point mutations and revealed that PPEY is necessary for effective release of virion from the cell membrane. Amino acid deletions and substitutions in PPEY motif reduce the infection spread. Recombinant viruses constructed on this basis demonstrated reduced virulence for mice, while causing strong immune responses [30]. Thus, significant substitutions in the matrix protein of the rabies virus can create gene constructs for production of modern rabies vaccines.

Significant mutations in the P-gene of the rabies virus. Phosphoprotein is the most important structural protein of the rabies virus and it has molecular weight of 260 kDa and a length of about 330 amino acid residues. The expanded N-terminal region of the P-protein interacts with RNA polymerase. The P-gene encodes at least four proteins synthesized in an infected cell. The phosphoprotein has a chaperone function and binds to N that is not yet bound to viral RNA. The C-terminal domain of the P-protein binds to the "nucleoprotein–RNA" complex and attaches the polymerase complex [29].

Schnell M. J. et al. detected immunodominant P-protein site, which is located in the range of 191–206 amino acid residues and is an interferon antagonist [9, 31, 32]. Jacob Y. et al. determined that the light chain of cytoplasmic dynein involved in the intracellular transport of organelles interacts with the rabies virus phosphoprotein [33]. Deletion of LC8 binding domain in the P-protein significantly inhibited replication and transcription of the virus in neurons. It shall be noted that such a recombinant virus was characterized by a decreased level of gene expression in neuronal cell cultures, while the growth pattern on non-neuronal cells remained unchanged [17, 26]. Thus, amino acid mutations in the dynein-binding domain of the P-protein make it possible to reduce the rabies virus virulence, and the created recombinant viruses make it possible to develop genetically modified rabies vaccines.

SYNTHESIS OF INFECTIOUS RECOMBINANT VIRAL PARTICLES USING MODIFIED cDNA

In 1994 for the first time, M. J. Schnell et al. constructed rabies virus using cloned cDNA [9]. The schematic diagram of this process included at least simultaneous transfection into the cell of four plasmids encoding N-, P-, L-proteins and full-size cDNA of the virus. Rabies virus (-)RNA was reverse transcribed into positive-sense antigenomic cDNA (Fig.). Using amplification of a specific gene from full-length cDNA, three plasmids *p-N*, *p-P*, *p-L* expressing N-, P- and L-proteins of the rabies virus were constructed. In order to avoid mutations, the L-gene was assembled from gradually cloned fragments. Similarly, a plasmid carrying information about the entire genome of the rabies virus (*p-genome*) was constructed.

Viral cDNA was gradually assembled with the help of restrictases (as part of a plasmid), to create a full matrix

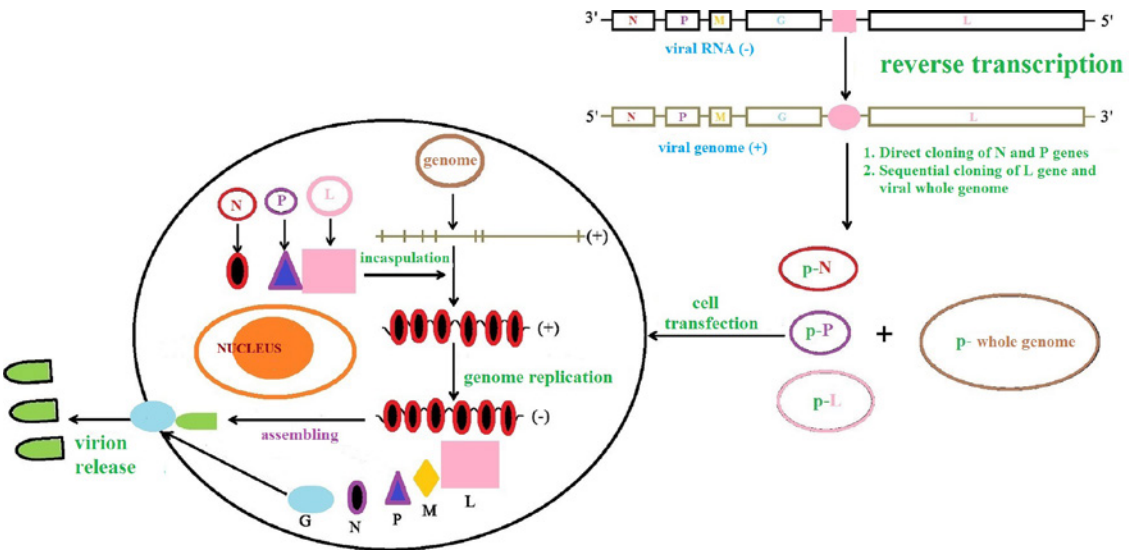

Fig. Synthesis of infectious recombinant rabies virus particles from cloned cDNA

for the rabies virus genetic material. For transcription of the constructed plasmids, T7-RNA polymerase or endogenous cellular RNA polymerases of type I or II were used. In order to obtain the exact 5′ and 3′ ends of the viral RNA, the cloned viral cDNA was flanked with autocatalytic ribozyme sequences. The N-, P- and L-proteins of the rabies virus expressed by three plasmids encapsulate the transcribed full-size antigenomic sense RNA to form a ribonucleoprotein, which functions as a matrix for further expression of structural viral proteins and amplification of rabies virus (-)RNA. The use of transcripts with antigenomic meaning is fundamental in this case, since only they can ensure the process of gene expression and avoid hybridization between genomic (-)RNA and plasmid (+)RNAs. The ribonucleoprotein shapes into a bullet using a matrix protein and separates from the cytoplasmic membrane, where glycosylated trimeric transmembrane compounds have accumulated. At the end of the process, infectious virions are formed, and the next cycle of infection begins [34–36].

Some researchers describe procedures that facilitate and increase effectiveness of creation of a viable rabies virus. Thus, to deliver T7 RNA polymerase, instead of infecting cells with recombinant smallpox vaccine virus, BSR-T7/5 cell line was created that expresses RNA polymerase [37]. Inoue K. et al. developed a segmented version of the virus and showed that additional nucleotides at the terminal end of the genome can affect the expression efficiency. Coding sequences of two ribozymes (HamRz and HdvRz) were "sewn" onto the 5′ and 3′ ends of the rabies virus genome to obtain full-sized virus transcripts with exact ends [35]. Such modifications significantly expand the possibilities of reverse genetics of the rabies virus nucleic acid for various cell lines and make it possible to quickly and efficiently generate a recombinant virus.

It should be noted that scientists also analyzed the ψ-region of the rabies pathogen genome. They found that the virus with a knocked out pseudogen was characterized by normal reproduction rates in biological test systems and did not differ from isolates or strains that have this region [9, 34]. Thus, the pseudogen ψ is an ideal target for insertions and makes it possible to carry out various genetic manipulations with the rabies virus genome.

CONCLUSION

Vaccination of animals is recommended in the Manual of the World Organization for Animal Health to control rabies. Currently, live, as well as cultural inactivated adsorbed and emulsion rabies vaccines are used for these purposes. At the same time, there is a number of disadvantages related to the vaccines, i.e. strict biosafety requirements for production laboratories and a fact that the attenuated strains may revert to virulence [3, 11, 14].

In order to reduce the virulence and increase the safety of rabies vaccines, methods of reverse genetics are currently used [38]. Some researchers demonstrate [9, 12, 31] that many properties of the rabies virus with non-segmented negative RNA are perfect to construct gene delivery vectors required for development of genetically modified rabies vaccines. The simple and conservative composition of the rabies virus genome makes it easy to use genetic engineering and to express modified genes.

The encapsidation of the rabies virus nucleic acid into a ribonucleoprotein has an advantage to significantly reduce the probability of recombination and thereby maintain genome stability [19, 38–41]. High frequency of reversion shall be taken into account during reproduction of the virus in an infected cell. It is associated with low accuracy of transcription with RNA polymerase, which indicates the need for simultaneous introduction of several modifications.

Based on the extensive research into modification of rabies virus genome using reverse genetics, it became possible to construct attenuated live strains which will help to develop modern genetically modified rabies vaccines, safe and effective. Currently, researchers focus on significant mutations introduced into the G-, M-, P-genes of rabies virus, leading to a loss of pathogenicity. Special attention is paid to gene constructs that include two or even three copies of the G-gene, which makes it possible to get suspensions with a high concentration of glycoprotein and induce a strong immune response when administered to animals. The application of reverse genetics for synthesis of infectious recombinant viral particles using modified rabies virus cDNA is also of great interest.

REFERENCES

1. Hidaka Y., Lim C. K., Takayama-Ito M., Park C. H., Kimitsuki K., Shiwa N., et al. Segmentation of the rabies virus genome. *Virus. Res*. 2018; 252: 68–75. DOI: 10.1016/j.virusres.2018.05.017.

2. Wu X., Smith T. G., Rupprecht C. E. From brain passage to cell adaptation: the road of human rabies vaccine development. *Expert. Rev. Vaccines*. 2011; 10 (11): 1597–1608. DOI: 10.1586/erv.11.140.

3. Briggs D. J., Nagarajan T., Rupprecht C. E. Rabies vaccines. *In: Rabies: Scientific Basis of the Disease and Its Management. Ed. by A. C. Jackson. 3rd ed*. Academic Press; 2013; Chapter 13: 497–526. DOI: 10.1016/B978-0-12-396547-9.00013-4.

4. Evans J. S., Horton D. L., Easton A. J., Fooks A. R., Banyard A. C. Rabies virus vaccines: is there a need for a pan-lyssavirus vaccine? *Vaccine*. 2012; 30 (52): 7447–7454. DOI: 10.1016/j.vaccine.2012.10.015.

5. Hicks D. J., Fooks A. R., Johnson N. Developments in rabies vaccines. *Clin. Exp. Immunol.* 2012; 169 (3): 199–204. DOI: 10.1111/j.1365-2249.2012.04592.x.

6. Hosokawa-Muto J., Ito N., Yamada K., Shimizu K., Sugiyama M., Minamoto N. Characterization of recombinant rabies virus carrying double glycoprotein genes. *Microbiol. Immunol*. 2006; 50 (3): 187–196. DOI: 10.1111/j.1348-0421.2006.tb03785.x.

7. Nel L. H. Vaccines for lyssaviruses other than rabies. *Expert. Rev. Vaccines*. 2005; 4 (4): 533–540. DOI: 10.1586/14760584.4.4.533.

8. Rupprecht C. E., Plotkin S. A. Rabies vaccines. *In: Vaccines. Ed. by S. A. Plotkin, W. A. Orenstein, P. A. Offit. 6th ed*. Elsevier Saunders; 2013: 646–668. DOI: 10.1016/B978-1-4557-0090-5.00036-7.

9. Schnell M. J., Mebatsion T., Conzelmann K. K. Infectious rabies viruses from cloned cDNA. *EMBO J*. 1994; 13 (18): 4195–4203. DOI: 10.1002/j.1460-2075.1994.tb06739.x.

10. Yin J., Wang X., Mao R., Zhang Z., Gao X., Luo Y., et al. Research advances on the interactions between rabies virus structural proteins and host target cells: accrued knowledge from the application of reverse genetics systems. *Viruses*. 2021; 13 (11):2288. DOI: 10.3390/v13112288.

11. Conzelmann K. K. Reverse Genetics of *Mononegavirales*: The Rabies Virus Paradigm. *In: Sendai Virus Vector. Ed. by Y. Nagai*. Tokyo: Springer; 2013: 1–20. DOI: 10.1007/978-4-431-54556-9_1.

12. Huang Y., Tang Q., Nadin-Davis S. A., Zhang S., Hooper C. D., Ming P., et al. Development of a reverse genetics system for a human rabies virus vaccine strain employed in China. *Virus. Res.* 2010; 149 (1): 28–35. DOI: 10.1016/j.virusres.2009.12.009.

13. Larsen D. D., Wickersham I. R., Callaway E. M. Retrograde tracing with recombinant rabies virus reveals correlations between projection targets and dendritic architecture in layer 5 of mouse barrel cortex. *Front. Neural Circuits*. 2008; 1:5. DOI: 10.3389/neuro.04.005.2007.

14. Conzelmann K. K., Cox J. H., Schneider L. G., Thiel H. J. Molecular cloning and complete nucleotide sequence of the attenuated rabies virus SAD B19. *Virology*. 1990; 175 (2): 485–499. DOI: 10.1016/0042-6822(90)90433-r.

15. Finke S., Conzelmann K. K. Recombinant rhabdoviruses: vectors for vaccine development and gene therapy. *Curr. Top. Microbiol. Immunol*. 2005; 292: 165–200. DOI: 10.1007/3-540-27485-5_8.

16. Finke S., Mueller-Waldeck R., Conzelmann K. K. Rabies virus matrix protein regulates the balance of virus transcription and replication. *J. Gen. Virol*. 2003; 84: 1613–1621. DOI: 10.1099/vir.0.19128-0.

17. Mebatsion T. Extensive attenuation of rabies virus by simultaneously modifying the dynein light chain binding site in the P protein and replacing Arg333 in the G protein. *J. Virol*. 2001; 75 (23): 11496–11502. DOI: 10.1128/JVI.75.23.11496-11502.2001.

18. Morimoto K., Shoji Y., Inoue S. Characterization of P gene-deficient rabies virus: propagation, pathogenicity and antigenicity. *Virus. Res*. 2005; 111 (1): 61–67. DOI: 10.1016/j.virusres.2005.03.011.

19. Wang Z. W., Sarmento L., Wang Y., Li X. Q., Dhingra V., Tseggai T., et al. Attenuated rabies virus activates, while pathogenic rabies virus evades, the host innate immune responses in the central nervous system. *J. Virol*. 2005; 79 (19): 12554–12565. DOI: 10.1128/JVI.79.19.12554-12565.2005.

20. Walker P. J., Dietzgen R. G., Joubert D. A., Blasdell K. R. Rhabdovirus accessory genes. *Virus. Res*. 2011; 162 (1–2): 110–125. DOI: 10.1016/j.virusres.2011.09.004.

21. Faber M., Faber M. L., Papaneri A., Bette M., Weihe E., Dietzschold B., Schnell M. J. A single amino acid change in rabies virus glycoprotein increases virus spread and enhances virus pathogenicity. *J. Virol.* 2005; 79 (22): 14141–14148. DOI: 10.1128/JVI.79.22.14141-14148.2005.

22. Etessami R., Conzelmann K. K., Fadai-Ghotbi B., Natelson B., Tsiang H., Ceccaldi P. E. Spread and pathogenic characteristics of a G-deficient rabies virus recombinant: an *in vitro* and *in vivo* study. *J. Gen. Virol.* 2000; 81: 2147–2153. DOI: 10.1099/0022-1317-81-9-2147.

23. Yan X., Prosniak M., Curtis M. T., Weiss M. L., Faber M., Dietzschold B., Fu Z. F. Silver-haired bat rabies virus variant does not induce apoptosis in the brain of experimentally infected mice. *J. Neurovirol*. 2001; 7 (6): 518–527. DOI: 10.1080/135502801753248105.

24. Zhang G., Wang H., Mahmood F., Fu Z. F. Rabies virus glycoprotein is an important determinant for the induction of innate immune responses and the pathogenic mechanisms. *Vet. Microbiol*. 2013; 162 (2–4): 601–613. DOI: 10.1016/j.vetmic.2012.11.031.

25. Tao L., Ge J., Wang X., Wen Z., Zhai H., Hua T., et al. Generation of a recombinant rabies Flury LEP virus carrying an additional G gene creates an improved seed virus for inactivated vaccine production. *Virol. J.* 2011; 8:454. DOI: 10.1186/1743-422X-8-454.

26. Tan Y., Liang H., Chen A., Guo X. Coexpression of double or triple copies of the rabies virus glycoprotein gene using a 'self-cleaving' 2A peptide-based replication-defective human adenovirus serotype 5 vector. *Biologicals*. 2010; 38 (5): 586–593. DOI: 10.1016/j.biologicals.2010.06.001.

27. Ito Y., Ito N., Saito S., Masatani T., Nakagawa K., Atoji Y., Sugiyama M. Amino acid substitutions at positions 242, 255 and 268 in rabies virus glycoprotein affect spread of viral infection. *Microbiol. Immunol*. 2010; 54 (2): 89–97. DOI: 10.1111/j.1348-0421.2009.00192.x.

28. Zandi F., Khalaj V., Goshadrou F., Meyfour A., Gholami A., Enayati S., et al. Rabies virus matrix protein targets host actin cytoskeleton: a protein-protein interaction analysis. *Pathog. Dis*. 2021; 79 (1):ftaa075. DOI: 10.1093/femspd/ftaa075.

29. Mebatsion T., Konig M., Conzelmann K. K. Budding of rabies virus particles in the absence of the spike

glycoprotein. *Cell.* 1996; 84 (6): 941–951. DOI: 10.1016/s0092-8674(00)81072-7.

30. Wirblich C., Tan G. S., Papaneri A., Godlewski P. J., Orenstein J. M., Harty R. N., Schnell M. J. PPEY motif within the rabies virus (RV) matrix protein is essential for efficient virion release and RV pathogenicity. *J. Virol*. 2008; 82 (19): 9730–9738. DOI: 10.1128/JVI.00889-08.

31. Schnell M. J., McGettigan J. P., Wirblich C., Papaneri A. The cell biology of rabies virus: using stealth to reach the brain. *Nat. Rev. Microbiol*. 2010; 8 (1): 51–61. DOI: 10.1038/nrmicro2260.

32. Schnell M. J., Tan G. S., Dietzschold B. The application of reverse genetics technology in the study of rabies virus (RV) pathogenesis and for the development of novel RV vaccines. *J. Neurovirol*. 2005; 11 (1): 76–81. DOI: 10.1080/13550280590900436.

33. Jacob Y., Badrane H., Ceccaldi P. E., Tordo N. Cytoplasmic dynein LC8 interacts with lyssavirus phosphoprotein. *J. Virol*. 2000; 74 (21): 10217–10222. DOI: 10.1128/jvi.74.21.10217-10222.2000.

34. Ceccaldi P. E., Fayet J., Conzelmann K. K., Tsiang H. Infection characteristics of rabies virus variants with deletion or insertion in the pseudogene sequence. *J. Neurovirol.* 1998; 4 (1): 115–119. DOI: 10.3109/13550289809113489.

35. Inoue K., Shoji Y., Kurane I., Iijima T., Sakai T., Morimoto K. An improved method for recovering rabies virus from cloned cDNA. *J. Virol. Methods*. 2003; 107 (2): 229–236. DOI: 10.1016/s0166-0934(02)00249-5.

36. Le Mercier P., Jacob Y., Tanner K., Tordo N. A novel expression cassette of lyssavirus shows that the distantly related Mokola virus can rescue a defective rabies virus genome. *J. Virol*. 2002; 76 (4): 2024–2027. DOI: 10.1128/jvi.76.4.2024-2027.2002.

37. Buchholz U. J., Finke S., Conzelmann K. K. Generation of bovine respiratory syncytial virus (BRSV) from cDNA: BRSV NS2 is not essential for virus replication in tissue culture, and the human RSV leader region acts as a functional BRSV genome promoter. *J. Virol*. 1999; 73 (1): 251–259. DOI: 10.1128/JVI.73.1.251-259.1999.

38. Wenqiang J., Yin X., Lan X., Li X., Liu J. Development of a reverse genetics system for the aG strain of rabies virus in China. *Arch. Virol*. 2014; 159 (5): 1033–1038. DOI: 10.1007/s00705-013-1919-9.

39. Wall N. R., Wickersham I. R., Cetin A., De La Parra M., Callaway E. M. Monosynaptic circuit tracing *in vivo* through Cre-dependent targeting and complementation of modified rabies virus. *Proc. Natl. Acad. Sci. USA*. 2010; 107 (50): 21848–21853. DOI: 10.1073/pnas.1011756107.

40. Wickersham I. R., Finke S., Conzelmann K. K., Callaway E. M. Retrograde neuronal tracing with a deletion-mutant rabies virus. *Nat. Methods*. 2007; 4 (1): 47–49. DOI: 10.1038/nmeth999.

41. Wickersham I. R., Sullivan H. A., Seung H. S. Production of glycoprotein-deleted rabies viruses for monosynaptic tracing and high-level gene expression in neurons. *Nat. Protoc*. 2010; 5 (3): 595–606. DOI: 10.1038/nprot.2009.248.

Received 03.11.2022
Revised 15.11.2022
Accepted 09.12.2022

INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Maksim I. Doronin, Candidate of Science (Biology), Head of Sector, Laboratory for FMD Prevention, FGBI "ARRIAH", Vladimir, Russia; *https://orcid.org/0000-0002-4682-6559, e-mail: doronin@arriah.ru.*

Ali Mazloum, Candidate of Science (Biology), Senior Researcher, Laboratory for African Swine Fever, FGBI "ARRIAH", Vladimir, Russia; *https://orcid.org/0000-0002-5982-8393, e-mail: mazlum@arriah.ru.*

Dmitry V. Mikhalishin, Doctor of Science (Veterinary Medicine), Head of Laboratory for FMD Prevention, FGBI "ARRIAH", Vladimir, Russia; *https://orcid.org/0000-0003-1718-1955, e-mail: mihalishin_dv@arriah.ru.*

Maria N. Mitrofanova, Candidate of Science (Veterinary Medicine), Junior Researcher, Information and Analysis Centre, FGBI "ARRIAH", Vladimir, Russia; *https://orcid.org/0000-0003-0126-9653, e-mail: mitrofanova@arriah.ru.*

Andrey Yu. Sukharkov, Candidate of Science (Biology), Head of Reference Laboratory for Rabies and BSE, FGBI "ARRIAH", Vladimir, Russia; *https://orcid.org/0000-0001-5326-2440, e-mail: suharkov@arriah.ru.*

Valeriya V. Kiseleva, Leading Biologist, Reference Laboratory for Rabies and BSE, FGBI "ARRIAH", Vladimir, Russia; *https://orcid.org/0000-0001-7114-6267, e-mail: kiseleva_vv@arriah.ru.*

Alexander V. Sprygin, Doctor of Science (Biology), Senior Researcher, Reference Laboratory for Bovine Diseases, FGBI "ARRIAH", Vladimir, Russia; *https://orcid.org/0000-0001-5982-3675, e-mail: sprygin@arriah.ru.*

Доронин Максим Игоревич, кандидат биологических наук, заведующий сектором лаборатории профилактики ящура ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *https://orcid.org/0000-0002-4682-6559, e-mail: doronin@arriah.ru.*

Мазлум Али, кандидат биологических наук, старший научный сотрудник референтной лаборатории по африканской чуме свиней ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *https://orcid.org/0000-0002-5982-8393, e-mail: mazlum@arriah.ru.*

Михалишин Дмитрий Валерьевич, доктор ветеринарных наук, заведующий лабораторией профилактики ящура ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *https://orcid.org/0000-0003-1718-1955, e-mail: mihalishin_dv@arriah.ru.*

Митрофанова Мария Николаевна, кандидат ветеринарных наук, младший научный сотрудник информационно-аналитического центра ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *https://orcid.org/0000-0003-0126-9653, e-mail: mitrofanova@arriah.ru.*

Сухарьков Андрей Юрьевич, кандидат биологических наук, заведующий референтной лабораторией по бешенству и BSE ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *https://orcid.org/0000-0001-5326-2440, e-mail: suharkov@arriah.ru.*

Киселева Валерия Владимировна, ведущий биолог лаборатории по бешенству и BSE ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *https://orcid.org/0000-0001-7114-6267, e-mail: kiseleva_vv@arriah.ru.*

Спрыгин Александр Владимирович, доктор биологических наук, старший научный сотрудник референтной лаборатории болезней крупного рогатого скота ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *https://orcid.org/0000-0001-5982-3675, e-mail: sprygin@arriah.ru.*

DOI: 10.29326/2304-196X-2023-12-1-13-22

Biological, cytomorphological and karyological heterogeneity of transformed cell lines derived from domestic pig (*Sus scrofa* L.) organs

B. L. Manin, E. A. Trofimova, V. L. Gavrilova, O. S. Puzankova
FGBI "Federal Centre for Animal Health" (FGBI "ARRIAH"), Vladimir, Russia

SUMMARY

The main advantage of transformed cell lines as compared to primary ones is that they allow generation of the stable material suitable for long-term research and practical use. Therefore, development of new continuous cell cultures from various animal tissues is of great practical importance. Results of examination of transformed cell lines derived from organs of domestic pigs (*Sus scrofa* L.) for their biological, cytomorphological and karyological features are described in the paper. The said cell cultures are confirmed to be susceptible to various animal viruses. Also, a procedure for preparation of new diploid cell culture from porcine spleen (SSs – *Spleen Sus scrofa*) is described. Based on the obtained data analysis it was concluded that the epithelial cells derived from trypsinized porcine spleens could be successfully immortalized. All transformed cell lines of porcine origin have similar morphology with predominated epithelium-like forms. Some of them – SPEV, A_4C_2, RSK – tend to adopt a spherical shape in suspension. Such cell lines as PSGK-30 and PPES cell lines form partial multilayer or they are characterized by significant monolayer compaction with pseudosyncytium formation. Only pseudodiploid cell culture (SPEV cell culture) tends to grow in suspension, it also grows in rotating culture flasks. Karyological transformations in different cell cultures stabilized at certain level. Spontaneous increase in chromosome numbers in the main population of transformed cell lines towards triploidy resulted in stabilization of culture properties and increase in proliferation. PSGK-30 cell culture has the highest modal class – 64 chromosomes. Near-diploid cultures (A_4C_2, RSK) demonstrate stable growth properties and are similar to SPEV cell culture in adopting spherical cell forms in medium, monolayer character and cell morphology. PK-15 cell culture having a distinct karyotype under different cultivation conditions while retaining other culture properties is found to be the most adaptive. A new transformed diploid SSs cell culture is developed by long-term incubation, subcultivation (more than 80 passages) and selection at the FGBI "ARRIAH" laboratory; it can remain diploid or may spontaneously become heteroploid-immortalized during further passaging. The cell hyperploidy is very likely to enhance telomerase activity, which in turn stabilizes immortalization and results in proliferative activity increase. The cell viability has been maintained so far by regular reseedings (split ratio – 1:2–1:3) performed 1–2 times a week.

Keywords: transformed cell line, continuous cell line, hybridoma, diploidy, heteroploidy, modal class of cells, proliferative activity

Acknowledgements: This work was funded by the FGBI "ARRIAH" within the scope of research activities "Veterinary Welfare".

For citation: Manin B. L., Trofimova E. A., Gavrilova V. L., Puzankova O. S. Biological, cytomorphological and karyological heterogeneity of transformed cell lines derived from domestic pig (*Sus scrof*a L.) organs. *Veterinary Science Today*. 2023; 12 (1): 13–22. DOI: 10.29326/2304-196X-2023-12-1-13-22.

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

For correspondence: Elena A. Trofimova, Head of Sector, Cell Cultivation Unit, FGBI "ARRIAH", 600901, Russia, Vladimir, Yur'evets, *e-mail: trofimova_ea@arriah.ru.*

УДК 619:576.535:636.4:57.082.26

Биологическая, цитоморфологическая и кариологическая гетерогенность постоянных линий клеток, полученных из органов домашней свиньи (*Sus scrofa* L.)

Б. Л. Манин, Е. А. Трофимова, В. Л. Гаврилова, О. С. Пузанкова
ФГБУ «Федеральный центр охраны здоровья животных» (ФГБУ «ВНИИЗЖ»), г. Владимир, Россия

РЕЗЮМЕ

Основным преимуществом постоянных линий клеток по сравнению с первичными является возможность наработки стабильного материала, пригодного для продолжительного использования в научно-исследовательских и практических целях. Поэтому важное прикладное значение имеет получение новых перевиваемых культур клеток из разнообразных тканей животных. В статье отражены результаты изучения биологических, цитоморфологических и кариологических особенностей постоянных линий клеток, полученных из органов домашней свиньи (*Sus scrofa* L.), подтверждена чувствительность данных культур к различным вирусам животных, а также описан процесс получения новой диплоидной культуры клеток из селезенки свиньи (SSs – *Splen Sus scrofa*). При анализе полученных данных пришли к выводу, что полноценной иммортализации подвергаются эпителиальные клетки, полученные из почек свиньи после трипсинизации. Все постоянные линии клеток свиного происхождения имеют схожую морфологию с преобладанием эпителиоподобных форм. Некоторые из них – СПЭВ, A_4C_2, RSK – имеют тенденцию переживания сферической формы в суспензии. Такие клеточные линии, как ПСГК-30 и ППЭС,

формируют частичный полислой либо для них характерно значительное уплотнение монослоя с образованием псевдосинцития. Только одна псевдодиплоидная клеточная культура СПЭВ имеет тенденцию к росту в суспензии, она также растет во вращающихся культуральных флаконах. Кариологические трансформации у разных культур стабилизировались на определенном уровне. Спонтанное увеличение количества хромосом в основной популяции постоянных линий клеток в сторону триплоидии привело к стабилизации культуральных свойств и увеличению пролиферации. Наивысший модальный класс – 64 хромосомы – имеет культура ПСГК-30. Околодиплоидные культуры (A_4C_2, RSK) характеризуются стабильными ростовыми параметрами и показывают сходство с культурой СПЭВ в отношении формирования переживающих сферических клеток в среде, качества монослоя и морфологии клеток. Наиболее пластичной клеточной линией оказалась РК-15, которая в разных условиях культивирования имеет отличительный кариотип при сохранении остальных культуральных свойств. В условиях лаборатории ФГБУ «ВНИИЗЖ» в результате длительного инкубирования, субкультивирования (свыше 80 пассажей) и отбора была получена новая постоянная диплоидная культура клеток SSs, которая при проведении дальнейших пассажей может остаться диплоидной или спонтанно стать гетероплоидной – иммортализованной. Велика вероятность того, что впоследствии гиперплоидность клеток спровоцирует увеличение теломеразной активности, что, в свою очередь, стабилизирует иммортализацию и приведет к увеличению пролиферативной активности. До настоящего времени жизнеспособность клеток поддерживается путем регулярных пересевов (коэффициент пересева – 1:2–1:3), осуществляемых 1–2 раза в неделю.

Ключевые слова: постоянная линия клеток, перевиваемая линия клеток, гибридома, диплоидность, гетероплоидность, модальный класс клеток, пролиферативная активность

Благодарность: Исследование проведено за счет средств ФГБУ «ВНИИЗЖ» в рамках научно-исследовательских работ по теме «Ветеринарное благополучие».

Для цитирования: Манин Б. Л., Трофимова Е. А., Гаврилова В. Л., Пузанкова О. С. Биологическая, цитоморфологическая и кариологическая гетерогенность постоянных линий клеток, полученных из органов домашней свиньи (*Sus scrofa* L.). *Ветеринария сегодня*. 2023; 12 (1): 13–22. DOI: 10.29326/2304-196X-2023-12-1-13-22.

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

Для корреспонденции: Трофимова Елена Александровна, заведующий сектором отдела культур клеток, ФГБУ «ВНИИЗЖ», 600901, Россия, г. Владимир, мкр. Юрьевец, *e-mail: trofimova_ea@arriah.ru.*

INTRODUCTION

Transformed cell lines (TCLs) including those originating from organs of domestic pig (*Sus scrofa* L.) are widely used in veterinary virology [1]. The cell lines demonstrating intensive proliferation were mainly derived from porcine kidney [2–9]. Attempted development of cell cultures easily propagating in matrix from the thyroid gland [10, 11], testicles [11, 12], intestines [10], spleen [13], synovial membrane [14] and other pig organs as well as attempted development of continuous macrophage/monocyte cell lines [15–23] were not successful due to their low proliferative potential with a split ratio of 1:2–1:3. Therefore, development of TCLs having high proliferative activity and applied significance is of current importance.

Normal karyotype of domestic pig well studied in veterinary medicine was used as a reference for karyological examinations [24, 25]. In contrast to molecular genetic analysis [26], karyological analysis allows identification of qualitative and quantitative changes in karyotypes of main porcine TCLs populations and comparison of their biological and cultural properties [27, 28].

The mains advantage of continuous cell cultures is their homogeneity and relevant stability whereas susceptibility of primary cell cultures to various viruses depends on individual features of the animal. Therefore, development of new continuous cell cultures from various animal tissues is an important task. Porcine spleen (*Spleen Sus scrofa* – SSs) is one of such tissues and development of diploid and continuous cell cultures from porcine spleen is of great practical importance.

A new continuous porcine spleen cell culture is developed as a result of long-term incubation, subcultivation and thorough selection; it has undergone more than 80 passages for two years. The diploid cell viability is maintained by regular re-seeding (split ratio– 1:2–1:3) 1–2 times a week.

The study was aimed at biological, cytomorphological and karyological examination of transformed cell lines derived from domestic pig organs (*Sus scrofa* L.), as well as description of the procedure for new diploid cell culture from swine spleen (SSs) development.

MATERIALS AND METHODS

The cell lines were phenotyped using Olympus CKX41 phase-contrast microscope (Japan) and ML-2B luminescent microscope (Russia).

Karyological method for metaphase plate preparation proposed by P. S. Moorhead et al. [24, 28, 29] was used for cell culture identification.

The cells were cultured in conventional media: MEM, DMEM, DMEM/F-12 supplemented with 10% bovine serum.

RESULTS AND DISCUSSION

The following porcine cell lines and sublines are used in Russian veterinary practice: IB-RS-2, SPEV, A_4C_2, A_4C_2/9k, A_4C, PK-15, SK-6, PPES, PPK, PSGK-30, RSK,

Table
Main characteristics of transformed cell lines of porcine origin

No.	Transformed cell lines	Split ratio	Karyology, modal class	Cell monolayer morphology
1	IB-RS-2 (porcine kidney)	1:3; 1:4	36	polygonal, epithelium-like
2	SPEV (porcine kidney)	1:4; 1:6	38	polygonal, epithelium-like, spherical
3	A_4C_2 (SPEV and porcine splenocyte hybrid)	1:3; 1:4	39	polygonal, epithelium-like, spherical
4	RSK (rabbit skin)	1:4; 1:6	40	polygonal, epithelium-like, spherical
5	PPES (porcine kidney)	1:4; 1:6	51	polygonal, epithelium-like
6	PK-15 (porcine kidney)	1:4; 1:6	53	polygonal, epithelium-like
7	PSGK-30 (Siberian ibex kidney)	1:4; 1:18	64	polygonal, epithelium-like
8	ST (swine testicles)	1:2	38	polygonal, epithelium-like
9	SSs (swine spleen)	1:2	38	polygonal, epithelium-like

KST [1, 2, 4, 5, 9, 25, 30–33] derived from kidneys, ST derived from testicles [11, 12], SSs derived from spleen [13].

There are 9 types of transformed porcine cell lines in the FGBI "ARRIAH" Collection (Table).

Examinations of lactate dehydrogenase isoenzyme spectra allowed us to identify RSK (rabbit skin) and PSGK-30 (Siberian ibex kidney) cell lines kept in the FGBI "ARRIAH" Collection as porcine ones. Data on the species identification of given cell cultures were supported by results of examination of their karyotypes, morphology and culture properties.

Marker medium-size metacentric chromosomes containing centromeres that were not stained during routine preparation procedure were used as a reference for confirming the cell culture species identification results (Fig. 1). The metacentric/acrocentric chromosome ratio in most cultures of porcine origin is about 2.2 (±5%): there are 26 metacentrics and 12 acrocentrics in primary porcine kidney cell line.

Description of transformed IB-RS-2 cell line. IB-RS-2 cell line is one of the "oldest" ones developed by M. P. de Castro from porcine kidneys in Sao Paulo, Brazil, in 1962.

The monolayer consists of polygonal epithelium-like cells. The overgrown monolayer forms a syncytium (Fig. 2). IB-RS-2 modal class of 36 chromosomes, 49% of populations (Fig. 3) is the lowest among all known porcine cell cultures (Table). This TCL is susceptible to foot-and-mouth disease virus (FMDV), Teschen disease virus, classical swine fever virus (CSFV), African swine fever virus (ASFV), swine vesicular disease virus (SVDV), vesicular exanthema of swine virus and other viruses.

It should be noted that this TCL has moderate proliferative potential (split ratio: 1:3; 1:4). Pseudosyncytium forms

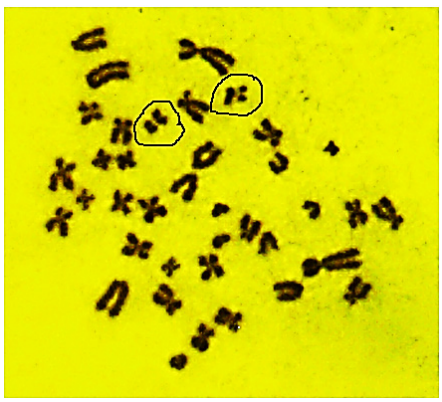

Fig. 1. Porcine diploid chromosome number with two markers and 26 metacentric and 12 acrocentric chromosome ratio

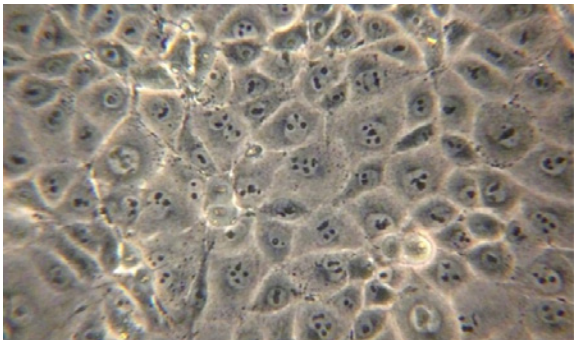

Fig. 2. IB-RS-2 cell line morphology, 40× lens

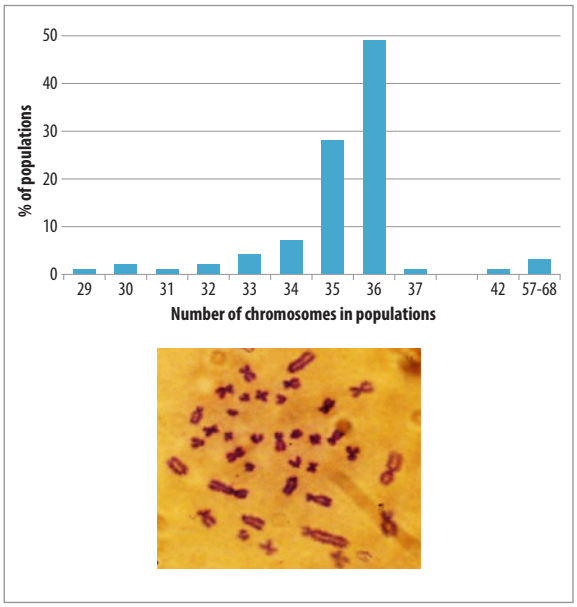

Fig. 3. Karyogram and metaphase plate of IB-RS-2 cell line, 36 chromosomes (26 metacentric and 10 acrocentric chromosomes)

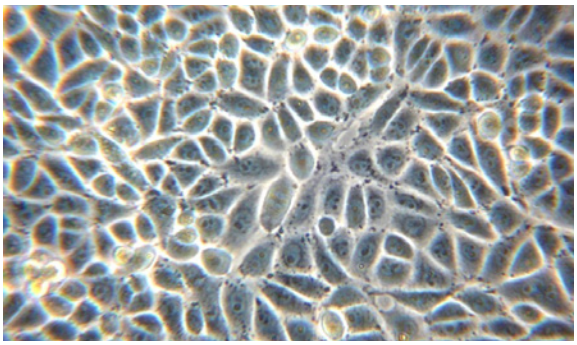

Fig. 4. SPEV cell line morphology, 40× lens

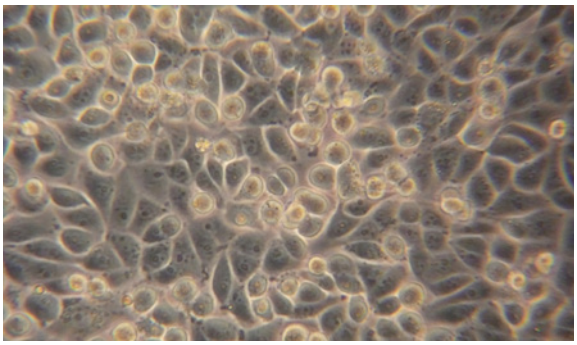

Fig. 6. A_4C_2 cell line morphology, 40× lens

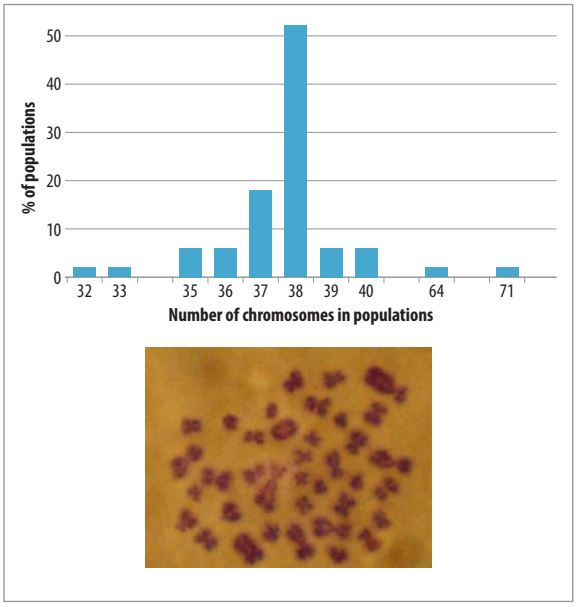

Fig. 5. Karyogram and metaphase plate of SPEV cell line, 38 chromosomes

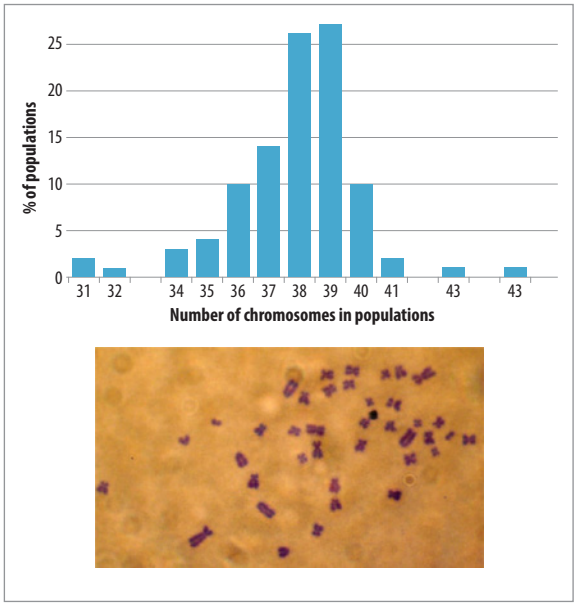

Fig. 7. Karyogram and metaphase plate of A_4C_2 cell line, 39 chromosomes

from stationary-phase monolayer, aggregates can be formed after trypsinization (culture re-seeding), with subsequent formation of colonies during adhesion. The stationary-phase monolayer desintegration is represented by degeneration.

Description of transformed SPEV cell line. SPEV cell line was developed by K. S. Kulikova et al. at the Moscow Research Institute of Virus Preparations in 1959. The cells have a polygonal and epithelial-like shape with rounded nuclei and 2–3 nucleoli (Fig. 4). The modal class – 38 chromosomes, 52% of populations (Fig. 5). Split ratio – 1:4–1:6. This is the only cell line of porcine origin that adapts to growth in suspension and easily cultivated in roller flasks. The cell line is susceptible to FMDV, rinderpest virus (RPV), CSFV, ASFV, transmissible gastroenteritis virus (TGEV), Aujeszky's disease virus (ADV) and other mammal disease agents. Contrary to IB-RS-2, stationary-phase SPEV cell line does not form pseudosyncytium, some cells become suspended and can exist in the suspension for a long time and divide if the limiting proliferation factors are not exhausted.

Description of transformed A_4C_2 cell line. Hybrid A_4C_2 cell line developed through co-cultivation of porcine splenocytes with SPEV cell line by L. P. Dyakonov et al. in the FSC VIEV (Moscow) in 1995 is one of the unique transformed cell lines. Monolayer cells similar to SPEV cell line are of polygonal and epithelium-like shape with rounded nuclei and 2–5 nucleoli (Fig. 6). Modal class – 39 chromosomes (Fig. 7). Its yield during cultivation in rotating flasks is lower than that one of SPEV cell line but similar tendency to cell detachment from the monolayer is observed. A_4C_2 cell line similar to SPEV cell line is susceptible to FMDV, RPV, CSFV, ASFV, TGEV, ADV and other mammal disease agents.

Cells of SPEV line were found to be predominant in hybrid A_4C_2 culture based on morphological and cultural characteristics. The karyotype was transformed, the modal class increased by one chromosome. The long-term co-cultivation with splenocytes appeared to result in proliferation decrease and karyotype transformation. At the same time, the susceptibility to viruses has not changed. Since proliferation intensity was lower than that one of SPEV the split ratio was 1:3; 1:4.

Description of transformed RSK cell line. The cell lines obtained from other institutions are subjected to tests for their morphological and karyological identification at the FGBI "ARRIAH". Thus, RSK cell culture (rabbit skin) obtained from the FSC VIEV (Moscow) was found to be non-susceptible to dermatotropic poxviruses and lumpy skin disease virus. Morphological and karyological examination of the cell line showed its significant similarity to SPEV cell line.

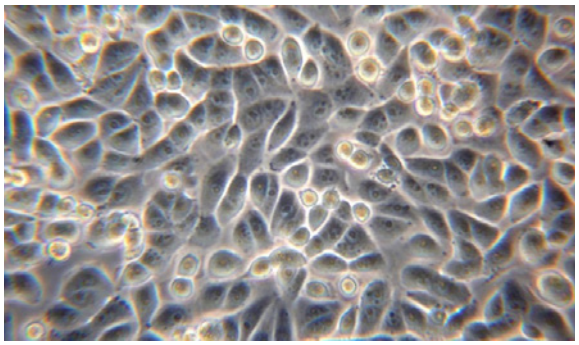

Fig. 8. RSK cell line morphology, 40× lens

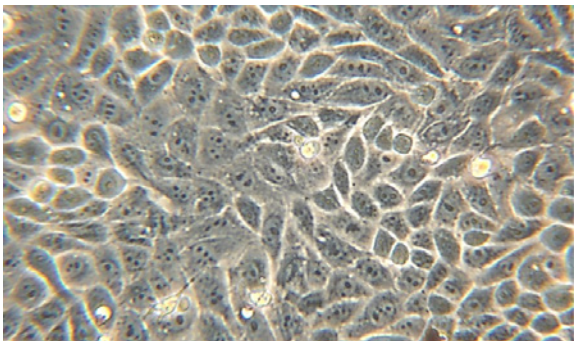

Fig. 10. PPES cell line morphology, 40× lens

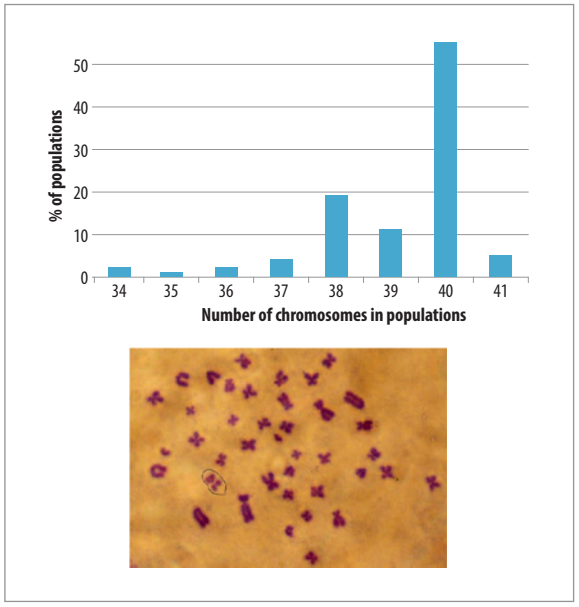

Fig. 9. Karyogram and metaphase plate of RSK cell line, 40 chromosomes

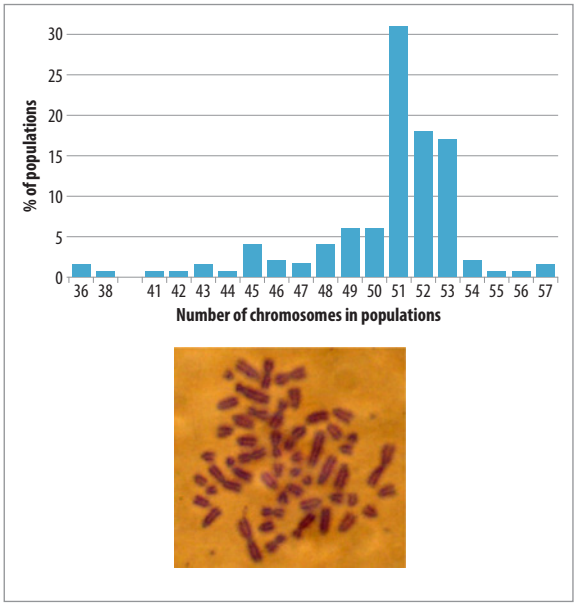

Fig. 11. Karyogram and metaphase plate of PPES cell line, 51 chromosomes

RSK monolayer consists of epithelium-like and rounded cells (Fig. 8). Modal class – 40 chromosomes, 55% of populations (Fig. 9). Chromosome morphology and some markers (medium-size submetacentric with non-stained metacentric bundle) indicate that given transformed cell line is of porcine origin. Split ratio is 1:4; 1:6. The cell line is also suitable for roller cultivation. It is found to be susceptible to TGEV, CSFV, as well as infectious bovine rhinotracheitis, equine rhinopneumonitis, porcine rotavirus infection agents.

We suppose that the rabbit skin cell line was contaminated by SPEV cells, which displaced RSK cells after long-term cultivation. At the same time, its karyotype transformed towards stable hyperploidy.

Description of transformed PPES cell line. PPES (continuous porcine embryo kidney) cell line developed by S. Kh. Khaertynov and G. N. Romanovich at the FSBSI "FCTRBS-ARRVI" (Kazan, Russia) in 1975 is one of the domestic promising and fast-growing transformed cell lines. The monolayer consists of polygonal epithelium-like cells and formed colonies of polylayer (Fig. 10). Modal class – 51 chromosomes, 31% of populations (Fig. 11).

This cell line is characterized by hyperdiploidy. The same tendency is observed in other cell lines of porcine origin. Increase in chromosome number in karyotype have had no effect on the proliferation intensity. On the contrary, the transformed PPES cell line split ratio is 1:4; 1:6. In particular, it is capable of growing in rotating (roller) vessels that is not typical for hyperdiploid cultures. PPES cell line is also characterized by absence of significant mycoplasma and virus contamination and therefore it is capable of long-term continuous passaging. Despite of its good culture and cytomorphological properties, the growth rate of porcine viruses, such as CSFV, TGEV, enterovirus, in this cell culture is low.

Continuous cell lines, PPK and PPK-66b (Kazan line), were prepared from PPES cell line. These cell lines have become more susceptible to porcine disease agents, for example, to porcine parvovirus, after long-term passaging in different media and sera but the cultures are found to be chronically contaminated with mycoplasmas and therefore have limited potential for continuous passaging without treatment with "strong" antibiotics (up to 10 passages). Continuous PPK and PPK-66b (Kazan line) are hyperploid and have modal class of 57 chromosomes in the karyotype. They are not capable of roller cultivation.

Description of transformed PK-15 cell line (FGBI "ARRIAH"). PK-15 cell culture is hyperdiploid. It was developed in the University of California, San-Diego (USA) in 1968. It was obtained by the FGBI "ARRIAH" from Friedrich Loeffler Institute (Germany) in 1986.

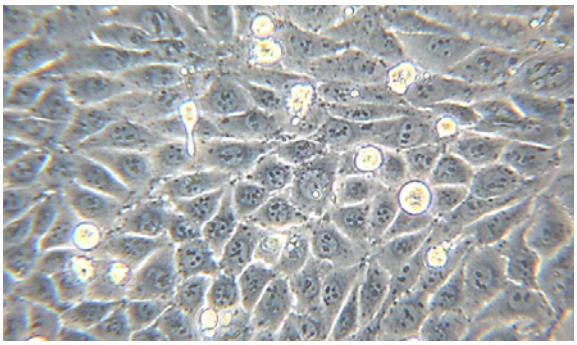

Fig. 12. PK-15 cell line (FGBI "ARRIAH") morphology, 40× lens

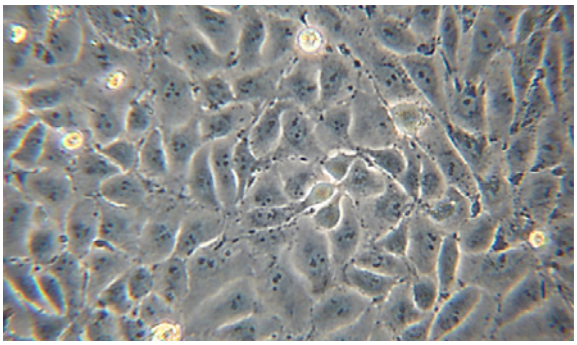

Fig. 14. PK-15 cell line (ATCC) morphology, 40× lens

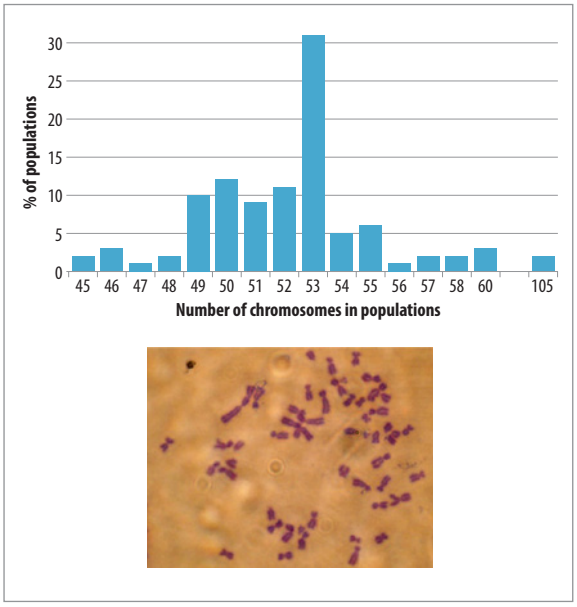

Fig. 13. Karyogram and metaphase plate of PK-15 cell line (FGBI "ARRIAH"), 53 chromosomes

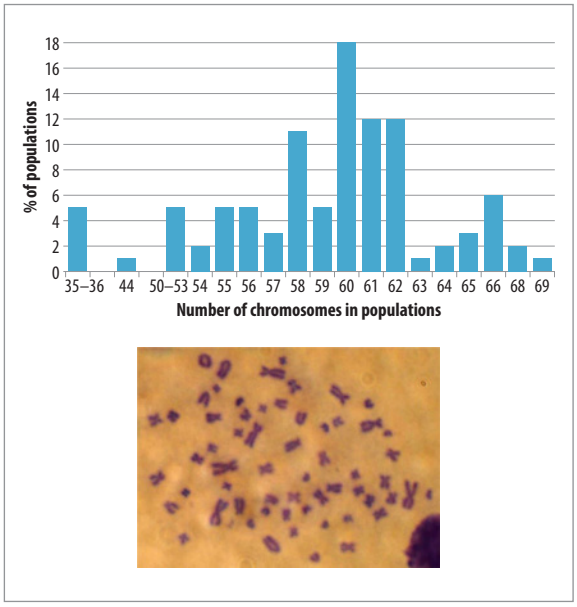

Fig. 15. Karyogram and metaphase plate of PK-15 cell line (ATCC), 60 chromosomes

The cell culture consists of epithelium-like cells, 10–14 mm in size (Fig. 12). Its modal class is 53 chromosomes (Fig. 13). Proliferation intensity is consistent with split ratio of 1:4 and 1:6. This transformed cell line is able to form a complete monolayer in rotating flasks. Its yield reaches 300 mln cells per roller flask (800 cm^2) in 3–4 days. Multinucleated cells (1–2%) form in the overgrown PK-15 monolayers in culture flasks.

The cell line is susceptible to ASFV, CSFV, ADV, vesicular stomatitis virus (VSV), Coxsackievirus, vaccinia virus (VACV), porcine circovirus (PCV), reovirus serotype 2 and 3, adenovirus serotype 4 and 5 and other mammal disease agents.

Description of transformed PK-15 cell culture (ATCC, American Type Culture Collection). PK-15 cell trophovariants obtained from Hungary and the ATCC have a modal class of 60 chromosomes (Fig. 14). Cell and monolayer morphology is identical to those of PK-15 culture described above (Fig. 15). Cell yield and proliferation intensity are also similar. Differences in the karyotype can be accounted for different cultivation conditions in different laboratories. In European laboratories full synthetic media are predominantly used for cultivation, whereas protein hydrolysates are often used at the FGBI "ARRIAH".

The cell line is susceptible to ASFV, CSFV, ADV, VSV, Coxsackievirus, VACV, PCV, reovirus serotype 2 and 3, adenovirus serotype 4 and 5, and other mammal disease agents.

Description of transformed PSGK-30 cell line. Continuous PSGK cell line (continuous Siberian ibex kidney cell line) was developed by I. G. Kekukh, L. P. Kiryukhina, Z. M. Lukyanova in the Research Institute of Agriculture (RIAC) of the MOA of USSR in 1976. There are the following trophovariants and sublines of the said cells: PSGK, PSGK-30, PSGK-60, PSGK-c60 and PSGK-c85.

Several researchers found that this cell culture was contaminated with the cells of porcine origin (V. G. Kostyuchenko, et al., 1985; N. Yu. Smyslova et al., 1996). Currently, PSGK-30 cell line is a highly transformed porcine culture that has formed as a result of contamination of primary Siberian ibex kidney cell culture with more viable transformed SPEV, PPK or PK-15 cell cultures.

PSGK-30 cell culture is one of the most active porcine cell cultures having high proliferation index up to 3.0. The split ratio can reach 1:20. High proliferation potential is achieved by optimization of the nutrient medium containing lactalbumin hydrolysate at concentration of 0.1%. The cell culture monolayer consists of epithelium-like cells (Fig. 16). The karyotype modal class is

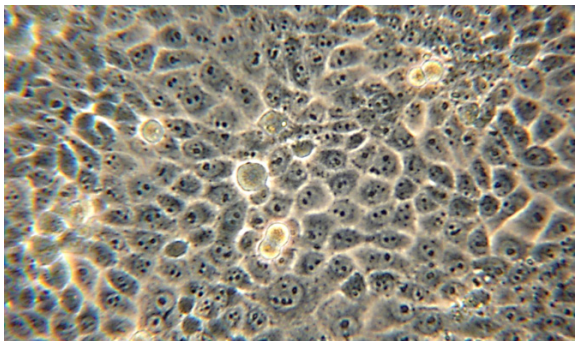

Fig. 16. PSGK-30 cell line morphology, 40× lens

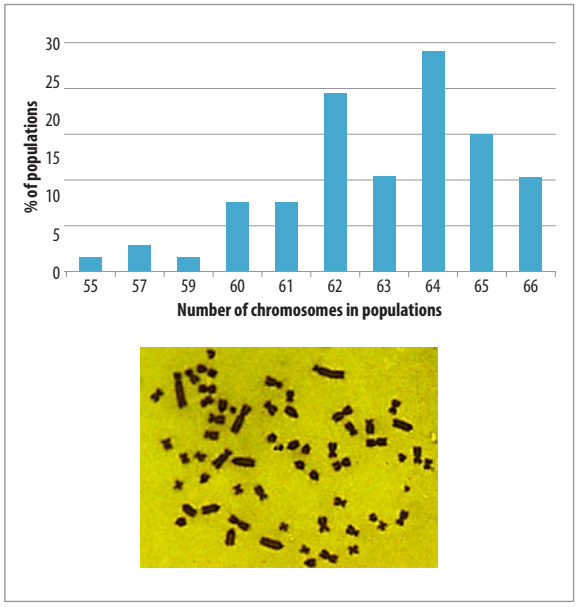

Fig. 17. Karyogram and metaphase plate of PSGK-30 cell line, 64 chromosomes

64 chromosomes, the highest one among the cultures of porcine origin (Fig. 17). The stationary-phase TCL monolayer is so dense that the cells become compacted with formation of epithelium-like polylayer in some layer sections. Conglomerates of the cells often form after trypsinization for reseeding that form growth colonies when sedimented and adhered. The cell yield from one cultivation flask is not higher than 120 mln cells. Transformed PSGK-30 is the main substrate for cultivation of master seed FMDV of all strains used for the vaccine production.

Description of transformed ST cell line. Cell lines derived from other pig organs, porcine testicles and spleen, form a specific group of TCLs. ST (swine testicles) cell line has low proliferative activity. At split ratio of 1:2 nonconfluent monolayer forms for 7–10 days. The cell cycle takes several days. The monolayer consists of large epithelium-like and spindle-shaped cells. Extracellular matrix develops in stationary-phase monolayer (Fig. 18).

Weak proliferative activity makes difficult the collection of the dividing cells used for karyological preparations. But even though its karyotype can be determined as diploid (Fig. 19).

ST cell culture has diploid karyotype with about 38 chromosomes. But it technically impossible to determine its modal class due to absence of sufficient number

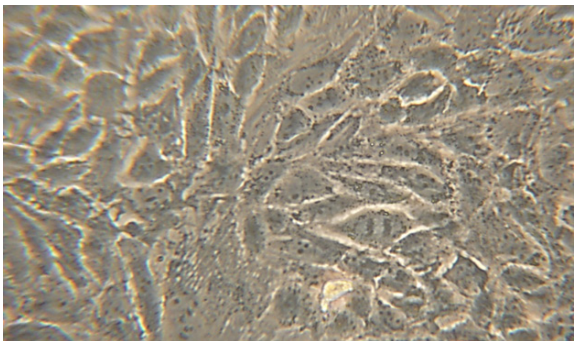

Fig. 18. ST cell line morphology, 40× lens

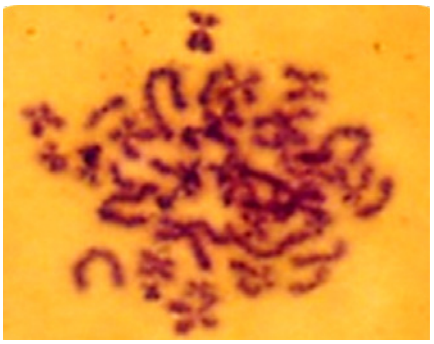

Fig. 19. Metaphase plate of ST cell line, about 38 chromosomes

of metaphase plates when standard karyotyping method is used.

Transformed ST cell line is susceptible to many viruses affecting pigs (*Sus scrofa*), but it is not used for production of diagnostica and specific vaccines due to its low proliferative activity (1:2), the cell monolayer forms within 1–1.5 weeks. Transformed ST cell line yielding potential has not been studied, and this is indicated in the data sheet for this cell line.

Development of diploid transformed SSs cell line. During standard operations for preparation of primary cells from animal organs at the Cell Cultivation Unit of the FGBI "ARRIAH", subcultivation of trypsinized piglet spleen cells using a semi-synthetic nutrient medium + DMEM/F-12, at ratio of 1:2–1:3 supplemented with 10% bovine serum treated with lanthanoides was attempted. This TCL was preliminary named as SSs (*Spleen Sus scrofa* – swine spleen).

At the first passages, the subculture consisted of mixed cell population with a predominating epithelium-like cells, which formed colonies evenly distributed over the entire culture surface of the flasks, then the colonies merged into a confluent cell monolayer. After trypsinization, the cells were large up to 20 μm, characterized by polymorphism and incomplete monolayer confluence (Fig. 20*a*).

As the diploid cells of the swine spleen were further passaged, their proliferative activity increased, the culture became morphologically more homogeneous, consisting of polygonal-shaped epithelium-like cells with clear, well-defined borders and rounded nuclei (with 1–3 nucleoli) and clear sometimes vacuolated cytoplasm.

Atypical dynamics was observed during subcultivation of the swine spleen cell line. The monolayer formed for 10 days by the 40th passage (in fact, by the 40th reseeding).

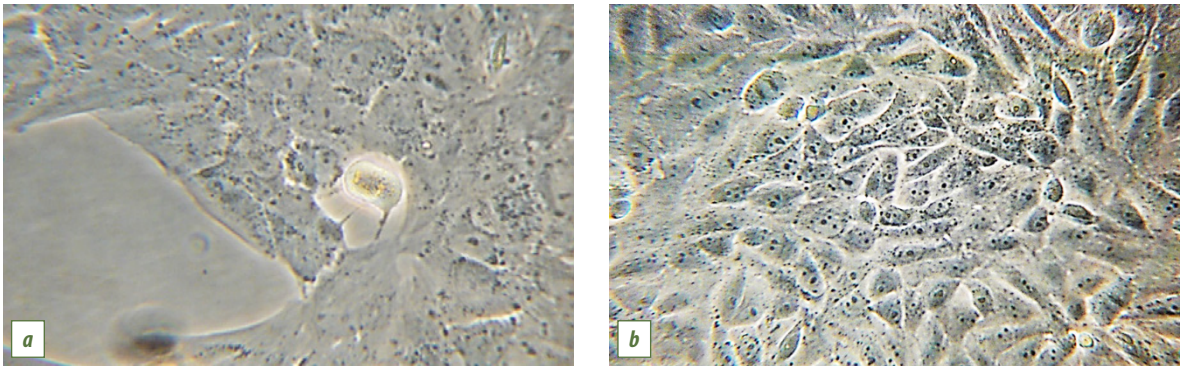

Fig. 20. SSs cell line morphology: a) at 6th passage; b) at 74th passage (40× lens)

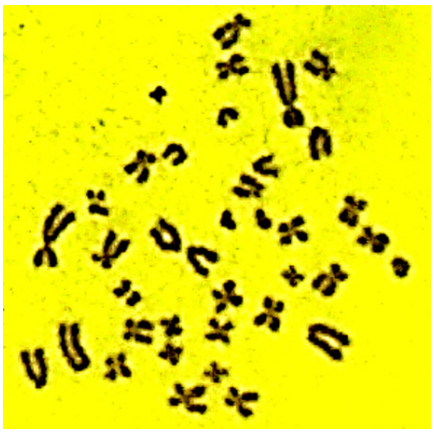

Fig. 21. Diploid metaphase plate of continuous SSs cell line, 74th passage, 38 chromosomes

By this passage, the size of the cells decreased, the density increased, but the number of mitoses remained at the same level. After reseeding (the cells were harvested from the substrate using trypsin-versene solution), sedimentation, adhesion and flattening of the cells took place within 24–36 hours. The medium was significantly acidified after 96 hours, but the monolayer did not form. Almost confluent monolayer formed when the medium was changed.

Transformed SSs cell line maintains reproduction of ASFV, CSFV, TGEV and porcine reproductive and respiratory syndrome virus.

The next step was to intensify proliferation through selection of nutrient media and cultivation conditions as well as to determine cell susceptibility to other animal viruses. SSs cell culture was adapted to minimum essential medium (MEM).

By 74th passage the cell became more morphologically homogenous with predominant epithelium-like cells. Cells in confluent monolayer were 15 μm in size (Fig. 20*b*). Proliferation intensity remained low: split ratio of 1:2; 1:3 after 4–6 days. Developed transformed SSs cell line was susceptible to CSFV, ASFV, ADV, TGEV and other porcine disease agents. The transformed SSs cell line was used for research only due to its low proliferative activity.

Karyological examinations showed that transformed SSs cell population contained mainly diploid cells (Fig. 21).

In our opinion, only cells of stromal origin in the transformed cell line population that *in vivo* did not demonstrate intensive proliferation were transformed towards immortalization.

CONCLUSION

Analysis of cyto-morphological and biological properties of transformed cell lines of porcine origin allows us to conclude that epithelial cells derived from trypcinized porcine kidney can be completely immortalized. All continuous porcine cell lines have similar morphology with predominate epithelium-like forms. Some of them, SPEV, A_4C_2, RSK, tend to adopt a spherical form in suspension. Some TLCs such as PSGK-30 and PPES, form a partial polylayer and characterized by significant monolayer compaction with pseudosyncitium formation. SPEV is the only one pseudodiploid cell culture that tends to grow in suspension, it also grows in rotating culture flasks.

Karyological transformations in different cultures stabilized at certain level. Spontaneous increase in chromosome numbers in main populations of transformed cell lines towards triploidy resulted in stabilization of culture properties and increased proliferation.

Near-diploid cultures (A_4C_2, RSK) also demonstrated stable growth parameters and trend for spherical cell formation in the medium as well as were similar to SPEV cell culture in monolayer quality and cell morphology.

Cell cultures derived from other pig organs: porcine testicles and spleen (ST, SSs) form a specific group. We suppose that these TCLs have originated from stromal cells and are characterized by low proliferative activity and diploid cell populations. Such TCLs are largely of interest to researchers.

REFERENCES

1. Animal cell in culture (methods and implementation in biotechnology). Ed. by L. P. Dyakonov; Russian Academy of Agricultural Sciences. 2nd ed., enlarged. Moscow: Sputnik+; 2009. 652 p. (in Russ.)

2. Belun O. V., Sokova V. A., Kurnosov A. N. Klonirovanie perevivaemoi linii kletok pochki porosenka = Cloning of continuous porcine cell line. *Voprosy veterinarnoi virusologii, mikrobiologii i epizootologii: tezisy dokladov nauchnoi konferentsii = Veterinary virology, microbiology and epidemiology aspects: Abstracts for scientific conference*. Pokrov: VNIIVViM; 1978; 16–17. (in Russ.)

3. Kolbasova O. L. Karyological and cytochemical characterization of continuous cell lines permissive and non-permissive (resistant) to classical swine fever virus: Author's abstract, Thesis for degree of Candidate of Science (Biology). Pokrov; 2000. 21 p. (in Russ.)

4. Filina A. Yu., Gerasimov V. N., Baibikov T. Z., Yegorova A. I. Cultivation of different classical swine fever

virus strains in continuous cell cultures. *Proceedings of the Federal Centre for Animal Health*. 2007; 5: 278–284. eLIBRARY ID: 14454067. (in Russ.)

5. Stroganova I. Ya., Trukhonenko A. A. Use of cell culture in virology: methodical guidelines. Krasnoyarsk: KrasSAU; 2013. 48 p. Available at: http://www.kgau.ru/sveden/2017/ipbivm/mu_360501_9.pdf. (in Russ.)

6. Pankova G. E. Cell population selection and morphologic characteristics of clones from the PP (RS) and PK-15 transplantable swine kidney cell lines. *Tsitologiya*. 1976; 18 (8): 1036–1039. PMID: 988662. (in Russ.)

7. Polyanskya G. G. Cell line generation, main characteristics and variability. *In: Cell cultivation methods*. Saint Petersburg: Polytechnic University; 2008; 22–40. (in Russ.)

8. Ruggli N., Summerfield A., Häni R. E. From pigs to cells: Virulence of classical swine fever virus is predicable in cell cultures. *3R-Info-Bulletin*. 2010; 44. Available at: https://www.forschung3r.ch/data/publications/Ruggli-Bul44.pdf.

9. Kasza L., Shadduck J. A., Christofinis G. J. Establishment, viral susceptibility and biological characteristics of a swine kidney cell line SK-6. *Res. Vet. Sci*. 1972; 13 (1): 46–51. PMID: 4336054.

10. Galnbek T. V. Porcine thyroid and intestinal cell cultures and their use in virology and biotechnology: Author's abstract, Thesis for degree of Candidate of Science (Biology). Moscow; 1991. 25 p. (in Russ.)

11. Dyakonov L. P., Subaev G. H., Galnbek T. V., Taktashev Sh. S., Nepoklonov E. A., Fedorova E. S., Rasulev O. Sh. Methodical recommendations for preparation of cells from porcine thyroid and other organs and their cultivation. Moscow; 1985. 19 p. Available at: https://meganorm.ru/Data2/1/4293737/4293737310.pdf. (in Russ.)

12. Zhdanova N. A. Preparation of suspension continuous porcine testicular cell line and optimization of its cultivation conditions: Author's abstract, Thesis for degree of Candidate of Science (Biology). Pokrov; 2009. 25 p. (in Russ.)

13. Makaryan E. A., Balayan O. R., Dichenskij A. V., Abylkasimov D. A., Degtyarev V. P., Fedotov S. V. Method for obtaining of preparation based on stem cells selected from pigs spleen tissue, for prevention and treatment of infectious and non-infectious diseases of domestic and farm animals. Patent No. 2611205 Russian Federation, Int. Cl. C12N 5/077 (2010.01), C12N 5/0797 (2010.01), A61P 19/02 (2006.01), A61P 31/00 (2006.01). FGBOU VO "Tverskaya gosudarstvennaya selskokhozyajstvennaya akademiya". No. 2015148589. Date of filing: 12.11.2015. Date of publication: 21.02.2017. Bull. No 6. Available at: https://patents.s3.yandex.net/RU2611205C1_20170221.pdf. (in Russ.)

14. Kolbasova O. L., Jurkov S. G., Neverovskaja N. S., Dmitrenko V. V., Lyska V. M. Strain of diploid cells of synovial membrane of young pig *Sus scrofa*, used for virology. Patent No. 2506310 Russian Federation, Int. Cl. C12N 5/077 (2010.01), G01N 33/569 (2006.01). GNU Vserossijskij nauchno-issledovatel'skij institute veterinarnoj virusologii i mikrobiologii Rossel'khozakademii. No. 012131176/10. Date of filing: 23.07.2012. Date of publication: 10.02.2014. Bull. No 4. Available at: https://patents.s3.yandex.net/RU2506310C1_20140210.pdf. (in Russ.)

15. Portugal R., Goatley L. C., Husmann R., Zuckermann F. A., Dixon L. K. A porcine macrophage cell line that supports high levels of replication of OURT88/3, an attenuated strain of African swine fever virus. *Emerg. Microbes Infect*. 2020; 9 (1): 1245–1253. DOI: 10.1080/22221751.2020.1772675.

16. Weingartl H. M., Sabara M., Pasick J., van Moorlehem E., Babiuk L. Continuous porcine cell lines developed from alveolar macrophages: partial characterization and virus susceptibility. *J. Virol. Methods*. 2002; 104 (2): 203–216. DOI: 10.1016/s0166-0934(02)00085-x.

17. Lee Y. J., Park C. K., Nam E., Kim S. H., Lee O. S., Lee du S., Lee C. Generation of a porcine alveolar macrophage cell line for the growth of porcine reproductive and respiratory syndrome virus. *J. Virol. Methods*. 2010; 163 (2): 410–415. DOI: 10.1016/j.jviromet.2009.11.003.

18. Chitko-McKown C. G., Chapes S. K., Miller L. C., Riggs P. K., Ortega M. T., Green B. T., McKown R. D. Development and characterization of two porcine monocyte-derived macrophage cell lines. *Results Immunol*. 2013; 3: 26–32. DOI: 10.1016/j.rinim.2013.03.001.

19. Kadoi K., Tsukise A., Shiba H., Ikeda K., Seki T., Ariga T. Establishment of a swine monocyte cell line. *New Microbiol*. 2001; 24 (3): 243–247. PMID: 11497081.

20. Takenouchi T., Kitani H., Suzuki S., Nakai M., Fuchimoto D. I., Tsukimoto M., et al. Immortalization and characterization of porcine macrophages that had been transduced with lentiviral vectors encoding the SV40 large T antigen and porcine telomerase reverse transcriptase. *Front. Vet. Sci*. 2017; 4:132. DOI: 10.3389/fvets.2017.00132.

21. Takenouchi T., Masujin K., Miyazaki A., Suzuki S., Takagi M., Kokuho T., Uenishi H. Isolation and immortalization of macrophages derived from fetal porcine small intestine and their susceptibility to porcine viral pathogen infections. *Front. Vet. Sci*. 2022; 9:919077. DOI: 10.3389/fvets.2022.919077.

22. Talbot N. C., Paape M., Worku M. Selective expansion and continuous culture of macrophages from adult pig blood. *Vet. Immunol. Immunopathol*. 1998; 64 (2): 173–190. DOI: 10.1016/s0165-2427(98)00128-7.

23. Wardley R. C., Lawman M. J., Hamilton F. The establishment of continuous macrophage cell lines from peripheal blood monocytes. *Immunology*. 1980; 39 (1): 67–73. PMID: 6769783.

24. Graphodatsky A. S., Radzhabli S. I. Farmed and laboratory mammal chromosomes: atlas. Novosibirsk: Nauka; 1988. 127 p. (in Russ.)

25. Dyakonov L. P., Galnbek T. V., Akinshina G. T., Abdrakhmanov I. K., Samuilenko A. Ya., Dagadanova A. V., et al. Specialized collection of continuous somatic cell lines of livestock and game animals, RCCC(V) (Livestock animals Russian Academy of Agricultural Sciences): catalogue. 2nd ed., enlarged. Moscow: VIEV; 2006. 115 p. (in Russ.)

26. Kuleshov K. V. Species identification of cell cultures with molecular-genetic methods: Author's abstract, Thesis for degree of Candidate of Science (Biology). Shchyolkovo; 2009. 24 p. (in Russ.)

27. Freshney R. Ian. Culture of Animal Cells: A Manual of Basic Technique. Hoboken: John Wiley and Sons, Inc.; 2005. 672 p.

28. Moorhead P. S., Nowell P. C., Mellman W. J., Battips D. M., Hungerford D. A. Chromosome preparations of leukocytes cultured from human peripheral blood. *Exp. Cell. Res*. 1960; 20: 613–616. DOI: 10.1016/0014-4827(60)90138-5.

29. Mamaeva S. E. Chromosomal analysis of cultured cells. *In: Methods of Cell Culture: Collection of Scientific*

Papers. Ed. by G. P. Pinaev. Leningrad: Nauka; 1988; 78–79. (in Russ.)

30. Prudnikova E. Ju., Balysheva V. I., Gal'nbek T. V., Balyshev V. M. Finite hybrid subline of cells A_4C_2/9k *Sus scrofa*, used for virological studies of African swine fever virus. Patent No. 2545720 Russian Federation, Int. Cl. C12N 5/073 (2010.01), C12N 7/00 (2006.01). GNU Vserossijskij nauchno-issledovatel'skij institute veterinarnoj virusologii i mikrobiologii Rossel'khozakademii. No. 2013153452/10. Date of filing: 03.12.2013. Date of publication: 04.10.2015. Bull. No. 10. Available at: https://patents.s3.yandex.net/RU2545720C1_20150410.pdf. (in Russ.)

31. D'jakonov L. P., Majdzhi E. V., Gerasimov V. N., Gal'nbek T. V., Dudar L. N., Soldatova N. V., Fedorova E. E., Egorova A. I. Strain of intraspecies hybrid cells of *Suis domestica* used for isolation and cultivation of swine classic plague virus. Patent No. 2082758 Russian Federation, Int. Cl. C12N 5/06. No. 94026140/13. Date of filing: 14.07.1994. Date of publication: 27.06.1996. Available at: https://patents.s3.yandex.net/RU94026140A1_19960710.pdf. (in Russ.)

32. The European Collection of Authenticated Cell Cultures. Cell Lines and Hybridomas. Available at: https://www.culturecollections.org.uk/products/celllines/index.aspx (date of access: 12.08.2022).

33. Pan I. C., Shimizu M., Hess W. R. Replication of African swine fever virus in cell cultures. *Am. J. Vet. Res*. 1980; 41 (9): 1357–1367. PMID: 7004279.

Received 18.01.2023
Revised 01.02.2023
Accepted 09.02.2023

INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Boris L. Manin, Candidate of Science (Biology), Vladimir, Russia; *https://orcid.org/0000-0002-5263-1491, e-mail: manin.boria@yandex.ru.*

Elena A. Trofimova, Head of Sector, Cell Cultivation Unit, FGBI "ARRIAH", Vladimir, Russia; *e-mail: trofimova_ea@arriah.ru.*

Vera L. Gavrilova, Candidate of Science (Biology), Researcher, Reference Laboratory for African Swine Fever, FGBI "ARRIAH", Vladimir, Russia; *https://orcid.org/0000-0001-5843-2779, e-mail: gavrilova_vl@arriah.ru.*

Olga S. Puzankova, Candidate of Science (Veterinary Medicine), Senior Researcher, Reference Laboratory for African Swine Fever, FGBI "ARRIAH", Vladimir, Russia; *https://orcid.org/0000-0003-1584-3169, e-mail: puzankova@arriah.ru.*

Манин Борис Леонидович, кандидат биологических наук, г. Владимир, Россия; *https://orcid.org/0000-0002-5263-1491, e-mail: manin.boria@yandex.ru.*

Трофимова Елена Александровна, заведующий сектором отдела культуры клеток ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *e-mail: trofimova_ea@arriah.ru.*

Гаврилова Вера Львовна, кандидат биологических наук, научный сотрудник референтной лаборатории по африканской чуме свиней ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *https://orcid.org/0000-0001-5843-2779, e-mail: gavrilova_vl@arriah.ru.*

Пузанкова Ольга Сергеевна, кандидат ветеринарных наук, старший научный сотрудник референтной лаборатории по африканской чуме свиней ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *https://orcid.org/0000-0003-1584-3169, e-mail: puzankova@arriah.ru.*

DOI: 10.29326/2304-196X-2023-12-1-23-28

Study of cultural properties of canine enteric coronavirus isolate in different cell cultures

A. A. Komarova, T. S. Galkina
FGBI "Federal Centre for Animal Health" (FGBI "ARRIAH"), Vladimir, Russia

SUMMARY

The etiology of the diseases affecting gastrointestinal tract of dogs is variable. The second most important enteric viral pathogen in the world after parvovirus is coronavirus (CCoV). Vast studies by scientists from different countries evidence the ubiquitous spread of coronavirus infection in dog populations. In this regard, the prevention of this disease is still an acute problem; firstly this means the development of effective vaccines, which can provide a reliable protection from the infection. The aim of this work was to study the CCoV isolate cultural properties, the selection of cell cultures most suitable for its reproduction and optimization of virus cultivation parameters in sensitive cell lines. The CCoV isolate recovered from the pathological material of a dead puppy with enteritis symptoms was used in the study. Seven continuous and two primary trypsinized cell cultures were tested during the study and it was established that the most sensitive cell culture for the reproduction of this isolate was continuous Crandell-Rees Feline Kidney Cells (CRFK) in which a typical cytopathic effect was noted on Day 2 of the cultivation. Virus infectivity titer in this culture was 3.58 ± 0.14 lg $TCID_{50}/cm^3$. It was established that such cell lines as feline spleen cells (FS) and primary trypsinized kitten spleen cells (KS) are also sensitive to the CCoV isolate but less than CRFK cells. Effect of such parameters as multiplicity of infection, cultivation time, inoculation technique, adsorption period and cell line age on coronavirus growth rate was studied for some selected cell cultures. It was concluded that CRFK and FS cells can be used for the propagation of viral material to develop diagnostic tools and vaccines against canine enteric coronavirus.

Keywords: canine coronavirus (CCoV), canine enteric coronavirus, canine intestinal infections, virus culture, continuous cell lines, primary trypsinized cells

Acknowledgements: The authors are deeply grateful to the employees of the FGBI "ARRIAH" Cell Culture Sector.

For citation: Komarova A. A., Galkina T. S. Study of cultural properties of canine enteric coronavirus isolate in different cell cultures. *Veterinary Science Today*. 2023; 12 (1): 23–28. DOI: 10.29326/2304-196X-2023-12-1-23-28.

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

For correspondence: Anna A. Komarova, Post-Graduate Student, Leading Veterinarian, Department for Pet Disease Prevention, FGBI "ARRIAH", 600901, Russia, Vladimir, Yur'evets, *e-mail: komarova_aa@arriah.ru.*

УДК 619:578.834.1:578.835.1:636.7:57.082.26

Изучение культуральных свойств изолята возбудителя коронавирусного энтерита собак в различных культурах клеток

А. А. Комарова, Т. С. Галкина
ФГБУ «Федеральный центр охраны здоровья животных» (ФГБУ «ВНИИЗЖ»), г. Владимир, Россия

РЕЗЮМЕ

Этиология заболеваний, сопровождающихся поражением желудочно-кишечного тракта у собак, многообразна. Вторым наиболее распространенным у собак энтеропатогеном вирусной природы в мире после парвовируса является коронавирус CCoV. Обширные исследования, проведенные учеными из разных стран, доказывают повсеместное распространение коронавирусной инфекции в популяции собак. В связи с этим не теряют актуальности вопросы профилактики данного заболевания, в первую очередь изготовление эффективных иммунобиологических препаратов, обеспечивающих надежную защиту от данной инфекции. Целью настоящей работы являлось изучение культуральных свойств изолята CCoV, выбор наиболее подходящей для его репродукции клеточной системы и оптимизация параметров культивирования вируса в чувствительных клеточных линиях. В опытах использовали изолят CCoV, выделенный из патологического материала, полученного от погибшего щенка с симптомами энтерита. В ходе исследований было испытано семь перевиваемых и две первично трипсинизированные культуры клеток и установлено, что наиболее чувствительной клеточной системой для репродукции данного изолята является перевиваемая культура клеток почки кошки CRFK, в которой на 2-е сут культивирования отмечалось характерное цитопатическое действие. Титр инфекционной активности вируса в данной культуре был на уровне 3,58 ± 0,14 lg $ТЦД_{50}/см^3$. Определено, что такие клеточные линии, как перевиваемая культура клеток селезенки кошки (FS) и первично трипсинизированная культура клеток селезенки котенка (СК), также чувствительны к изоляту CCoV, но в меньшей степени, чем CRFK. Также в ходе опытов изучалось влияние на накопление коронавируса таких показателей, как множественность заражения, время культивирования, способ заражения, срок предварительного контакта (адсорбции) вируса с культурой клеток и возраст клеточной системы для некоторых выбранных культур клеток. Сделан вывод, что линии клеток CRFK и FS могут быть использованы для получения вирусного материала с целью разработки средств диагностики и специфической профилактики коронавирусного энтерита собак.

Ключевые слова: коронавирус собак (CCoV), коронавирусный энтерит собак, кишечные инфекции собак, культивирование вирусов, перевиваемые культуры клеток, первично трипсинизированные культуры клеток

Благодарности: Авторы выражают глубокую благодарность сотрудникам сектора культуры клеток ФГБУ «ВНИИЗЖ».

Для цитирования: Комарова А. А., Галкина Т. С. Изучение культуральных свойств изолята возбудителя коронавирусного энтерита собак в различных культурах клеток. *Ветеринария сегодня*. 2023; 12 (1): 23–28. DOI: 10.29326/2304-196X-2023-12-1-23-28.

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

Для корреспонденции: Комарова Анна Александровна, аспирант, ведущий ветеринарный врач отдела профилактики болезней мелких домашних животных ФГБУ «ВНИИЗЖ», 600901, Россия, г. Владимир, мкр. Юрьевец, *e-mail: komarova_aa@arriah.ru.*

INTRODUCTION

Symptoms of gastrointestinal disorders in puppies are a frequent reason for visits to veterinary clinics. The etiological factors responsible for their occurrence are diverse. Canine coronavirus (CCoV) is the second most common enteric pathogen in the world after canine parvovirus (CPV) responsible for the viral diseases [1–3].

Serological and virological tests have shown that CCoV is widespread in the dog population, especially in kennels and animal shelters [4]. It is also reported in wild canines, including foxes, raccoon dogs and wolves [5–7]. Since the late 1990s and in the 2000s, scientists from different countries have repeatedly identified highly virulent strains of the coronavirus in dogs [8–10]. Pantropic CCoV (pCCoV) isolated in Italy in 2005 causes systemic disease in puppies followed by death [11]. In 2008, pantropic CCoV-associated disease was reported in France and Belgium [12], in 2010 in Greece [13]. In 2020, a large number of gastroenteritis cases caused by canine coronavirus infection were reported in the UK [14, 15].

The results of molecular tests conducted in Italy and Australia suggest that the virus is changing and its virulence is gradually increasing [16]. In this regard, the development of vaccines that provide reliable protection against new, including pantropic, strains of canine coronavirus remains critical, since existing vaccines have shown limited effectiveness [17].

The purpose of this work was to study the cultural properties of the canine coronavirus isolate in primary and continuous cell cultures for further use in immunobiological production.

MATERIALS AND METHODS

The CCoV isolate recovered from pathological samples from a dead puppy with enteritis symptoms was used in the tests.

To study the cultural properties of the virus, the following cell cultures were selected:

– trypsinized primary cells and subcultures: kitten kidney (KK) cells and kitten spleen (KS) cells;

– continuous cell cultures: feline kidneys (CRFK), feline spleen (FS), canine kidneys (MDCK), as well as African green monkey kidney (Vero-76), mouse neuroblastoma (Neuro-2a), porcine kidney (IB-RS-2) and domestic goat gonad (YADK-04).

Cell cultures were obtained from the Cell Culture Sector of the FGBI "ARRIAH".

Two methods of cell culture inoculation were used:

– inoculation of a cell culture monolayer: the virus was inoculated in a completely formed monolayer of primary trypsinized and continuous cell cultures;

– inoculation of cell suspension: the virus was inoculated in cell suspension during seeding.

The virus was cultured in 25 cm^2 polystyrene cell culture flasks (T25).

Before inoculation of the cell monolayer with the virus, the nutrient medium was removed from the flasks. Then the virus was inoculated and cell culture flasks were incubated for 60 minutes in CO_2 incubator at (37.0 ± 0.5) °C for contact (adsorption) of the virus to the cell monolayer. After contact (adsorption), a maintenance medium was added to the flasks. For maintenance a semi-synthetic nutrient medium was used, prepared according to the formula by the FGBI "ARRIAH", with the addition of 2% fetal bovine serum, 2% L-glutamine, streptomycin (100 μg/cm^3) and penicillin (100 U/cm^3).

At the end of the cultivation time, each flask was frozen at a temperature of minus (45 ± 5) °C and thawed at (20 ± 2) °C. Monolayer was disaggregated by periodic shaking of the flask, then samples were taken from the flasks to determine the titer of the CCoV isolate infectivity.

The CCoV isolate infectivity was determined in CRFK cell line grown in 96-well plates Costar® (Corning, USA) by microtitration using a generally accepted procedure. Microplates were examined daily using Olympus SKX53 inverted microscope (Japan), the number of wells with a typical cytopathic effect (CPE) was recorded. The infectivity titer was calculated using the Kerber method and expressed in lg $TCID_{50}$/cm^3. Samples were additionally tested for canine enteric coronavirus using commercial chromatographic immunoassay kits Asan Easy Test® CCV Ag (Asan Pharmaceutical Co., Ltd., Republic of Korea).

RESULTS AND DISCUSSION

To study the susceptibility of various cell cultures to the CCoV isolate, five serial passages were made in continuous (CRFK, MDCK, FS, Vero-76, YADK-04, Neuro-2a, IB-RS-2) and trypsinized primary cells and sub-cultures (KK, KS). The presence of the CCoV antigen in the culture fluid after each passage was determined

Table 1
Sensitivity of different continuous cell lines to the CCoV isolate ($n = 3$)

Passage	Infectivity titer, lg $TCID_{50}/cm^3$						
	CRFK	MDCK	FS	Vero-76	YADK-04	Neuro-2a	IB-RS-2
1	3.08 ± 0.14	1.25 ± 0.25	2.50 ± 0.25	< 1.0	< 1.0	< 1.0	< 1.0
2	3.33 ± 0.14	< 1.0	2.92 ± 0.14	< 1.0	< 1.0	< 1.0	< 1.0
3	3.58 ± 0.14	< 1.0	3.08 ± 0.14	–	–	–	–
4	3.58 ± 0.14	–*	3.08 ± 0.14	–	–	–	–
5	3.58 ± 0.14	–*	3.08 ± 0.14	–	–	–	–

* not tested.

by immunochromatographic test kits (ICT). The results of the tests performed are given in Table 1.

It was found that CCoV isolate actively replicated and accumulated only in CRFK and FS lines. In other cell cultures, CCoV did not replicate. The maximum viral growth rate was observed from Passage 3 in CRFK cell culture (3.58 ± 0.14 lg $TCID_{50}/cm^3$), and microscopic examination revealed typical CPE in the cell culture starting from Passage 1 (Fig. 1–4). It was manifested by cell rounding, cytoplasm degeneration, symplast formation and gradual destruction of the monolayer.

In FS cell lines, the virus accumulated in smaller quantities, starting from Passage 3 the titer was 3.08 ± 0.14 lg $TCID_{50}/cm^3$, no typical CPE was observed during microscopic examination. When culturing the virus in MDCK cell line, minimal viral growth rate in Passage 1 was noted equal to 1.25 ± 0.25 lg $TCID_{50}/cm^3$, however, further microtitration in CRFK cell line did not reveal a typical CPE, and at Passage 4 ICT gave a negative result. In other cell lines, the passaging of the CCoV isolate turned out to be impractical after Passage 2 since microtitration in CRFK-containing wells showed no typical CPE, and the ICT demonstrated the lack of the viral antigen.

Results of the CCoV isolate cultural properties analysis in KS and KK trypsinized primary and subculture cells are presented in Table 2.

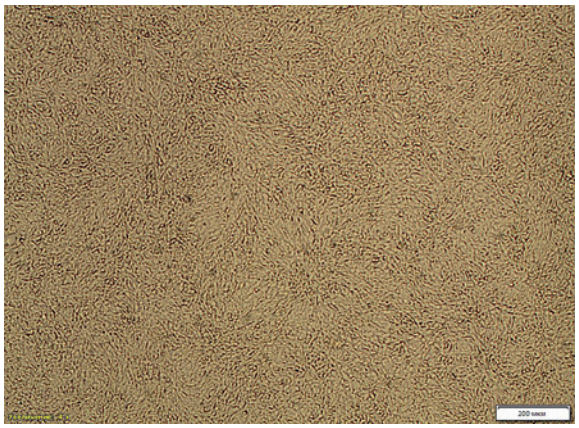

Fig. 1. CCoV not-inoculated CRFK monolayer after 3 days (40× magnification)

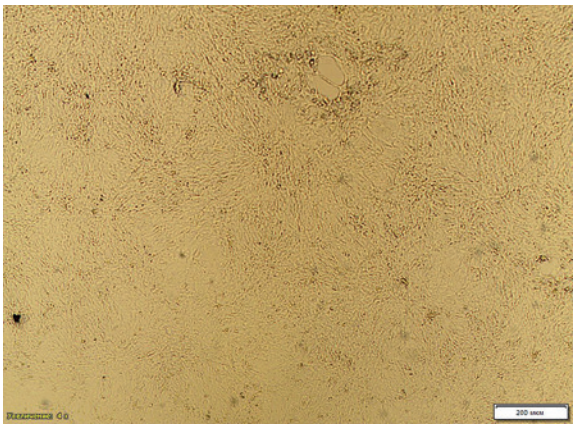

Fig. 2. CRFK monolayer 2 days post inoculation with CCoV isolate (40× magnification)

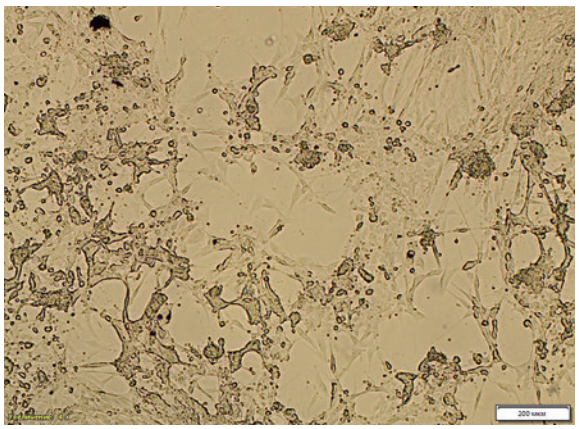

Fig. 3. CRFK monolayer 3 days post inoculation with CCoV isolate (40× magnification)

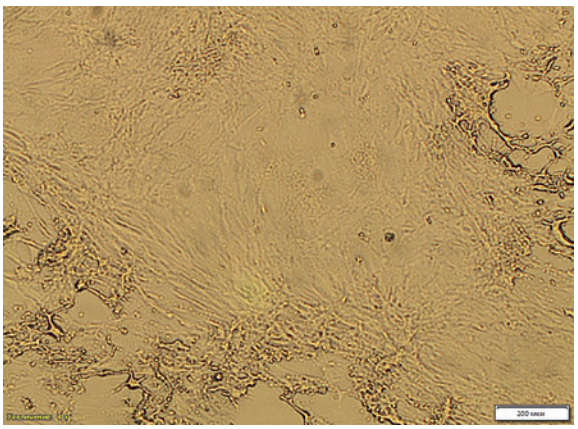

Fig. 4. CRFK monolayer 3 days post inoculation with CCoV isolate (100× magnification)

Virus cultivation in KK trypsinized primary and subculture cells led to a gradual decrease in its infectivity. At Passage 2, ICT showed a positive result for the canine enteric coronavirus antigen. However, the titration in CRFK cell line did not demonstrate typical CPE in the plate. At Passage 3, the ICT result was negative, therefore, further cultivation of the virus in the KK trypsinized primary and subculture cells became impractical. Cultivation of CCoV isolate in KS trypsinized primary and subculture cells resulted in a gradual viral growth, with the infectivity titer of 3.42 ± 0.14 lg $TCID_{50}/cm^3$ at Passage 5. At the same time, no typical CPE was observed in this cell culture.

It should be noted that continuous CRFK and FS cell lines can be considered most suitable of all the tested cell cultures for this CCoV isolate reproduction.

Table 2
CCoV isolate reproduction in primary trypsinized and subculture cells (KS and KK), *n* = 3

Cell culture	Passage	Cultivation time, h	Infectivity titer, lg $TCID_{50}/cm^3$	ICT result
KS	1	72	1.50 ± 0.25	+
	2	72	2.58 ± 0.14	+
	3	72	2.92 ± 0.14	+
	4	72	2.92 ± 0.14	+
	5	72	3.42 ± 0.14	+
KK	1	72	2.33 ± 0.14	+
	2	72	< 1.0	+
	3	72	–*	–

* not tested.

Table 3
CCoV isolate growth dynamics depending on culture time in continuous, primary and subculture cells (*n* = 3)

Cell culture	Infectivity titer, lg $TCID_{50}/cm^3$			
	48 hours	72 hours	96 hours	120 hours
FS	2.75 ± 0.25	3.17 ± 0.14	2.83 ± 0.29	2.67 ± 0.14
KS	3.00 ± 0.25	3.42 ± 0.14	3.17 ± 0.14	3.08 ± 0.29

Table 4
Effect of multiplicity of infection on CCoV isolate infectivity (*n* = 3)

Cell culture	MOI, $TCID_{50}$/cell	Cultivation time, hours	Infectivity titer, lg $TCID_{50}/cm^3$
CRFK	0.1	48	3.25 ± 0.25
	0.01	72	3.58 ± 0.14
	0.001	96	1.50 ± 0.25
	0.0001	96	1.08 ± 0.14
FS	0.1	72	3.17 ± 0.14
	0.01	72	3.08 ± 0.14
	0.001	72	2.92 ± 0.38
	0.0001	72	2.08 ± 0.14

MOI – multiplicity of infection.

Further studies included the analysis of the effect of the following factors on viral growth rate: the time of virus cultivation in cell cultures in which the CCoV isolate did not produce CPE, the multiplicity of infection, inoculation method, the period of viral contact (adsorption) to cells and the age of cell lines.

To study the dynamics of the CCoV isolate growth rate depending on the cultivation time, cell cultures in which the CCoV isolate did not produce typical CPE and the monolayer remained compact throughout the entire cultivation were used. The above mentioned cultures included continuous FS cell line and KS trypsinized primary and subculture cells. CCoV isolate was cultured for 48, 72, 96 and 120 hours. The results of the tests performed are given in Table 3.

Analysis of the results showed that the cultivation of the CCoV isolate for 48 hours is insufficient for its maximum growth rate, the infectivity titer after Day 2 was the lowest. It was found that the most optimal time for coronavirus cultivation is 72 hours (3 days). The infectivity titer of the CCoV isolate was the highest and was equal to 3.17 ± 0.14 and 3.42 ± 0.14 lg $TCID_{50}/cm^3$ in FS and KS cell lines, respectively. With further cultivation after 96 and 120 hours in both cell cultures, the infectivity titer of the virus decreased.

To study the effect of the multiplicity of infection on the infectivity of the CCoV isolate, the following infection doses were used: 0.0001; 0.001; 0.01 and 0.1 $TCID_{50}$/cell. The tests were performed using two continuous CRFK and FS cell lines. Incubation of the virus in CRFK cell culture was stopped when 80% of the monolayer was destructed and cells detached from the glass. In FS cell lines, the virus was incubated for 72 hours, since earlier experiments showed that this time is optimal for maximum growth rate of the CCoV isolate in the specified cell culture. The results of the tests performed are given in Table 4.

It found that the CCoV isolate infectivity in CRFK cell culture with a multiplicity of infection of 0.01 $TCID_{50}$/cell was 3.58 ± 0.14 $TCID_{50}$/cell. At lower inoculation doses (0.0001 and 0.001 $TCID_{50}$/cell), the infectivity decreased significantly, and the time of CPE manifestation increased to 96 hours. At a multiplicity of infection of 0.1 $TCID_{50}$/cell, a faster CPE manifestation and 80% destruction of the monolayer was observed. However, the titer of the virus was lower and was at the level of 3.25 ± 0.25 lg $TCID_{50}/cm^3$, which is probably due to too rapid destruction of the monolayer, as a result of which the virus did not have enough time to accumulate in maximum concentration. Thus, the optimal multiplicity of infection of CRFK cell line with the CCoV isolate is 0.01 $TCID_{50}$/cell.

The infectivity of CCoV isolate in FS cell culture at multiplicity of infection of 0.1 and 0.01 $TCID_{50}$/cell was 3.17 ± 0.14 and 3.08 ± 0.14 lg $TCID_{50}/cm^3$, respectively. At lower inoculation doses (0.0001 and 0.001 $TCID_{50}$/cell), the infectivity of the virus decreased, therefore it is advised to inoculate FS cell line with a dose of 0.01 $TCID_{50}$/cell.

At the next stage of the work, two methods of inoculation of CRFK and FS cell lines were compared: direct inoculation of cell suspension with the virus and adsorption on the formed cell monolayer for an hour.

The results presented in Table 5 demonstrate that the titer of the CCoV isolate infectivity was maximal when it was

inoculated on the cell monolayer. In CRFK and FS cell lines, it was 3.58 ± 0.14 and 3.17 ± 0.14 lg $TCID_{50}/cm^3$, respectively. Inoculation of CRFK and FS cell suspension gave a lower infectivity of the virus.

Also in this study, the effect of such a factor as the time of preliminary contact (adsorption) of the virus with the CRFK monolayer on the dynamics of CCoV isolate accumulation was analysed. The results of the tests performed are given in Table 6.

It was found that the cultivation of the CCoV isolate without prior contact (adsorption) with the CRFK monolayer was accompanied by a low viral growth, the infectivity titer was at the level of 2.42 ± 0.14 lg $TCID_{50}/cm^3$. With an increase in the time of preliminary contact (adsorption) of the virus with the monolayer, the infectivity titer of the CCoV isolate gradually increased from 3.08 ± 0.14 lg $TCID_{50}/cm^3$ if adsorption lasted for 30 minutes and to 3.33 ± 0.14 lg $TCID_{50}/cm^3$ with 60 minute adsorption. Subsequently an increase in the adsorption time to 90 minutes led to a slight decrease in the infectivity. Thus, 60 minutes can be considered the optimal time of the viral contact (adsorption) with the monolayer.

The final stage of the work was to study the effect of the cell culture age on the CCoV isolate infectivity. For this purpose, CRFK cell line was chosen, since this is the line in which the virus accumulates in maximum concentration and manifests visible CPE. For testing, a cell culture with a fully formed monolayer was used.

As it can be seen from Table 7, inoculation of the CCoV isolate on a one- and two-day CRFK monolayer, the infectivity titers of the virus did not differ and was equal to 3.08 ± 0.14 lg $TCID_{50}/cm^3$. When the virus was inoculated into three-day CRFK cells, the infectivity of the CCoV isolate increased insignificantly and amounted to 3.17 ± 0.14 lg $TCID_{50}/cm^3$. When a four-day cell culture was inoculated with the CCoV isolate, the growing of the virus decreased to 2.92 ± 0.14 lg $TCID_{50}/cm^3$, and the cultivation time increased to 96 hours. A decrease in the viral infectivity may be associated with aging of the CRFK cells and with a slowdown of metabolic processes in cells. Thus, to accumulate the CCoV isolate in the maximum amount, it is advisable to use CRFK cells with a fully formed 1–3-day monolayer.

CONCLUSION

In this work, the possibility of the CCoV isolate reproduction in various continuous and trypsinized primary cell lines of homologous and heterologous origin was studied. The study of the susceptibility of continuous cells to CCoV isolate revealed that CRFK and FS cell lines are effective for the preparation of a highly active viral suspension. These cell lines can be used to prepare viral material for developing diagnostic tools and vaccines against canine enteric coronavirus. Studies have shown that the optimal dose of CRFK cell inoculation is 0.01 $TCID_{50}$/cell, the viral infectivity titer was 3.58 ± 0.14 lg $TCID_{50}/cm^3$. For FS cells, it is advisable to use an infection dose of 0.01 $TCID_{50}$/cell. It was established that the virus accumulates in maximum amounts when CRFK cells are inoculated into a fully formed 1–3-day cell monolayer and cultured for 48–72 hours. For FS cell culture, the optimal conditions included inoculation of the virus in the formed cell monolayer and cultivation for 72 hours.

Table 5
Effect of inoculation technique on CCoV isolate infectivity in continuous cells ($n = 3$)

Inoculation method	MOI, $TCID_{50}$/cell	Cultivation time, hours	Cell culture	Infectivity titer, lg $TCID_{50}/cm^3$
Monolayer	0.01	72	CRFK	3.58 ± 0.14
Suspension	0.01	48		3.17 ± 0.14
Monolayer	0.01	72	FS	3.17 ± 0.14
Suspension	0.01	72		2.75 ± 0.25

MOI – multiplicity of infection.

Table 6
Effect of contact (adsorption) period on CCoV isolate growth rate in CRFK cells ($n = 3$)

Parameters	Adsorption time, minutes			
	no contact (adsorption)	30	60	90
MOI, $TCID_{50}$/cell	0.01			
Infectivity titer, lg $TCID_{50}/cm^3$	2.42 ± 0.14	3.08 ± 0.14	3.33 ± 0.14	3.25 ± 0.25

MOI – multiplicity of infection.

Table 7
Effect of CRFK cell age on infectivity of CCoV isolate ($n = 3$)

Age of culture, days	Time of cultivation, hours	Infectivity titer, lg $TCID_{50}/cm^3$
1	48	3.08 ± 0.14
2	72	3.08 ± 0.14
3	72	3.17 ± 0.14
4	96	2.92 ± 0.14

REFERENCES

1. Alves C. D. B. T., Granados O. F. O., Budaszewski R. D. F., Streck A. F., Weber M. N., Cibulski S. P., et al. Identification of enteric viruses circulating in a dog population with low vaccine coverage. *Braz. J. Microbiol*. 2018; 49 (4): 790–794. DOI: 10.1016/j.bjm.2018.02.006.

2. McElligott S., Collins P. J., Sleator R. D., Martella V., Decaro N., Buonavoglia C., O'Shea H. Detection and genetic characterization of canine parvoviruses and coronaviruses in southern Ireland. *Arch. Virol*. 2011; 156 (3): 495–503. DOI: 10.1007/s00705-010-0861-3.

3. Decaro N., Desario C., Billi M., Mari V., Elia G., Cavalli A., et al. Western European epidemiological survey for parvovirus and coronavirus infections in dogs. *Vet. J*. 2011; 187 (2): 195–199. DOI: 10.1016/j.tvjl.2009.10.027.

4. Decaro N., Buonavoglia C. An update on canine coronaviruses: viral evolution and pathobiology. *Vet. Microbiol*. 2008; 132 (3–4): 221–234. DOI: 10.1016/j.vetmic.2008.06.007.

5. Ma G. G., Lu C. P. Two genotypes of canine coronavirus simultaneously detected in the fecal samples of healthy foxes and raccoon dogs. *Wei Sheng Wu Xue Bao*. 2005; 45 (2): 305–308. PMID: 15989282. (in Chinese)

6. Rosa G. M., Santos N., Grøndahl-Rosado R., Fonseca F. P., Tavares L., Neto I., et al. Unveiling patterns of viral

pathogen infection in free-ranging carnivores of northern Portugal using a complementary methodological approach. *Comp. Immunol. Microbiol. Infect. Dis*. 2020; 69:101432. DOI: 10.1016/j.cimid.2020.101432.

7. Zarnke R. L., Evermann J., Ver Hoef J. M., McNay M. E., Boertje R. D., Gardner C. L., et al. Serologic survey for canine coronavirus in wolves from Alaska. *J. Wildl. Dis*. 2001; 37 (4): 740–745. DOI: 10.7589/0090-3558-37.4.740.

8. Sanchez-Morgado J. M., Poynter S., Morris T. H. Molecular characterization of a virulent canine coronavirus BGF strain. *Virus Res*. 2004; 104 (1): 27–31. DOI: 10.1016/j.virusres.2004.02.038.

9. Evermann J. F., Abbott J. R., Han S. Canine coronavirus-associated puppy mortality without evidence of concurrent canine parvovirus infection. *J. Vet. Diagn. Invest*. 2005; 17 (6): 610–614. DOI: 10.1177/104063870501700618.

10. Escutenaire S., Isaksson M., Renström L. H., Klingeborn B., Buonavoglia C., Berg M., et al. Characterization of divergent and atypical canine coronaviruses from Sweden. *Arch. Virol*. 2007; 152 (8): 1507–1514. DOI: 10.1007/s00705-007-0986-1.

11. Buonavoglia C., Decaro N., Martella V., Elia G., Campolo M., Desario C., et al. Canine coronavirus highly pathogenic for dogs. *Emerg. Infect. Dis*. 2006; 12 (3): 492–494. DOI: 10.3201/eid1203.050839.

12. Zicola A., Jolly S., Mathijs E., Ziant D., Decaro N., Mari V., Thiry E. Fatal outbreaks in dogs associated with pantropic canine coronavirus in France and Belgium. *J. Small Anim. Pract*. 2012; 53 (5): 297–300. DOI: 10.1111/j.1748-5827.2011.01178.x.

13. Ntafis V., Mari V., Danika S., Fragkiadaki E., Buonavoglia C. An outbreak of canine coronavirus in puppies in a Greek kennel. *J. Vet. Diagn. Invest*. 2010; 22 (2): 320–323. DOI: 10.1177/104063871002200231.

14. Radford A. D., Singleton D. A., Jewell C., Appleton C., Rowlingson B., Hale A. C., et al. Outbreak of severe vomiting in dogs associated with a canine enteric coronavirus, United Kingdom. *Emerg. Infect. Dis*. 2021; 27 (2): 517–528. DOI: 10.3201/eid2702.202452.

15. Collins M., Singleton D. A., Noble P. J. M., Pinchbeck G. L., Smith S., Brant B., et al. Small animal disease surveillance 2020/21: SARS-CoV-2, syndromic surveillance and an outbreak of acute vomiting in UK dogs. *Vet. Rec*. 2021; 188 (8):304. DOI: 10.1002/vetr.427.

16. Pratelli A., Elia G., Martella V., Tinelli A., Decaro N., Marsilio F., et al. M gene evolution of canine coronavirus in naturally infected dogs. *Vet. Rec*. 2002; 151 (25): 758–761. PMID: 12521247.

17. Wang X., Li C., Guo D., Wang X., Wei S., Geng Y., et al. Co-circulation of canine coronavirus I and IIa/b with high prevalence and genetic diversity in Heilongjiang Province, Northeast China. *PLoS One*. 2016; 11 (1):e0146975. DOI: 10.1371/journal.pone.0146975.

Received 22.12.2022
Revised 25.01.2023
Accepted 10.02.2023

INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Anna A. Komarova, Post-Graduate Student, Leading Veterinarian, Department for Pet Disease Prevention, FGBI "ARRIAH", Vladimir, Russia; *https://orcid.org/0000-0002-1876-2025, e-mail: komarova_aa@arriah.ru.*

Tatyana S. Galkina, Candidate of Science (Veterinary Medicine), Head of Department for Pet Disease Prevention, FGBI "ARRIAH", Vladimir, Russia; *https://orcid.org/0000-0001-9494-8537, e-mail: galkina_ts@arriah.ru.*

Комарова Анна Александровна, аспирант, ведущий ветеринарный врач отдела профилактики болезней мелких домашних животных, ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *https://orcid.org/0000-0002-1876-2025, e-mail: komarova_aa@arriah.ru.*

Галкина Татьяна Сергеевна, кандидат ветеринарных наук, заведующий отделом профилактики болезней мелких домашних животных, ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *https://orcid.org/0000-0001-9494-8537, e-mail: galkina_ts@arriah.ru.*

DOI: 10.29326/2304-196X-2023-12-1-29-37

FeLV-induced feline leukemia as a natural model for leukemia pathophysiology study (review)

T. V. Tabakaeva[1,2], A. V. Tsybulsky[1], K. A. Baranchugova[1], I. V. Galkina[1], M. Yu. Shchelkanov[1,2,3]

[1] Far Eastern Federal University, Vladivostok, Russia

[2] FSBSI "Research Somov Institute of Epidemiology and Microbiology" (Somov Institute of Epidemiology and Microbiology), Vladivostok, Russia

[3] Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far Eastern Branch of the Russian Academy of Sciences (FSCEATB FEB RAS), Vladivostok, Russia

SUMMARY

Leukemia is a large group of diseases different in etiopathogenetic mechanisms and common in almost all mammalian species. The review focuses on feline leukemia, a common disease of domestic and wild felids (*Carnivora*, *Felidae*), being one of the main causes of their deaths. Feline leukemia pathogenesis and etiology are described; possible methods for the infection treatment and prevention, as well as possibility of using cats as a model for feline leukemia study are assessed. Feline leukemia etiological agent is a feline leukemia virus (FeLV), having single-stranded RNA genome surrounded with icosahedral capsid formed by p27 capsid protein monomers. Leukemia clinical manifestations in felids depend on high virulence of the virus and the disease is characterized with pronounced clinical picture and multiple organ dysfunction. Treatment of leukemia in cats is ineffective and is mainly aimed at maintaining the functions of the body organs and systems. Immunomodulators and chemotherapy are also used. Vaccination is used as a preventive measure, but commercially available adjuvanted and non-adjuvanted vaccines do not confer effective protection from the infection. The leukemia virus is reported in wild felids including rare and endangered feline species that is undoubtedly affects their population sizes. Despite very few data on leukemia, the reported cases show that leukemia in large cats is also severe and fatal. Feline leukemia, despite the accumulated of data, remains an ongoing serious and unresolved problem not only for veterinarians, but also for ecologists, zoologists and virologists involved in the research related to the feline family, study of retroviruses and biodiversity conservation on the planet. Further applied and fundamental research and verification thereof in the field of feline leukemia virus study, leukemia treatment and prevention are required.

Keywords: review, feline leukemia, retroviruses, feline leukemia virus, experimental models

Acknowledgements: The study was performed with support of the Russian Foundation for Basic Research (RFBR) in the framework of project 18-34-00075 "Study of interferon system state in domestic cats with retro- and parvovirus pathology associated with ecto- and endoparasites and development of effective programmes on interferon therapy of concomitant viral-parasitic diseases in cats".

For citation: Tabakaeva T. V., Tsybulsky A. V., Baranchugova K. A., Galkina I. V., Shchelkanov M. Yu. FeLV-induced feline leukemia as a natural model for leukemia pathophysiology study (review). *Veterinary Science Today*. 2023; 12 (1): 29–37. DOI: 10.29326/2304-196X-2023-12-1-29-37.

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

For correspondence: Tatyana V. Tabakaeva, Researcher, Laboratory of Ectoparasitology, Somov Institute of Epidemiology and Microbiology, 690087, Russia, Primorsky Krai, Vladivostok, ul. Sel'skaya, 1, *e-mail: rabchan1992@gmail.com.*

УДК 619:616.155.392:636.8:616-085.371

FeLV-индуцированный лейкоз кошачьих как естественная модель для изучения патофизиологии лейкозов (обзор)

Т. В. Табакаева[1,2], А. В. Цыбульский[1], К. А. Баранчугова[1], И. В. Галкина[1], М. Ю. Щелканов[1,2,3]

[1] ФГАОУ ВО «Дальневосточный федеральный университет» (Дальневосточный федеральный университет), г. Владивосток, Россия

[2] ФГБНУ «Научно-исследовательский институт эпидемиологии и микробиологии имени Г. П. Сомова» Роспотребнадзора (ФГБНУ «НИИ эпидемиологии и микробиологии им. Г. П. Сомова» Роспотребнадзора), г. Владивосток, Россия

[3] ФГБУН «Федеральный научный центр биоразнообразия наземной биоты Восточной Азии» Дальневосточного отделения Российской академии наук (ФНЦ Биоразнообразия ДВО РАН), г. Владивосток, Россия

РЕЗЮМЕ

Лейкозы – большая группа различных по этиопатогенетическим механизмам заболеваний, распространенных практически у всех видов млекопитающих. В обзоре внимание уделено лейкозу кошек, или лейкемии кошачьих, – часто встречающемуся заболеванию домашних и диких представителей

семейства кошачьих (*Carnivora*, *Felidae*), которое является одной из основных причин их смертности. Дана характеристика этиологии и патогенеза лейкемии кошачьих, оцениваются возможные способы лечения и профилактики инфекции, а также возможность использования домашней кошки как модели для изучения лейкозов семейства кошачьих. Этиологический агент лейкемии кошачьих – вирус лейкемии кошачьих (FeLV – Feline leukemia virus), геном которого представлен одноцепочечной РНК, упакованной в капсид икосаэдрической симметрии, формируемый мономерами капсидного белка p27. Клинические проявления лейкемии у кошачьих связаны с высокой вирулентностью вируса и характеризуются ярко выраженной клинической картиной и развитием полиорганной недостаточности. Лечение лейкемии у кошек малоэффективно и направлено в основном на поддержание функционирования органов и систем. Также применяются иммуномодуляторы и химиотерапия. В качестве превентивной меры используется вакцинопрофилактика, однако существующие на рынке адъювантные и безадъювантные вакцины не обеспечивают надежной защиты от инфекции. Вирус лейкемии встречается у диких кошачьих, в том числе у редких и исчезающих видов, что, несомненно, влияет на численность их популяций. Несмотря на то что данных по лейкемии у диких кошачьих крайне мало, отдельные зарегистрированные случаи свидетельствуют о том, что заболевание у крупных кошек также имеет тяжелое течение и приводит к летальному исходу. Лейкемия кошачьих, несмотря на накопленный массив данных, и по сей день остается серьезной, нерешенной проблемой не только для специалистов ветеринарной практики, но также для экологов, зоологов и вирусологов, чья область исследования так или иначе связана с семейством кошачьих, изучением ретровирусов и сохранением биоразнообразия на планете. Необходимы дальнейшие прикладные и фундаментальные исследования и их верификация в области изучения вируса лейкемии кошачьих, лечения и профилактики лейкоза.

Ключевые слова: обзор, лейкоз кошачьих, ретровирусы, вирус лейкемии кошачьих, экспериментальная модель

Благодарности: Исследование выполнено при поддержке РФФИ в рамках проекта 18-34-00075 «Изучение состояния системы интерферона у домашних кошек с сочетанной ретро- и парвовирусной патологией и различными экто- и эндопаразитозами и разработка эффективных программ интерферонотерапии сочетанных вирусно-паразитарных заболеваний кошек».

Для цитирования: Табакаева Т. В., Цыбульский А. В., Баранчугова К. А., Галкина И. В., Щелканов М. Ю. FeLV-индуцированный лейкоз кошачьих как естественная модель для изучения патофизиологии лейкозов (обзор). *Ветеринария сегодня*. 2023; 12 (1): 29–37. DOI: 10.29326/2304-196X-2023-12-1-29-37.

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

Для корреспонденции: Табакаева Татьяна Владимировна, научный сотрудник лаборатории эктопаразитологии ФГБНУ «НИИ эпидемиологии и микробиологии им. Г. П. Сомова» Роспотребнадзора, 690087, Россия, Приморский край, г. Владивосток, ул. Сельская, д. 1, *e-mail: rabchan1992@gmail.com.*

INTRODUCTION

Leukemia is a large group of diseases that differ in etiology and pathogenesis, being a type of hemoblastoses (from ancient Greek *αἷμα* – blood and *βλαστός* – growth), i.e. malignant hematopoietic and lymphatic diseases. Hemoblastoses can be systemic diseases (leucosis/leukemia) as well as regional nodular tumor neoplasms (lymphoma and hematosarcoma). All hematoblastoses of this group have a clonal nature [1, 2]. Moreover, a malignantly transformed clone can originate both from immature hematopoietic cells of the bone marrow, and from maturing and mature blood cells.

Malignant blood and lymph cell clones can be of two main origins-myeloid and lymphoid. The myelogenesis products are erythrocytes, granulocytes, platelets, monocytes, dendritic cells, macrophages and mast cells, and the lymphopoiesis products are B-lymphocytes and plasmacytes, T-lymphocytes, NK cells. The particular cell line undergoing malignant transformation gives rise to particular variant of hemoblastosis, and there can obviously be a lot of such variants. Variable leukemia morphological forms and clinical courses are also accounted for rather broad spectrum of malignantly transformed blood cell maturity – from slightly-differentiated cells to cells that have practically lost normal phenotype signs (blast crisis is typical for this leukemia stage).

Leukemias and lymphomas differ not only in presence and absence of systemic lesions. It is known that lymphoma in its terminal stage can spread with extensive metastases in various tissues and organs, including the bone marrow [3], i.e. the disease becomes systemic. However, the bone marrow is affected primarily in case of leukemia, and secondarily as a result of metastasis in case of lymphomas and hematosarcomas.

This review focuses on leukemias developing in all species of feline family (*Carnivora*, *Felidae*). This pathology is increasingly prevalent and the main cause of death in felids. The study of leukemia in domestic cats (*Felis catus*) is of importance [4–6] from the point of view of pet animal life quality improvement and biomedical ethics [7], but also as an accessible model for rare and endangered felids, limited populations of which are extremely vulnerable. The situation is the most critical in the south of Russian Far East. Forward-looking economic development of the region poses objective threats for Far Eastern leopards (*Panthera pardus orientalis*) and Amur tigers (*Panthera tigris altaica*) [8]. There are specialized animal shelters where leukemia-affected felids are kept. Our practice shows that it is possible to organize full-fledged and well-randomized studies of both the fundamental mechanisms of the feline leukemia development and effectiveness of certain experimental treatment methods in case of cooperation with such shelters.

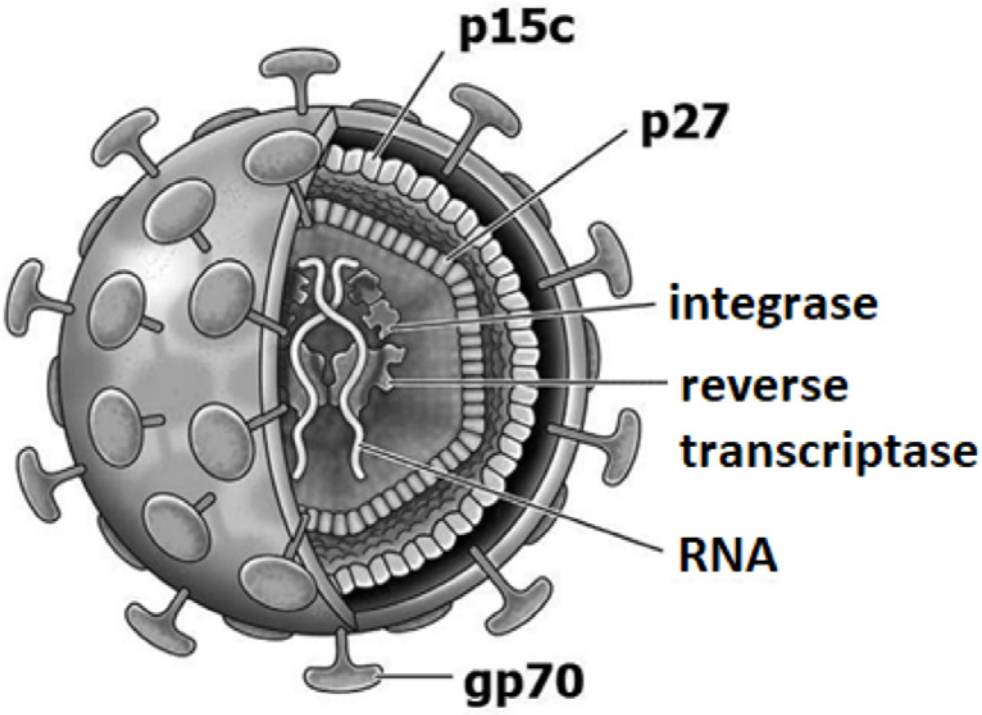

Fig. Morphology of feline leukemia virus virion

Moreover, the studies can be carried out for a long time including monitoring of the survival of animals within their natural lifespan.

Feline leukemia virus (FeLV) belongs to *Ortervirales* order, *Retroviridae* family, *Orthoretrovirinae* subfamily, *Gammaretrovirus* genus. In addition to FeLV, this genus of orthoretroviruses includes chick syncytial virus (CSV), Finkel-Biskis-Jinkins murine sarcoma virus (FBJ MuSV), Gardner-Arnstein feline sarcoma virus (GA-FeSV), gibbon ape leukemia virus (GaLV), Guinea pig type-C oncovirus (GPCOV), Hardy-Zuckerman feline sarcoma virus (HZ-FeSV), Harvey murine sarcoma virus (Ha-MuSV), Kirsten murine sarcoma virus (Ki-MuSV), koala retrovirus (KoRV), Moloney murine sarcoma virus (Mo-MSV), murine leukemia virus (MuLV), porcine type-C oncovirus (PCOV), reticuloendotheliosis virus (REV), Snyder-Theilen feline sarcoma virus (ST-FeSV), Trager duck spleen necrosis virus (TDSNV), viper retrovirus (VRV), woolly monkey sarcoma virus (WMSV)[1].

In accordance with D. Baltimore's classification, the *Retroviridae* family belongs to group VI (ssRNA-RT), i.e. to the viruses containing the enzyme called reverse transcriptase (revertase), which allows for generating a double-stranded DNA replica (cDNA) on the viral genomic RNA template. The viral genome-encoded integrase, being a part of the nucleoprotein, enables cDNA incorporation into host cell chromosome (cDNA incorporated in the chromosome is called provirus or pvDNA). In the infected cell nucleus some cDNAs become circulized (ccDNAs) and acquire cosmid functions [9, 10].

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

Virion morphology. Feline leukemia virus virion is a spherical enveloped particle 100–110 nm in diameter. The virion structure is given in figure below. The virion contains two copies of single-stranded positive-sense RNA and nucleoprotein-associated enzymes: revertase, integrase and protease. The nucleoprotein is packed in an icosahedral capsid formed by capsid protein p27 monomers. The outer lipid envelope is impregnated by p15c matrix protein on the inside and contains p15e transmembrane protein and gp70 surface glycoprotein receptor.

At the virion level, *FeLV genome* consists of two identical copies of positive-sense single-stranded RNA 9.6 kb in size. The virus contains untranslated regulatory long terminal repeats (LTR) at the 5′ and 3′ ends, flanking three viral genes: *gag* (encoding structural group-specific capsid proteins), *pol* (encoding protease, reverse transcriptase and integrase) and *env* (encoding virion surface proteins). FeLV genome has two open reading frames (ORF): 1) *gag* + *pol*; 2) *env* [11].

Like other retroviruses, FeLV genome is highly variable. This leads to the emergence of new genotypes and antigenic variants causing various clinical disease forms, and eventually hinders development of effective medicines and vaccines [12]. Lack of 3′–5′-exonuclease activity in the retrovirus reverse transcriptase is one of the most important mechanisms for the point nucleotide substitutions emergence, that prevents this enzyme from correction of its own errors during strand elongation [13, 14]. There are numerous recombinants between different FeLV variants, including those with the endogenous enFeLV virus. The pathogen genetic and antigenic variability is associated with large range of FeLV virulent properties, various pathogenetic

mechanisms giving rise to various clinical manifestations of FeLV infection [15]. The concept that RNA-containing viruses, and especially retroviruses, exist only in the form of populations representing complex topological objects in the space of their variable digital images [16] has been known since the end of the last century. In the paper of L. L. Bolin and L. S. Levy [17], this concept has been applied to FeLV infection: there is a complex of genetically heterogeneous virus populations in the body – FeLV itself, that has initiated the disease and the products generated due to its natural variability, that may increase virus aggressiveness and ability to evade the immune system.

There are several FeLV variants classified to subgroups: A, B, C, D, T and TG35 that also comprise several variants. It should be noted that each of them has own specific mechanisms of the virus attachment to target cells [18, 19].

Clinical manifestations of FeLV infection depend on the virus variant, virus burden and host immune status. FeLV is highly virulent therefore most of infected animals demonstrate apparent clinical manifestations [20]. Kittens at the age of 4 months are the most susceptible to FeLV, whereas aging cats are usually being the source of infection, in which the virus can be found in the blood and various tissues of internal organs, and excreted with urine, saliva and nasopharyngeal mucus. The virus can be transmitted both horizontally and vertically: kittens from infected cat can be infected during their prenatal development.

The infection is much more common in urban (domestic and stray) cats than in rural cats. It is believed that this is accounted for higher frequency of contacts between cats in urban settings.

The virus is characterized by low resistance to environmental factors. FeLV is inactivated outside the animal within 8 hours. Therefore, FeLV-infected cats are the main source of infection. Animals can transmit the virus among themselves through casual close contacts, and there is also evidence of possible horizontal virus transmission by cat fleas of the *Ctenocephalides felis* species [21].

FeLV circulating in domestic cat populations is also found in populations of wild felids (tigers, lions, leopards, cougars, jaguars, lynxes, cheetahs, etc.) [12, 15, 22–26]. All the species of the *Felidae* family examined so far have been found to be more or less susceptible to FeLV. However, it appeared that there are certain interspecies differences in the susceptibility and clinic courses of pathological conditions developing in infected cats. For example, T. M. Harrison et al. [15] performed an interesting study involving 11 African lions affected by malignant lymphoma. In all the examined animals, lymphomas were of T-cell genesis, unlike B-cell lymphomas characteristic of other feline species, including *Felis catus*. No FeLV was detected in any of the lions examined; although one diseased animal was found to be seropositive to this virus, i.e. it had specific antibodies to the virus antigens. The results of such study evidenced that the pathogenesis mechanisms and malignancy processes in infected animals are currently insufficiently investigated. Also, the study results have supported the opinion that the virus initiating carcinogenesis (leukogenesis) may not be detected further by current diagnostic methods. At a minimum, this is indicative of the need for a combination of immunochemical and molecular genetic analysis methods for viral feline leukemia diagnosis to detect both virus-specific seroconversion and viral genome replicative and expression activity.

The feline leukemia virus is a specific member of the *Felidae* family and does not infect other animals, in particular, it is not transmitted to humans or dogs being in frequent and prolonged contact with domestic cats [11, 20, 21].

The mechanisms of the progression of feline leukemia-associated pathological conditions are currently insufficiently investigated. Leukemias and lymphomas (or lymphosarcomas) are considered to be the final stage of feline leukemia. Moreover, disease transformation into leukemic form may not require the presence of virus-specific antigens [27]. The detection of FeLV-infected cats, seronegative to virus-specific antigens, indicates that the virus can fulfil its leukogenic potential not only through expression of FeLV provirus genetic material at the translation level, but also through variations in proviral genome integration into various sites of the host cell genome, induction of chromosomal rearrangements, activation of c-oncogenes and other genomic alterations in host cells [28].

There are six main stages of FeLV infection [29]:

1. The virus enters the cat's body orally or parenterally (usually by bite and other skin wounds, as well as a result of "friendly" contacts: mutual licking, shared bowl, etc.). After oronasal invasion the virus at first infects epithelial cells and leucocytes (mainly tonsil B-lymphocytes and macrophages). Infected leucocytes can recirculate, but in most cases they are retained in the nearest regional lymph nodes, where the virus actively replicates.

2. *De novo*-generated virus progeny enters to blood-lymph circulation systems, i.e. infectious agents disseminate throughout the body. Since FeLV like other retroviruses has a cytolytic potential the virus can be released from the infected cell by lysis. Pools of lymphocytes and monocytes are depleted and the host immunity becomes apparently compromised.

3. Number of infected peripheral lymphoid cells and circulating lymphocytes increases. This stage of the infectious process is characterized by the active production of anti-FeLV antibodies enabling monitoring of the disease dynamics, as well as evaluation of the therapy effectiveness.

4. Pronounced viremia develops. Total infection of hemolymphatic system is followed by the infection of intestine epithelial cells.

5. The disease takes leukemic form characterized by bone marrow cell infection and malignant transformation of bone marrow progenitor cells. At this stage, spontaneous eradication of the virus is practically

impossible and the disease becomes fatal. The virus actively replicates in all leukocytes produced in the bone marrow (in lymphocytes, neutrophils, monocytes, eosinophils). As a result, the immunity becomes largely and severely compromised. This leads to the development of various secondary infectious processes and increasingly aggravated multiple organ dysfunction.

6. Terminal stage (blast crisis). At this stage cells of almost all organs and tissues of the diseased animals are infected. Mucous membrane and glandular epithelial cells demonstrating the highest metabolic and proliferative activity are the most affected. The virus actively replicates in oral, nasal and pharyngeal epithelial tissues, salivary glands, gastric and intestinal mucosa, tracheal cells, cells of renal tubules, bladder, pancreas, sebaceous glands. The terminal stage is considered to be untreatable stage of the disease.

The virus can be vertically transmitted from a pregnant female to its kittens, in this case the kittens immediately enter the second stage of infection. The probability of a newborn kitten become infected depends on the viral load in the mother's body during pregnancy.

Feline leukemia-associated non-oncological pathologies are mediated by the effect of the virus on the immune system. In addition to systemic blood disorders (anemia and leukopenia), the infection can cause various organ lesions in cats, for example, myocarditis. The clinical manifestations of the infection developing in affected cats are very variable: loss of appetite, skin and coat disorders, prolonged and recurrent bacterial, fungal, viral infections of the skin and other tissues, eye lesions, otitis, inflammatory lesions of the bladder and respiratory tract, lymphadenopathy, fatigue, fever, weight loss, stomatitis, gingivitis, changes in behavior, diarrhea, jaundice, immunodeficiency of various types, leukemic manifestations, development of thymus lymphomas and other manifestations in the form of lymphomas and sarcomas of various localization [30–32].

In some cases, the infection can be prolonged asymptomatic and latent, i.e. animals carrying the virus do not show any signs of the disease for many years [31]. Is it associated with specific nature of the virus only, or with more effective immunological antiviral and antitumor surveillance mechanisms? The answers to these questions are of obvious fundamental and extremely practical importance.

There are some very interesting (but insufficiently verified) reports on the infected cats that can effectively fight the infection by developing strong immunity and becoming completely resistant to the diseases associated with this virus. However, the virus is not completely eradicated and such "healthy" virus carriers can infect other cats at contact, facilitating the virus spread in the population [33, 34]. Nevertheless, the above data on the clinical feline leukemia extreme polymorphism and, in particular, on the leukemic process variability indicate the importance of studying immunological mechanism features, involvement of the interferon system and other factors of antiviral and antitumor innate immunity for development of techniques and technologies enabling improvement of the effectiveness of treatment of at least these hemoblastosis forms in cats.

Treatment of feline leukemia is a difficult task: firstly, due to the retroviral nature of the infection (which implies the insertion of the provirus into the host cell chromosome), and secondly, due to nonspecific symptoms and ability to affect many organs and systems that results in multiple organ failure at later stages. For this reason, there is currently no single and effective treatment pattern for leukemia in cats. The treatment includes symptomatic therapy aimed, on the one hand, at maintaining the functioning of the cardiovascular system, kidneys, liver and other vital organs, on the other hand, at containment of the secondary infections associated with immunodeficiency.

Immunomodulating drugs have the greatest effectiveness and less side effects as manifested by improved general condition and reduced mortality in diseased animals [18]. However, this treatment fails to cure the disease in animal and completely eradicate the virus. Chemotherapy based on a combination of vincristine, cyclophosphamide and prednisolone or involving use of L-asparaginase and doxorubicin is another effective type of treatment. Chemotherapy achieves the remission, but it does not exceed 10 months. Moreover, this therapy is associated with various side effects that preclude its use in animals with hepatic or renal failures [35].

The vaccines were proposed for the disease prevention as early as at the beginning of the XXI century, but no highly effective vaccine has been developed so far. Currently, there are several polyvalent vaccines – both adjuvanted (for example, Nobivak® Feline 2-FeLV, Merck & Co., Inc., USA) and vector non-adjuvanted vaccines, but none of them confers strong protection against feline leukemia.

Non-adjuvanted Purevax® FeLV vaccine (also known as Eurifel FeLV) produced by Merck & Co. (France) and Biokema SA (Switzerland) is currently the most popular vaccine against FeLV infection. This is a recombinant DNA vaccine that contains Canarypox virus (*Chitovirales: Poxviridae, Avipoxvirus*) as a genetic vector carrying two FeLV genes, *gag* and *env*, incorporated in its genome. The vaccine is a living whole-virion construct that does not replicate in feline cells. This vaccine is the most preferred, as it has practically no side effects [5, 36, 37].

The leukemia has a significant impact on the populations of specially protected wild felids due to their small sizes and therefore increased susceptibility to infectious agents, especially those capable of circulating in latent form.

The conservation and reproduction of rare and endangered large predator species occupying the top of the food pyramid is an important task for maintaining biodiversity on the planet. The wild large felids are the most diverse in the Russian Far East, habitat of the Amur tigers and the Far Eastern leopards, whose populations still require comprehensive protection despite their significant increase over the past two decades owing to conservation measures. The following

ecological and biological factors have a significant impact on the large wild felid population sizes: climatic changes, anthropogenic impact, feed availability (deer and wild boars) [38–40]. Infectious diseases can significantly reduce populations, causing mass deaths in animals, and affect the survival and reproduction of animals. Even a slight decrease in survival can be critical for slow-breeding and small-numbered feline species, such as the Amur tiger and the Far Eastern leopard. However, data on infectious and parasitic diseases of these animals are scarce [8, 41–45]. Available epidemiological data do not allow full assessment of the infectious disease impact on the rare feline species population and the clinical picture described for the isolated cases does not give the understanding of the infection pathogenesis in wild large felids [41, 46].

FeLV cases in large cats kept in zoos are reported. Most often, the infection is latent, and animals do not demonstrate any clinical symptoms [47–49]. In some cases, secondary infections and organ lesions associated with FeLV infection develop in tigers. For example, fatal amyloidosis associated with feline leukemia virus was reported in a Bengal tiger in Mexico [50]. Similar to domestic cats, FeLV infection in wild cats is manifested by acute leukemia and leads to the development of immunodeficiency and secondary infections [51, 52].

At the moment, there are no uniform procedures for the leukemia diagnosis, treatment and vaccination in large felids [47]. Due to the fact that both wild and domestic cats are susceptible to FeLV infection, leukemia pathogenesis and clinical course are similar that enables use of infected domestic cats as a model for the development of anti-feline leukemia treatment and vaccination methods [6, 44, 53], as well as putting the obtained results into veterinary practice when working with large felids, at first zoo felids, and then wild felids.

CONCLUSION

Feline leukemia, despite the accumulated data, remains a serious and unresolved problem for veterinary practitioners, as well as for ecologists, zoologists and virologists involved in investigations related to the feline family, retroviruses and maintaining biodiversity on the planet. Further applied and fundamental research and verification thereof in the field of feline leukemia virus study, leukemia treatment and prevention are required.

REFERENCES

1. Hotta T. Clonality in hematopoietic disorders. *Int. J. Hematol.* 1997; 66 (4): 403–412. DOI: 10.1016/s0925-5710(97)00092-3.

2. Smirnova S. Yu., Sidorova Yu. V., Ryzhikova N. V., Sychevskaya K. A., Parovichnikova E. N., Sudarikov A. B. Evolution of tumor clones in adult acute lymphoblastic leukemia. *Acta Naturae*. 2016; 8 (4): 100–109. DOI: 10.32607/20758251-2016-8-4-100-109.

3. Roug I. K., McCartney L. B. Metastatic non-Hodgkin lymphoma presenting as low back pain and radiculopathy: a case report. *J. Chiropr. Med*. 2012; 11 (3): 202–206. DOI: 10.1016/j.jcm.2012.05.008.

4. Moskvina T. V., Shchelkanov M. Yu., Tsybulski A. V. FeLV-infection: problems and prospects of vaccine prevention and interferon-therapy of feline leukemia. *Russian Journal of Infection and Immunity*. 2021; 11 (4): 624–634. DOI: 10.15789/2220-7619-FPA-882. (in Russ.)

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

6. Tsybulsky A., Tabakaeva T., Klimovich A., Shchelkanov M., Kostetsky E., Aliev M., Degtyarenko A. Interferon status and expression of *p53* and *gadd45g* genes in leukaemogenic FeLV infection in domestic cats. *Adv. Anim. Vet. Sci*. 2022; 10 (3): 565–572. DOI: 10.17582/journal.aavs/2022/10.3.565.572.

7. Shchelkanov M. Yu., Yarygina M. V., Galkina I. V., Kiku P. F. The dialectic approach to biomedical ethics as a foundation of its implementation in actual social cultural traditions. *Problems of Social Hygiene, Public Health and History of Medicine*. 2019; 27 (4): 414–417. DOI: 10.32687/0869-866X-2019-27-4-414-417. (in Russ.)

8. Shchelkanov M. Yu., Galkina I. V., Aramilev S. V., Surovyi A. L., Fomenko P. V., Zhuravlev Yu. N. Far Eastern Bank of biological materials from big cats (*Pantherinae*) as an improvement tool of the practice of enforcement of Articles 226.1 and 258.1 of the Criminal Code of the Russian Federation. *Russian Journal of Criminology*. 2017; 11 (1): 146–153. DOI: 10.17150/25004255.2017.11(1).146-153. (in Russ.)

9. Szilasi A., Dénes L., Balka G. Feline leukemia virus (FeLV): literature review. *Magyar Állatorvosok Lapja*. 2018; 140 (8): 457–472. (in Hungarian)

10. Weiss A. T., Klopfleisch R., Gruber A. D. Prevalence of feline leukaemia provirus DNA in feline lymphomas. *J. Feline Med. Surg*. 2010; 12 (12): 929–935. DOI: 10.1016/j.jfms.2010.07.006.

11. Brown M. A., Cunningham M. W., Roca A. L., Troyer J. L., Johnson W. E., O'Brien S. J. Genetic characterization of feline leukemia virus from Florida panthers. *Emerg. Infect. Dis*. 2008; 14 (2): 252–259. DOI: 10.3201/eid1402.070981.

12. Filoni C., Helfer-Hungerbuehler A. K., Catão-Dias J. L., Marques M. C., Torres L. N., Reinacher M., Hofmann-Lehmann R. Putative progressive and abortive feline leukemia virus infection outcomes in capative jaguarundis (*Puma yagouaroundi*). *Virol. J*. 2017; 14 (1):226. DOI: 10.1186/s12985-017-0889-z.

13. Shchelkanov M. Yu., Soinov L. A., Zalunin V. V., Gumennyi D. A., Yudin A. N., Natan A. A., et al. One-parameter discrete model of the genetic diversity. *J. Biomol. Struct. Dyn*. 1998; 15 (5): 887–894. DOI: 10.1080/07391102.1998.10508210.

14. Shchelkanov M. Yu., Yudin A. N., Antonov A. V., Soinov L. A., Zalunin V. V., Vedenov A. A., Karamov E. V. Variability analysis of HIV-1 gp120 V3 region: II. Hierarchy of taxons. *J. Biomol. Struct. Dyn*. 1997; 15 (2): 231–241. DOI: 10.1080/07391102.1997.10508188.

15. Harrison T. M., McKnight C. A., Sikarskie J. G., Kitchell B. E., Garner M. M., Raymond J. T., et al. Malignant lymphoma in African lions (*Panthera leo*). *Vet. Pathol*. 2010; 47 (5): 952–957. DOI: 10.1177/0300985810375054.

16. Shchelkanov M. Y., Soinov L. A., Yudin A. N., Denisov M. V., Slavsky A. A., Petrenko M. S., Vedenov A. A. Stochastic properties of one-parameter discrete model of genetic diversity in the unique random parameter case. *J. Biomol. Struct. Dyn*. 1999; 17 (3): 597–605. DOI: 10.1080/07391102.1999.10508389.

17. Bolin L. L., Levy L. S. Viral determinants of FeLV infection and pathogenesis: lessons learned from analysis of a natural cohort. *Viruses*. 2011; 3 (9): 1681–1698. DOI: 10.3390/v3091681.

18. Anderson M. M., Lauring A. S., Robertson S., Dirks C., Overbaugh J. Feline Pit2 functions as a receptor for subgroup B feline leukemia viruses. *J. Virol*. 2001; 75 (22): 10563–10572. DOI: 10.1128/JVI.75.22.10563-10572.2001.

19. Mendoza R., Anderson M. M., Overbaugh J. A putative thiamine transport protein is a receptor for feline leukemia virus subgroup A. *J. Virol*. 2006; 80 (7): 3378–3385. DOI: 10.1128/JVI.80.7.3378-3385.2006.

20. Phipps A. J., Chen H., Hayes K. A., Roy-Burman P., Mathes L. E. Differential pathogenicity of two feline leukemia virus subgroup A molecular clones, pFRA and pF6A. *J. Virol*. 2000; 74 (13): 5796–5801. DOI: 10.1128/jvi.74.13.5796-5801.2000.

21. Vobis M., D'Haese J., Mehlhorn H., Mencke N. Evidence of horizontal transmission of feline leukemia virus by the cat flea (*Ctenocephalides felis*). *Parasitol. Res*. 2003; 91 (6): 467–470. DOI: 10.1007/s00436-003-0949-8.

22. Cunningham M. W., Brown M. A., Shindle D. B., Terrell S. P., Hayes K. A., Ferree B. C., et al. Epizootiology and management of feline leukemia virus in the Florida puma. *J. Wildl. Dis*. 2008; 44 (3): 537–552. DOI: 10.7589/0090-3558-44.3.537.

23. Guimaraes A. M., Brandão P. E., de Moraes W., Cubas Z. S., Santos L. C., Villarreal L. Y., et al. Survey of feline leukemia virus and feline coronaviruses in captive neotropical wild felids from Southern Brazil. *J. Zoo Wildl. Med*. 2009; 40 (2): 360–364. DOI: 10.1638/2008-0067.1.

24. Sleeman J. M., Keane J. M., Johnson J. S., Brown R. J., Woude S. V. Feline leukemia virus in a captive bobcat. *J. Wildl. Dis.* 2001; 37 (1): 194–200. DOI: 10.7589/0090-3558-37.1.194.

25. Leutenegger C. M., Hofmann-Lehmann R., Riols C., Liberek M., Worel G., Lups P., et al. Viral infections in free-living populations of the European wildcat. *J. Wildl. Dis*. 1999; 35 (4): 678–686. DOI: 10.7589/0090-3558-35.4.678.

26. Tangsudjai S., Malidang S., Phonarknguen R., Boonyarittichaikit R., Pattanarangsan R., Buddhirongawatr R., Chaichoun K. Feline leukemia virus (FeLV) in captive wild felids in Thailand during 2004–2005. *J. Appl. Anim. Sci*. 2010; 3 (1–3): 25–31.

27. Moskvina T., Klimovich A., Stenkova A., Tsybulsky A., Tabakaev A., Shchelkanov M. The prevalence of FeLV and FIV infection in cats and hematological changes and clinical signs in FeLV/FIV infected cats from Vladivostok, Russia. *Adv. Anim. Vet. Sci*. 2019: 7 (7): 570–573. DOI: 10.17582/journal.aavs/2019/7.7.570.573.

28. Weiss R. A. The discovery of endogenous retroviruses. *Retrovirology*. 2006; 3:67. DOI: 10.1186/1742-4690-3-67.

29. Levy J. K., Burling A. Feline leukemia virus and related diseases in cats – overview. *Generalized Conditions. MSD Veterinary Manual*. 2018; 9 p.

30. Hisasue M., Nagashima N., Nishigaki K., Fukuzawa I., Ura S., Katae H., et al. Myelodysplastic syndromes and acute myeloid leukemia in cats infected with feline leukemia virus clone33 containing a unique long terminal repeat. *Int. J. Cancer*. 2009; 124 (5): 1133–1141. DOI: 10.1002/ijc.24050.

31. Marker L., Munson L., Basson P. A., Quackenbush S. Multicentric T-cell lymphoma associated with feline leukemia virus infection in a captive Namibian cheetah (*Acinonyx jubatus*). *J. Wildl. Dis*. 2003; 39 (3): 690–695. DOI: 10.7589/0090-3558-39.3.690.

32. Neil J. C., Fulton R., Rigby M., Stewart M. Feline leukaemia virus: generation of pathogenic and oncogenic variants. *Curr. Top. Microbiol. Immunol*. 1991; 171: 67–93. DOI: 10.1007/978-3-642-76524-7_4.

33. Jarrett O. Pathogenicity of feline leukemia virus is commonly associated with variant viruses. *Leukemia*. 1992; 6 (Suppl 3): 153S–154S. PMID: 1318467.

34. Rojko J. L., Kociba G. J. Pathogenesis of infection by the feline leukemia virus. *J. Am. Vet. Med. Assoc*. 1991; 199 (10): 1305–1310. PMID: 1666072.

35. Hartmann K. Efficacy of antiviral chemotherapy for retrovirus-infected cats: What does the current literature tell us? *J. Feline. Med. Surg*. 2015; 17 (11): 925–939. DOI: 10.1177/1098612X15610676.

36. Patel M., Carritt K., Lane J., Jayappa H., Stahl M., Bourgeois M. Comparative efficacy of feline leukemia virus (FeLV) inactivated whole-virus vaccine and canarypox virus-vectored vaccine during virulent FeLV challenge and immunosuppression. *Clin. Vaccine Immunol*. 2015; 22 (7): 798–805. DOI: 10.1128/CVI.00034-15.

37. Wardley R. C., Berlinski P. J., Thomsen D. R., Meyer A. L., Post L. E. The use of feline herpesvirus and baculovirus as vaccine vectors for the *gag* and *env* genes of feline leukaemia virus. *J. Gen. Virol*. 1992; 73 (7): 1811–1818. DOI: 10.1099/0022-1317-73-7-1811.

38. Pikunov D. G., Miquelle D. G., Abramov V. K., Nikolaev I. G., Seredkin I. V., Murzin A. A., Korkishko V. G. A survey of Far Eastern leopard (*Panthera pardus orientalis*) and Amur tiger (*Panthera tigris altaica*) populations in southwest Primorski Krai, Russian Far East (February 2003). Vladivostok; 2003. 66 p. Available at: https://www.save-leopard.ru/_ld/0/61_7-download.pdf. (in Russ.)

39. Seryodkin I. V., Goodrich D. M., Lewis D., Miquelle D. G., Esaulova N. V., Konyaev S. V., et al. Infectious and endoparasitic diseases of the Amur tiger. *Bulletin of KrasGAU*. 2015; 12: 185–191. eLIBRARY ID: 25054295. (in Russ.)

40. Gilbert M., Miquelle D. G., Goodrich J. M., Reeve R., Cleaveland S., Matthews L., Joly D. O. Estimating the potential impact of canine distemper virus on the Amur

tiger population (*Panthera tigris altaica*) in Russia. *PLoS One*. 2014; 9 (10):e110811. DOI: 10.1371/journal.pone.0110811.

41. Lyubchenko E. N., Korotkova I. P., Ivanchuk G. B., Kukharenko N. C. Pathomorphological changes in organs under alimentary dystrophy of wild feline of the Far East. *Far Eastern Agrarian Herald.* 2019; 1 (49): 66–72. DOI: 10.24411/1999-6837-2019-11010. (in Russ.)

42. Jung I., Kim Y. S., Jee H., Sohn S. Y., Yoo H. S., Kim D. Y., et al. Feline panleukopenia virus infection in a Siberian tiger (*Panthera tigris altaica*). *J. Vet. Clin*. 2009; 26 (5): 504–507.

43. Lewis J., Tomlinson A., Gilbert M., Alshinetski M., Arzhanova T., Goncharuk M., et al. Assessing the health risks of reintroduction: The example of the Amur leopard, *Panthera pardus orientalis*. *Transbound. Emerg. Dis*. 2020; 67 (3): 1177–1188. DOI: 10.1111/tbed.13449.

44. Moskvina T. V., Schelkanov M. Yu., Begun M. A. Endoparasites of the Siberian tiger (*Panthera tigris altaica*). *Integrative Zoology*. 2018; 13 (5): 507–516. DOI: 10.1111/1749-4877.12342.

45. Voronova A. N., Vainutis K. S., Tabakaeva T. V., Sapotsky M. V., Kakareka N. N., Volkov Y. G., et al. Molecular identification of the trematode *P. ichunensis* stat. n. from lungs of siberian tigers justified reappraisal of *Paragonimus westermani* species complex. *J. Parasit. Dis*. 2022; 46 (3): 744–753. DOI: 10.1007/s12639-022-01481-7.

46. Lyubchenko E. N., Korotkova I. P., Fomenko P. V. Lymphoma of the Amur tigris. *Aktual'nye voprosy i innovatsionnye tekhnologii v veterinarnoi meditsine, zhivotnovodstve i prirodookhrannom komplekse: materialy Mezhdunarodnoi nauchno-prakticheskoi konferentsii, posvyashchennoi 40-letnemu yubileyu so dnya obrazovaniya veterinarnogo fakul'teta* (*6–8 noyabrya 2019 g.*). *Ch. 1. = Current aspects and innovative technologies in veterinary field, animal farming industry and environmental field. Proceedings of the International Scientific and Practical Conference devoted to the 40th anniversary of the Veterinary Faculty establishment* (*November 6–8, 2019*). *Part 1*. Ussuriysk: FSBEI HE Primorskaya SAA; 2019; 177–180. eLIBRARY ID: 42374757. (in Russ.)

47. Reyes M. F., Barrios E. R., Favila M. J. B., Moralde K. I. A., Flores M. L. S. Prevalence of feline immunodeficiency virus, feline leukemia virus and *Toxoplasma gondii* in captive tigers (*Panthera tigris*) in a wildlife facility in the Philippines. *Philipp. J. Vet. Anim. Sci*. 2017; 43 (1): 38–43.

48. Huang S., Li X., Guo L., You D., Xie W., Xu H., et al. Prevalence of four viruses in captive Siberian tigers from Northeastern China. *Transbound. Emerg. Dis*. 2022; 69 (5): e1434–e1444. DOI: 10.1111/tbed.14475.

49. Liu E., Ma L., Huang S., You D., Guo L., Li X., et al. The first feline immunodeficiency virus from Siberian tigers (*Panthera tigris altaica*) in Northeastern China. *Arch. Virol*. 2022; 167 (2): 545–551. DOI: 10.1007/s00705-022-05370-5.

50. De la Cruz-Hernández N. I., Merino-Charres J. O., Salinas-Navarrete E. M., Monreal Garcia A. E., Martinez Burnes J., Rangel Lucio J. A. R., Venegas-Barrera C. Amyloidosis associated with feline leukemia virus in a white bengal tiger (*Panthera tigris tigris*). *Thai. J. Vet. Med.* 2016; 46 (4): 679–683.

51. Rolim V. M., Casagrande R. A., Wouters A. T., Driemeier D., Pavarini S. P. Myocarditis caused by feline immunodeficiency virus in five cats with hypertrophic cardiomyopathy. *J. Comp. Pathol*. 2016; 154 (1): 3–8. DOI: 10.1016/j.jcpa.2015.10.180.

52. Keller D. L., Steinberg H., Sladky K. K. Disseminated histoplasmosis in a Bengal tiger (*Panthera tigris*). *J. Zoo. Wildl. Med*. 2011; 42 (4): 727–731. DOI: 10.1638/2010-0206.1.

53. Tabakaeva T. V., Klimovich A. A., Tsybulsky A. V., Tabakaev A. V., Shchelkanov M. Yu. Retroviral infections of cats in Vladivostok, Russia. *The 2nd International Conference on Northeast Asia Biodiversity (August 27–31, 2019): Abstract Gather.* Baishan, China; 2019; 30. Available at: https://www.biosoil.ru/files/publications/00018494.pdf.

Received 28.10.2022
Revised 07.12.2022
Accepted 16.01.2023

INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Tatyana V. Tabakaeva, Researcher, Laboratory of Ectoparasitology, Somov Institute of Epidemiology and Microbiology, Vladivostok, Russia; *https://orcid.org/0000-0002-9517-7495, e-mail: rabchan1992@gmail.com.*

Alexander V. Tsybulsky, Candidate of Science (Medicine), Associate Professor, Department of Biochemistry, Microbiology and Biotechnology, Far Eastern Federal University, Vladivostok, Russia; *https://orcid.org/0000-0002-5408-1454, e-mail: avt_botech@mail.ru.*

Ksenya A. Baranchugova, Post-Graduate Student, Veterinarian, Far Eastern Federal University, Vladivostok, Russia; *https://orcid.org/0000-0003-3617-9711, e-mail: baranchugovaka@dvfu.ru.*

Табакаева Татьяна Владимировна, научный сотрудник лаборатории эктопаразитологии, ФГБНУ «НИИ эпидемиологии и микробиологии им. Г. П. Сомова» Роспотребнадзора, г. Владивосток, Россия; *https://orcid.org/0000-0002-9517-7495, e-mail: rabchan1992@gmail.com*.

Цыбульский Александр Васильевич, кандидат медицинских наук, доцент кафедры биохимии, микробиологии и биотехнологии, Дальневосточный федеральный университет, г. Владивосток, Россия; *https://orcid.org/0000-0002-5408-1454, e-mail: avt_botech@mail.ru*.

Баранчугова Ксения Александровна, аспирант, ветеринарный врач, Дальневосточный федеральный университет, г. Владивосток, Россия; *https://orcid.org/0000-0003-3617-9711, e-mail: baranchugovaka@dvfu.ru*.

Irina V. Galkina, Candidate of Science (Medicine), Leading Researcher, Laboratory for Microorganism Ecology, Far Eastern Federal University, Vladivostok, Russia; *https://orcid.org/0000-0002-7899-555X, e-mail: galkina333@mail.ru*.

Mikhail Yu. Shchelkanov, Doctor of Science (Biology), Director, Somov Institute of Epidemiology and Microbiology, Vladivostok, Russia; *https://orcid.org/0000-0001-8610-7623, e-mail: adorob@mail.ru.*

Галкина Ирина Вячеславовна, кандидат медицинских наук, ведущий научный сотрудник лаборатории экологии микроорганизмов, Дальневосточный федеральный университет, г. Владивосток, Россия; *https://orcid.org/0000-0002-7899-555X, e-mail: galkina333@mail.ru*.

Щелканов Михаил Юрьевич, доктор биологических наук, директор ФГБНУ «НИИ эпидемиологии и микробиологии им. Г. П. Сомова» Роспотребнадзора, г. Владивосток, Россия; *https://orcid.org/0000-0001-8610-7623, e-mail: adorob@mail.ru.*

DOI: 10.29326/2304-196X-2023-12-1-38-44

Features of model coronaviruses distribution in feline organs and tissues in the context of COVID-19 pathogenesis study

V. N. Afonyushkin[1,2,3], O. E. Sysoeva[1], O. S. Kozlova[1], D. V. Shamovskaya[3]

[1] FSSFEI HE "Novosibirsk State Agricultural University" (FSSFEI HE Novosibirsk SAU), Novosibirsk, Russia

[2] Siberian Federal Research Center of Agrobiotechnologies of the Russian Academy of Sciences (SFSCA RAS), Krasnoobsk, Novosibirsk Oblast, Russia

[3] Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of the Russian Academy of Sciences (ICBFM SB RAS), Novosibirsk, Russia

SUMMARY

To date, there is reason to believe that, unlike classical acute respiratory virus infections caused by adenoviruses, rhinoviruses, orthomyxoviruses, COVID-19 behaves completely differently. Firstly, the pathological processes are likely to be immune-mediated and the immune system quite slowly ensures the elimination of the virus from the organism. Secondly, the dynamics of the disease symptom development and the duration of intestinal virus shedding after recovery give reason to believe that the SARS-CoV-2 infection is mainly localized in the intestine. A possible reason is that in the presence of proteolytic enzymes, viral particles mature, hydrophilic amino acids are removed from the surface of the virion, making it more hydrophobic and able to adhere to cells due to hydrophobic interactions. The presence of the ACE2 receptor mainly in the enterocytes of the ileum does not exclude the accumulation of coronavirus in lymphocytes, given that there are more lymphocytes in the gastrointestinal tract than anywhere else, this fact can be considered as another justification for the predominant accumulation of coronaviruses, including SARS-CoV-2 in the intestine. A distinctive feature of feline coronavirus infection and, in particular, infectious feline peritonitis, from human COVID-19 infection was considered to be the presence of effusion peritonitis as the main complication leading to death, while respiratory and cardiovascular insufficiency is more characteristic for humans. Nevertheless, cases of serous peritonitis in humans infected with COVID-19 have already been described. In the context of the analyzed model, the clinical case described in the study allows principal possibility of exacerbation of chronic coronavirus infection in case of re-infection (superinfection) and development of a predominantly local infection.

Keywords: cats, coronavirus, COVID-19, FCoV, SARS-CoV-2, pathoanatomical examination, histological examination

Acknowledgements: This work was supported by the Russian state-funded project for ICBFM SB RAS (grant number 121031300043-8).

For citation: Afonyushkin V. N., Sysoeva O. E., Kozlova O. S., Shamovskaya D. V. Features of model coronaviruses distribution in feline organs and tissues in the context of COVID-19 pathogenesis study. *Veterinary Science Today*. 2023; 12 (1): 38–44. DOI: 10.29326/2304-196X-2023-12-1-38-44.

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

For correspondence: Olga S. Kozlova, Senior Lecturer, Department of Veterinary and Sanitary Examination and Parasitology, FSSFEI HE Novosibirsk SAU, 630039, Russia, Novosibirsk, ul. Dobrolyubova, 160, *e-mail: loi-2005@yandex.ru*.

УДК 619:578.834.1:636.8:616-036.12:616.9-022.39

Изучение особенностей распределения модельных коронавирусов в органах и тканях кошачьих в контексте изучения патогенеза COVID-19

В. Н. Афонюшкин[1,2,3], О. Е. Сысоева[1], О. С. Козлова[1], Д. В. Шамовская[3]

[1] ФГБОУ ВО «Новосибирский государственный аграрный университет» (ФГБОУ ВО Новосибирский ГАУ), г. Новосибирск, Россия

[2] ФГБУН Сибирский федеральный научный центр агробиотехнологий Российской академии наук (СФНЦА РАН), р. п. Краснообск, Новосибирская обл., Россия

[3] ФГБУН Институт химической биологии и фундаментальной медицины Сибирского отделения Российской академии наук (ИХБФМ СО РАН), г. Новосибирск, Россия

РЕЗЮМЕ

На сегодняшний день есть основание считать, что в отличие от классических острых респираторных вирусных инфекций, вызываемых аденовирусами, риновирусами, ортомиксовирусами, COVID-19 ведет себя совершенно по-другому. Во-первых, патологические процессы скорее являются иммуноопосредованными, и иммунитет довольно медленно обеспечивает элиминацию вируса из организма. Во-вторых, динамика развития симптомов заболевания, длительность вирусовыделения из кишечника после переболевания дают основание считать, что SARS-CoV-2 находится преимущественно в кишечнике. Возможной причиной этого является то, что в присутствии протеолитических ферментов происходит созревание вирусных частиц, удаление гидрофильных аминокислот с поверхности вириона делает его более гидрофобным и способным прилипать к клеткам за счет гидрофобных взаимодействий.

Наличие рецептора ACE2 главным образом в энтероцитах подвздошной кишки не исключает накопление коронавируса в лимфоцитах. Учитывая, что лимфоцитов в желудочно-кишечном тракте больше, чем где-либо, этот факт можно рассматривать как еще одно обоснование преимущественного накопления коронавирусов, в т. ч. SARS-CoV-2, в кишечнике. Отличительной чертой коронавирусной инфекции кошек, и в частности инфекционного перитонита кошек, от COVID-19 человека считалось наличие выпотного перитонита в качестве основного осложнения, ведущего к смерти, в то время как для людей более характерна дыхательная и сердечно-сосудистая недостаточность. Тем не менее уже описаны случаи развития серозного перитонита у людей на фоне COVID-19. В контексте анализируемой модели описанный в работе клинический случай допускает принципиальную возможность обострения хронической коронавирусной инфекции при повторном заражении (суперинфекции) с развитием преимущественно локальной инфекции.

Ключевые слова: кошки, коронавирус, COVID-19, FCoV, SARS-CoV-2, патолого-анатомическое исследование, гистологическое исследование

Благодарности: Исследование поддержано в рамках государственного задания ИХБФМ СО РАН № 121031300043-8.

Для цитирования: Афонюшкин В. Н., Сысоева О. Е., Козлова О. С., Шамовская Д. В. Изучение особенностей распределения модельных коронавирусов в органах и тканях кошачьих в контексте изучения патогенеза COVID-19. *Ветеринария сегодня*. 2023; 12 (1): 38–44. DOI: 10.29326/2304-196X-2023-12-1-38-44.

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

Для корреспонденции: Козлова Ольга Сергеевна, старший преподаватель кафедры ветеринарно-санитарной экспертизы и паразитологии ФГБОУ ВО Новосибирский ГАУ, 630039, Россия, г. Новосибирск, ул. Добролюбова, д. 160, *e-mail: loi-2005@yandex.ru.*

INTRODUCTION

The global coronavirus pandemic has changed life and affected not only human but animal health as well. The circulation of SARS-CoV-2 was most often detected in domestic cat population. It should be noted that among pets with confirmed laboratory diagnosis there were animals that had contacts with SARS-CoV-2-infected owners and sheltered (stray) animals [1].

Two serotypes (I and II) of feline coronaviruses (FCoVs) are circulating in feline population. Each serotype is represented by two biotypes with different pathogenicity: feline enteric coronavirus (FECV) and feline infectious peritonitis virus (FIPV) [2]. The leading theory of feline infectious peritonitis (FIP) development is based on the assumption that FIPV emerges as a result of somatic mutations in the *de novo* FECV genome, leading to changes in the FECV tropism for enterocytes to tropism for monocytes and macrophages. The more pathogenic FIPV replicates in macrophages [3].

Infectious peritonitis causes animal mortality in 100% cases and is accompanied with liver dysfunction, often pulmonary edema and damage to the central nervous system [4]. Although the prevalence of FCoV infection in feline population is high, the FIP infection rate is quite low and rarely exceeds 5% of FCoV-infected cats raised in multiple-cat households [5].

The FCoV genome is represented by a single-stranded RNA encoding four structural proteins (the spike (S), envelope (E), membrane (M) and nucleocapsid (N) proteins) and seven non-structural proteins [6]. The FCoV spike (S) protein is considered the viral regulator of binding and entry to the cell, and it is also involved in FCoV tropism and virulence. Mutations in the S protein coding sequence of FCoVs appear to be responsible for the change in viral tropism from enterocytes to monocytes and macrophages. As a result, FCoV enters the bloodstream and causes peritonitis [7].

There are similarities between FCoV and SARS-CoV-2:

1. Not all cats exposed to FCoV become infected, not all infected cats remain FCoV carriers for a long time, only a few cats infected with FCoV die from infectious peritonitis.

2. Severe damage to the respiratory system in humans with COVID-19, as well as multiple organ failure in cats with FIP, are associated with the immune system response when immunocompetent cells damage organs and tissues [8].

The data published by a group of Chinese researchers show that cats are able to develop an immune response to SARS-CoV-2 [9]. Moreover, SARS-CoV-2 can be transmitted from cat to cat [10]. The question of whether this is followed by the development of an infectious process in a cat or whether it leads to a change in the course of an already existing coronavirus infection remains open. Besides, it is not clear, whether cats play a role in the spread of SARS-CoV-2 among humans, whether humans are able to develop an immunological response to FCoV and, if possible, whether it affects the course of COVID-19-associated infection?

The dry form of infectious feline peritonitis appears to be most similar to the acute form of COVID-19 in humans [11]. Similarities include vasculitis, the immune-mediated nature of organ and tissue damage (including antibody-dependent infection enhancement, ADE), dissemination of the pathogen from the gastrointestinal tract [12].

The aim of the paper is to study the distribution of model coronaviruses in feline organs and tissues in the context of COVID-19 pathogenesis study.

MATERIALS AND METHODS

The concept of the pathogenesis of combined coronavirus superinfection in humans was considered based on the example of studying the pathogenetic mechanisms of both SARS-CoV-2 and FCoV infections in cats.

The research was conducted in the animal facility of the SFSCA RAS. The study was aimed at the cats that died from various forms of infectious peritonitis after contact with infected owners, as well as at the cats that were PCR-positive for FCoV and SARS-CoV-2 genome RNA. The qDNA and samples of formaline-fixed feline and human biomaterial were tested using reverse transcription polymerase chain reaction (RT-PCR) and immunofluorescence microscopy.

The study of the FCoV distribution in FIP-affected animals was carried out using the real-time RT-PCR. RNA was isolated from internal organs using phenol-chloroform extraction method, a cloned fragment of a targeted gene was used as a control. Quantification of the control DNA was carried out using FCoV-specific digital PCR. For control of RNA expression and normalization, the bacteriophage MS2 RNA (an external control sample that was added to each reaction) and the mRNA of feline housekeeping genes (GAPDH) were evaluated.

The pathology specimens were fixed in 10% buffered formalin. After histological treatment, the paraffin sections were dewaxed in xylene, then the xylene was removed with ethanol and the sections were immersed in 100 mM Tris-HCl solution. Antigens were unmasked by heating in a microwave oven in a citrate buffer solution (pH 6.0).

The cell nuclei were stained with Hoechst 33258 (Invitrogen, USA), the S-protein fragment was detected using rabbit polyclonal antibodies SARS Coronavirus Spike Protein Antibody Cat. No. PA1-41375 (Invitrogen, USA). The reaction was carried out in 0.05 M phosphate buffered saline (pH 7.2) with the addition of 0.02% Tween-20 (PBS-T) and 0.3% bovine serum albumin. Donkey Anti-Rabbit IgG (H+L), Mouse/Rat/Human SP ads-AF555, Cat. No. 6440-32 (SouthernBiotech, USA) were used as a conjugate in the same buffer solution. The reaction was carried out at room temperature for an hour. The unbound PBS-T antibodies (pH 7.2) were washed with distilled water, enclosed in glycerin and subjected to microscopy using an Imager D1 fluorescent microscope (Zeiss, Germany) and AxioVision software. Luminescence was detected using light filter systems FS 49, FS 10, FS 20. Similar histological products containing no primary antibodies were used for control of nonspecific binding of the conjugate.

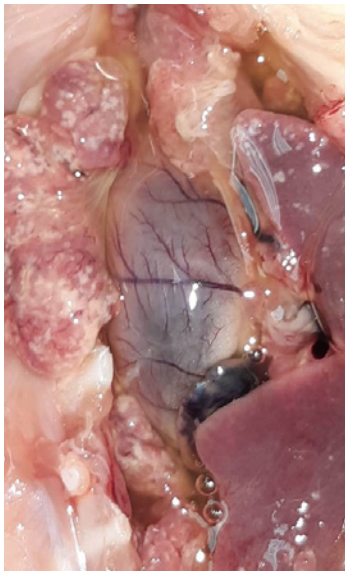

Fig. 1. Lesions in the thymus, pulmonary edema, hydrothorax

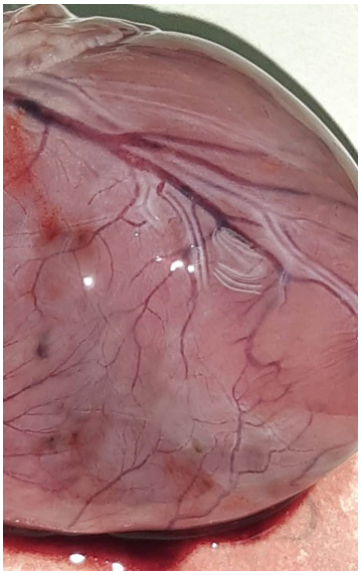

Fig. 2. Right-sided myocarditis, myogenic dilatation and hemorrhages with areas of focal hyperemia and ischemia

RESULTS AND DISCUSSION

Study of the FCoV distribution in organs and tissues of a kitten with a dry form of FIP (clinical case). Case history: all kittens from the same litter at the age of two months got diseased showing signs of rhinorrhea, diarrhea and depression. One of the kittens began to lose weight at the age of five months and was euthanized with a diagnosis of a dry form of FIP. The diagnosis was based on the detection of genomic RNA of FCoV in a pleural transudate. Also, at the time of diagnosis establishment, the concentration of urea in sera decreased to 3.5 mmol/L, the level of triglycerides increased to 95 g/L, globulins – up to 70 g/L, the ratio of albumin/globulin was 0.4. Other biochemical parameters (glucose, creatinine, albumin, alanine aminotransferase, alkaline phosphatase) were within normal limits. Neutrophils, single macrophages and lymphocytes were identified in a pleural smear. Hematocrit was lowered to 21.6%, hemoglobin was 9% below normal, eosinophils were 90% below normal, platelet count – 2% lower (171 cells/μL). The other hematological parameters were within the normal range. Tomography showed a large amount of fluid in the thoracic cavity, areas of higher-density infiltration, increased X-ray density in cranial lobes of the lungs with areas of consolidation. Pulmonary interstitial density was observed mainly in the area of the cranial lobe of the left lung, bronchiectases were also noted.

The animal carcass was subjected to necropsy immediately after euthanasia. The following organs were collected for testing: the brain (pituitary gland, frontal lobe of the cerebral cortex, corpus callosum, olfactory bulb, parietal region of the brain, cerebellum, dura mater), lymph nodes (mesenteric, pharyngeal, mediastinal, axillary, submandibular), blood samples (from the portal vein, left and right heart ventricles), lung, choanae, larynx, trachea, thoracic transudate, organs of the gastrointestinal tract (duodenum, colon, liver), thymus, spleen, parotid salivary gland.

Analysis of pathophysiological and pathologic-anatomical mechanisms of organ system damage in cats with FIP in combination with SARS-CoV-2 infection. Pulmonary edema, inflammatory changes in the thymus, hyperplasia of mediastinal lymph nodes, accumulation of fluid in the thoracic cavity were observed during the necropsy. Noteworthy is also myogenic dilatation and hemorrhages in the myocardium of the right heart ventricle (Fig. 1, 2). The trachea and bronchi had no changes, but the choanae were hyperemic.

Necropsy of abdominal organs showed inflammatory changes mainly in the mesenteric lymph nodes (Fig. 3). No inflammatory changes in the gastrointestinal mucosa or signs of pyogranulomatous polyserasitis were detected. No fluid was present in the abdomen. The spleen was also uneven in colour, splenitis manifestations were observed.

Assessment of FCoV distribution in a kitten. Anatomical mapping of the FCoV distribution made it possible

to detect viral RNA in the intestinum colon, blood of the right and left heart ventricles, mediastinal lymph node, portal vein, thoracic transudate, thymus and lung.

The virus was not detected in other parts of the gastrointestinal tract and lymph nodes, choanae, brain, liver and kidneys, or its concentration was below the detection threshold.

The largest amount of FCoV was found in the colon, the viral load was 3.02 times less in the lung, and the concentration of the pathogen was 1.46 times less in the thymus than in the lung. The amount of the viral RNA was 2.8 times larger in the blood of the left heart ventricle than the right one, and 5.7 times less in the portal vein than in the blood of the right ventricle (Fig. 4).

Correlation of the distribution of the SARS-CoV-2 antigen in human lungs and internal organs of a cat with combination of COVID-19 and FIP infection. As the study results by some authors have shown, from the histological picture observed when the human lungs are affected by the SARS-CoV-2 virus, it follows that the viral antigen (a fragment of S-protein between the sites of proteolytic cleavage by furin and TMPRSS2), in addition to the affected cells of the respiratory epithelium and macrophages, is detected on erythrocytes [13]. A fragment of a coronavirus spike protein is not necessarily associated with viral particles. Nevertheless, in the context of immune-mediated lung damage, the presence of this antigen may have pathogenetic significance.

It is an interesting fact that not all red blood cells in the lung tissue are stained with antibodies to SARS-CoV-2 (they are intensely black under luminescent microscopy) [14]. Also, the immune staining reaction was absent in preparations containing no primary antibodies, which reduces the likelihood of an artifact (but does not definitively exclude it).

The SARS-CoV-2 S-protein antigen is also visualized in the lungs of a cat that is PCR-positive for the presence of SARS-CoV-2 genomic RNA (Fig. 5), however, it is observed mainly on the apical surface of the cells of the respiratory epithelium, but not within the interstitium or blood vessels. Thickening of the interalveolar septa and signs of karyolysis, including in the absence of localization of this antigen, do not allow us to assume a topological relationship between the localization of the antigen and the pathological process (see also RT-PCR results for FCoV, shown in Figure 4).

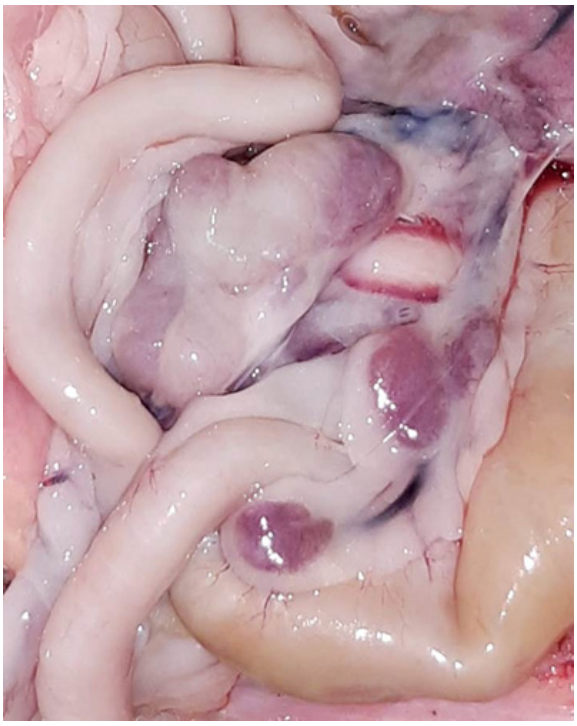

Fig. 3. Inflammation of mesenteric lymph nodes

Despite the fact that based on PCR data there are quite large amounts of FCoV in the intestine, the SARS-CoV-2-related virus was not detected in the intestine either using PCR or immunofluorescence technique (Fig. 6). The absence of a signal also makes it possible to exclude non-specific binding of fluorescently labeled antibodies to erythrocytes.

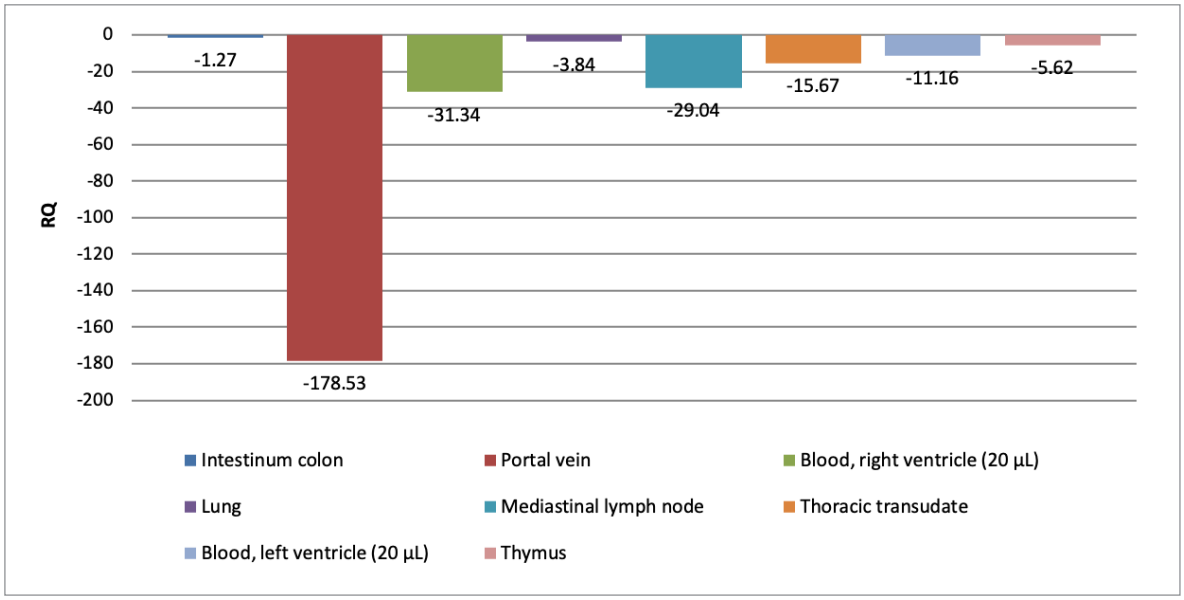

Fig. 4. Relative quantity of FCoV genomic RNA (ddCt) in different organs of a kitten

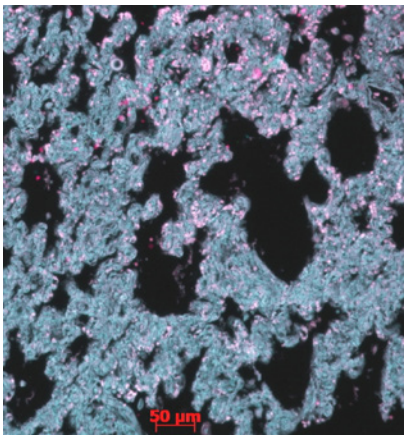

Fig. 5. SARS-CoV-2 antigen distribution in feline lung tissue (immunofluorescence, Hoechst 33258 staining of anti-SARS-CoV-2 S-protein antibody and AlexaFluor 555-labeled conjugate, 150× magnification)

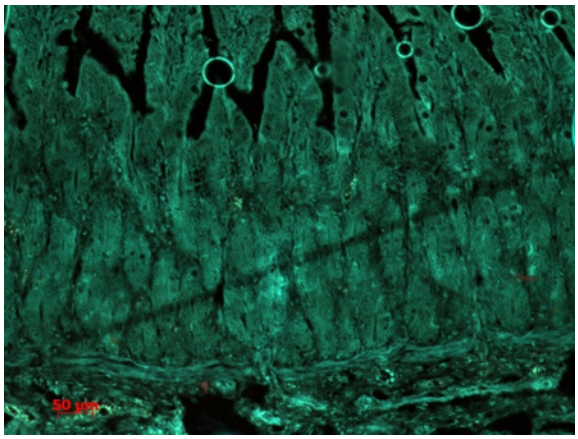

Fig. 6. Distribution of SARS-CoV-2 antigen in feline jejunal tissue (immunofluorescence, Hoechst 33258 staining of anti-SARS-CoV-2 S protein antibody and AlexaFluor 555-labeled conjugate, 150× magnification)

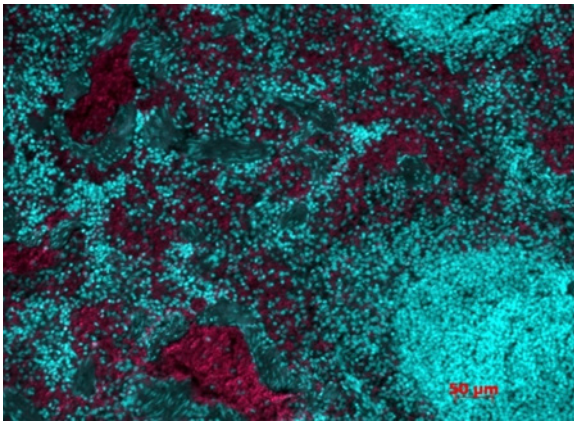

Fig. 7. Distribution of SARS-CoV-2 antigen in feline spleen (immunofluorescence, Hoechst 33258 staining of anti-SARS-CoV-2 S protein antibody and AlexaFluor 555-labeled conjugate, 150× magnification)

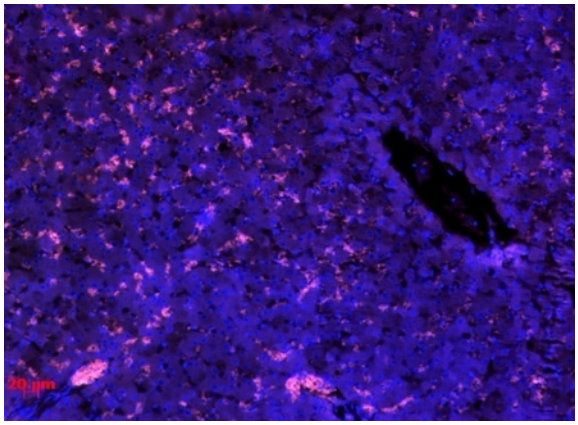

Fig. 8. Distribution of SARS-CoV-2 antigen in liver (immunofluorescence, Hoechst 33258 staining of anti-SARS-CoV-2 S protein antibody and AlexaFluor 555-labeled conjugate, 150× magnification)

The largest amount of SARS-CoV-2 S-antigen is observed in the spleen, but mainly in the composition of red blood cells in the lumen of blood vessels, red pulp and macrophages. No antigen was detected in the lymphoid nodules of the spleen (Fig. 7).

The data of immunofluorescence analysis of the liver present the greatest interest. The SARS-CoV-2 S-protein antigen is distributed in capillaries mainly located perilobularly (Fig. 8). It is also found in the lumen of the hepatic arteries, but not in the branches of the portal vein. These data are consistent with the results of the detection of the analyzed virus protein in the lungs, but not in the intestine. The uneven microcirculation of blood from the hepatic artery and portal vein suggests the formation of centrilobular hypoxia due to immune-mediated aggregation of erythrocytes (entering the liver from the lungs) or vasoconstriction caused by the interaction of S-protein with ACE2 receptors of blood vessels.

Analysis of the distribution of viral RNA in blood vessels allows us to speculate about the possibility of both hematogenic and lymphogenic transport of coronaviruses from the intestine to the small pulmonary circulation (Fig. 4). The unequal amount of viral RNA in the portal vein, liver and right heart ventricle gives only two possibilities for a sharp increase in the concentration of the virus in the right ventricle: 1) a large amount of viral particles can form in the liver (despite the fact that viral RNA was not detected by RT-PCR), 2) a significant part of the viral particles enters the lymphogenic pathways either from the intestine or from mesenteric lymph nodes (however, viral RNA was not detected by PCR). The uneven nature of inflammatory processes in mesenteric lymph nodes should be noted.

The asymmetric myocardial injury (right-sided myocarditis and myogenic dilatation, Fig. 2) suggests a longer-term entry of the viral agent to the lungs from the large circulatory circle, and not from the upper respiratory tract, where the virus is not detected. Despite the lung injury due to coronavirus causing a condition incompatible with life, RT-PCR proved to be an insufficiently sensitive method for detecting the virus in the nose. This fact makes it possible to doubt the high efficacy of PCR diagnosis of coronavirus pneumonia, including in humans, when trying to detect the virus in the nasal passages or oral cavity.

Various forecasts can be made based on the above assumptions. The virus has to shed in the intestine for

a long time (and this forecast has been realized), including in humans with antibodies. The incubation period for this type of virus reproduction and distribution can be quite long and/or accompanied with asymptomatic carrying (it would be difficult to imagine a long-term asymptomatic virus carrying in the bronchi and trachea).

This hypothesis also explains that intestinal lesions are recognized as the earliest COVID-19 manifestations. A sharp increase in the virus concentration in the lungs may occur when the virus is identified in the intestine by immune cells, intestinal inflammation develops, intestinal perfusion increases and, accordingly, the viruses, activated T cells and neutrophils massively enter the right atrium and ventricle through the caudal vena cava (which should mainly cause right-sided heart damage).

In order for the SARS-CoV-2 RNA to get packaged into the capsid of another virus, it should specifically bind to the N nucleocapsid protein. M protein is likely to participate in this process [15]. That is, this process cannot occur with all types of coronaviruses. However, it seems possible to compare the RNA secondary structures of different coronaviruses and N amino acid sequences (preferably, tertiary structures) and predict the risks of spillover between different species using bioinformatics methods. If we take into account the similarity of FCoV and SARS-CoV-2, the formation of a viral RNA complex of *Betacoronavirus* genus representatives with the nucleocapsid protein of the genome of the infectious bronchitis virus (IBV) is unlikely.

CONCLUSION

The results obtained in the study helped to specify some epizootological characteristics of the disease, confirm and supplement the available clinical data on animals infected with FCoV and SARS-CoV-2, as well as facilitated the study of pathologic and anatomical picture and histological changes typical of this pathology.

The study provided evidence for the mechanisms of coronavirus distribution and spread from the organs where the greatest replication of the virus occurs (thymus and intestines) to the lungs, where there is an accumulation of viral particles trapped by the hematogenic pathway in a smaller volume relative to other target organs. Intestinal lesions are considered among the COVID-19 earliest manifestations [16]. A sharp increase in the virus concentration in the lungs may occur when the virus is identified in the intestine by immune cells, intestinal inflammation develops, intestinal perfusion increases and, accordingly, viruses, activated T cells and neutrophils massively enter the right atrium and ventricle through the caudal vena cava (which should mainly cause right-sided heart damage).

In the context of the analyzed model, the clinical case described in the study allows principal possibility of exacerbation of chronic coronavirus infection in case of re-infection (superinfection) and development of a predominantly local infection. The presented data also shed a new light on red blood cells. The unevenness of the erythrocyte population in relation to the presence of the S-protein antigen gives reason to consider them as false targets when interacting with the virus or as carriers of adsorbed virus antigens. The revealed facts require further studies.

REFERENCES

1. Kupriianov I. I. Circulation of SARS-CoV-2 and manifestation of COVID-19 in house cat (*Felis catus*). *Animal Agriculture and Veterinary Medicine*. 2022; 2 (45): 59–65. eLIBRARY ID: 49056737. (in Russ.)

2. Jaimes J. A., Millet J. K., Stout A. E., André N. M., Whittaker G. R. A tale of two viruses: the distinct spike glycoproteins of feline coronaviruses. *Viruses*. 2020; 12 (1):83. DOI: 10.3390/v12010083.

3. Kulikov E. V., Vatnikov Y. A., Sakhno N. V., Popova I. A., Gnezdilova L. A., Kuznetsov V. I., Strizhakov A. A. Pathologicoanatomical characteristics of feline infectious peritonitis. *RJOAS*. 2017; 4 (64): 270–280. DOI: 10.18551/rjoas.2017-04.34. (in Russ.)

4. Chang H. W., Egberink H. F., Halpin R., Spiro D. J., Rottier P. J. Spike protein fusion peptide and feline coronavirus virulence. *Emerg. Infect. Dis*. 2012; 18 (7): 1089–1095. DOI: 10.3201/eid1807.120143.

5. Jaimes J. A., Whittaker G. R. Feline coronavirus: Insights into viral pathogenesis based on the spike protein structure and function. *Virology*. 2018; 517: 108–121. DOI: 10.1016/j.virol.2017.12.027.

6. Khaitovich A. B. Coronavirus (genome structure, replication). *Crimea Journal of Experimental and Clinical Medicine*. 2020; 4 (10): 78–95. DOI: 10.37279/2224-6444-2020-10-4-78-95. (in Russ.)

7. Myrrha L. W., Silva F. M., Peternelli E. F., Junior A. S., Resende M., de Almeida M. R. The paradox of feline coronavirus pathogenesis: a review. *Adv. Virol*. 2011; 2011:109849. DOI: 10.1155/2011/109849.

8. Giordano A., Spagnolo V., Colombo A., Paltrinieri S. Changes in some acute phase protein and immunoglobulin concentrations in cats affected by feline infectious peritonitis or exposed to feline coronavirus infection. *Vet. J*. 2004; 167 (1): 38–44. DOI: 10.1016/s1090-0233(03)00055-8.

9. Zhang Q., Zhang H., Huang K., Yang Y., Hui X., Gao J., et al. SARS-CoV-2 neutralizing serum antibodies in cats: a serological investigation. *bioRxiv*. 2020. DOI: 10.1101/2020.04.01.021196.

10. Halfmann P. J., Hatta M., Chiba S., Maemura T., Fan S., Takeda M., et al. Transmission of SARS-CoV-2 in domestic cats. *N. Engl. J. Med*. 2020; 383 (6): 592–594. DOI: 10.1056/NEJMc2013400.

11. Barsegyan L. S., Sukharev O. I., Kulikov Y. V. Feline infectious peritonitis virus infection (literature review). *Actual Questions of Veterinary Biology*. 2015; 1 (25): 16–23. eLIBRARY ID: 23118810. (in Russ.)

12. Terekhova Yu. O., Tsibezov V. V., Rakhmanina N. A., Verkhovsky O. A. Immunoblotting assay for detection of coronaviruses antibodies for feline infectious peritonitis (FIP) diagnosis. *Russian Veterinary Journal. Small Pets and Wild Animals*. 2012; 4: 26–28. eLIBRARY ID: 17863566. (in Russ.)

13. Massoth L. R., Desai N., Szabolcs A., Harris C. K., Neyaz A., Crotty R., et al. Comparison of RNA *in situ* hybridization and immunohistochemistry techniques for the detection and localization of SARS-CoV-2 in human tissues. *Am. J. Surg. Pathol*. 2021; 45 (1): 14–24. DOI: 10.1097/PAS.0000000000001563.

14. Buryachkovskaya L. I., Melkumyants A. M., Lomakin N. V., Antonova O. A., Ermishkin V. V. Injury of vascular endothelium and erythrocytes in COVID-19 patients. *Consilium Medicum*. 2021; 23 (6): 469–476. DOI: 10.26442/20751753.2021.6.200939. (in Russ.)

15. Jackwood M. W., Hall D., Handel A. Molecular evolution and emergence of avian gammacoronaviruses. *Infect. Genet. Evol*. 2012; 12 (6): 1305–1311. DOI: 10.1016/j.meegid.2012.05.003.

16. Sarsenbaeva A. S., Lazebnik L. B. Features of intestinal damage in COVID-19. *Experimental and clinical gastroenterology*. 2020; 184 (12): 16–22. DOI: 10.31146/1682-8658-ecg-184-12-16-22. (in Russ.)

Received 08.01.2023
Revised 20.01.2023
Accepted 26.01.2023

INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Vasiliy N. Afonyushkin, Candidate of Science (Biology), Head of Sector, SFSCA RAS, Krasnoobsk, Novosibirsk Oblast, Russia; Researcher of the ICBFM SB RAS, Novosibirsk, Russia; Associate Professor of the Department of Epizootology and Microbiology, FSSFEI HE Novosibirsk SAU, Novosibirsk, Russia; *https://orcid.org/0000-0001-5177-4733, e-mail: lisocim@mail.ru.*

Olga E. Sysoeva, 4th year Undergraduate Student of Veterinary and Sanitary Expert, Faculty of Veterinary Medicine, FSSFEI HE Novosibirsk SAU, Novosibirsk, Russia; *https://orcid.org/0000-0002-6361-7214, e-mail: olya.sysoeva@internet.ru*.

Olga S. Kozlova, Senior Lecturer, Department of Veterinary and Sanitary Examination and Parasitology, FSSFEI HE Novosibirsk SAU, Novosibirsk, Russia; *https://orcid.org/0000-0002-3191-7597, e-mail: loi-2005@yandex.ru.*

Daria V. Shamovskaya, Junior Researcher, ICBFM SB RAS, Novosibirsk, Russia; *https://orcid.org/0000-0003-2934-5451, e-mail: shamovskaya@gmail.ru.*

Афонюшкин Василий Николаевич, кандидат биологических наук, заведующий сектором СФНЦА РАН, р. п. Краснообск, Новосибирская обл., Россия; сотрудник ИХБФМ СО РАН, г. Новосибирск, Россия; доцент кафедры эпизоотологии и микробиологии ФГБОУ ВО Новосибирский ГАУ, г. Новосибирск, Россия; *https://orcid.org/0000-0001-5177-4733, e-mail: lisocim@mail.ru.*

Сысоева Ольга Евгеньевна, студентка 4-го курса бакалавриата ветеринарно-санитарной экспертизы факультета ветеринарной медицины, ФГБОУ ВО Новосибирский ГАУ, г. Новосибирск, Россия; *https://orcid.org/0000-0002-6361-7214, e-mail: olya.sysoeva@internet.ru.*

Козлова Ольга Сергеевна, старший преподаватель кафедры ветеринарно-санитарной экспертизы и паразитологии ФГБОУ ВО Новосибирский ГАУ, г. Новосибирск, Россия; *https://orcid.org/0000-0002-3191-7597, e-mail: loi-2005@yandex.ru.*

Шамовская Дарья Вадимовна, младший научный сотрудник ИХБФМ СО РАН, г. Новосибирск, Россия; *https://orcid.org/0000-0003-2934-5451, e-mail: shamovskaya@gmail.ru.*

DOI: 10.29326/2304-196X-2023-12-1-45-51

Development of polymerase chain reaction kit for detection of SARS-CoV-2 RNA in biological samples collected from animals

A. M. Timina, A. S. Yakovleva, M. V. Timanov, M. V. Biryuchenkova, Ye. S. Orlova
FGBI "Federal Centre for Animal Health" (FGBI "ARRIAH"), Vladimir, Russia

SUMMARY

Today, global attention is drawn to the same common problem – spread of the novel COVID-19 infection. From the end of December 2019 novel SARS-CoV-2 virus spread over the majority of the countries and on 11 March 2020 the World Health Organization announced pandemic. Global spread of COVID-19 was not limited to the human population and there was a need to test pets and farm animals, who were in contact with the humans. There are more and more reports on SARS-CoV-2 detected in minks, ferrets, dogs, cats, tigers, lions and other animals. Today the key method of COVID-19 diagnosis is polymerase chain reaction, but all currently available test-kits are intended for the virus detection in humans. The paper demonstrates data on the development of the real-time PCR-based method for SARS-CoV-2 RNA detection in the biological samples collected from animals. During the research, an optimal system of primers and a probe were selected, reaction conditions were tested, basic validation specifications (sensitivity, specificity, reproducibility) were set. The validation results demonstrated that the method met all the criteria of the high-quality measurement/test methods and it can be used for diagnostic tests. The test-kit was based of the method intended for SARS-CoV-2 RNA detection in animal biological samples and it was put into the veterinary practice. Animal populations in different regions of the Russian Federation were subjected to the screening tests in order to detect the novel coronavirus genome. No SARS-CoV-2 was reported in herbivorous animals in the Russian Federation. The FGBI "ARRIAH" experts detected only one positive pet animal.

Keywords: novel SARS-CoV-2, real-time polymerase chain reaction, test-kit, animal biological sample

Acknowledgements: The work was funded by the FGBI "ARRIAH" as a part of the research activities "Animal Health and Welfare". The authors are grateful to the specialists of the Smorodintsev Research Institute of Influenza (Ministry of Health of the Russian Federation) for the kindly provided human biological samples.

For citation: Timina A. M., Yakovleva A. S., Timanov M. V., Biryuchenkova M. V., Orlova Ye. S. Development of polymerase chain reaction kit for detection of SARS-CoV-2 RNA in biological samples collected from animals. *Veterinary Science Today*. 2023; 12 (1): 45–51. DOI: 10.29326/2304-196X-2023-12-1-45-51.

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

For correspondence: Anna M. Timina, Candidate of Science (Veterinary Medicine), Senior Researcher, Reference Laboratory for Highly Dangerous Diseases, FGBI "ARRIAH", 600901, Russia, Vladimir, Yur'evets, *e-mail: timina@arriah.ru.*

УДК 619:578.834.1:616-076

Разработка тест-системы для обнаружения РНК вируса SARS-CoV-2 в биоматериале от животных методом полимеразной цепной реакции

А. М. Тимина, А. С. Яковлева, М. В. Тиманов, М. В. Бирюченкова, Е. С. Орлова
ФГБУ «Федеральный центр охраны здоровья животных» (ФГБУ «ВНИИЗЖ»), г. Владимир, Россия

РЕЗЮМЕ

Сегодня внимание всего мирового сообщества приковано к одной общей проблеме – распространению новой коронавирусной инфекции (COVID-19). С конца декабря 2019 г. коронавирус SARS-CoV-2 охватил большинство стран мира, и 11 марта 2020 г. Всемирной организацией здравоохранения была объявлена пандемия. Глобальное распространение COVID-19 не ограничилось человеческой популяцией, и возникла необходимость тестирования домашних и сельскохозяйственных животных, находящихся в контакте с человеком. Появляется все больше сообщений о выявлении SARS-CoV-2 у норок, хорьков, собак, кошек, тигров, львов и других животных. Основным методом диагностики COVID-19 на сегодняшний день является полимеразная цепная реакция, однако все существующие в настоящее время тест-системы предназначаются для выявления вируса у людей. В статье представлены данные по разработке метода обнаружения РНК вируса SARS-CoV-2 в биоматериале от животных с помощью полимеразной цепной реакции в реальном времени. В процессе проведенных исследований выбрана оптимальная система праймеров и зонд, отработаны условия реакции, определены основные валидационные характеристики метода (чувствительность, специфичность, воспроизводимость). В результате проведения валидации установлено, что он отвечает требованиям, предъявляемым к качественным методам измерений/испытаний, и может применяться в диагностических исследованиях. На основе разработанного метода создана тест-система для выявления РНК вируса SARS-CoV-2 в биоматериале от животных, которая внедрена в вете-

ринарную практику. Проведены скрининговые исследования популяций животных из различных регионов Российской Федерации с целью выявления генома нового коронавируса. Показано, что среди травоядных животных Российской Федерации вирус SARS-CoV-2 не встречается. Среди домашних животных-компаньонов специалистами ФГБУ «ВНИИЗЖ» был зафиксирован лишь один положительный случай.

Ключевые слова: новый коронавирус SARS-CoV-2, полимеразная цепная реакция в реальном времени, тест-система, биоматериал от животных

Благодарности: Работа выполнена за счет средств ФГБУ «ВНИИЗЖ» в рамках тематики научно-исследовательских работ «Ветеринарное благополучие». Авторы благодарят сотрудников ФГБУ «НИИ гриппа им. А. А. Смородинцева» Минздрава России за предоставленные образцы биоматериала, полученного от людей.

Для цитирования: Тимина А. М., Яковлева А. С., Тиманов М. В., Бирюченкова М. В., Орлова Е. С. Разработка тест-системы для обнаружения РНК вируса SARS-CoV-2 в биоматериале от животных методом полимеразной цепной реакции. *Ветеринария сегодня*. 2023; 12 (1): 45–51. DOI: 10.29326/2304-196X-2023-12-1-45-51.

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

Для корреспонденции: Тимина Анна Михайловна, кандидат ветеринарных наук, старший научный сотрудник референтной лаборатории по особо опасным болезням ФГБУ «ВНИИЗЖ», 600901, Россия, г. Владимир, мкр. Юрьевец, *e-mail: timina@arriah.ru.*

INTRODUCTION

Similar to MERS-CoV and SARS-CoV novel coronavirus SARS-CoV-2 induces severe pneumonia in humans (COVID-19). It is one of the RNA-viruses of family *Coronaviridae*, lineage Beta-CoV B.

The first human cases of pneumonia of unknown origin were reported in Wuhan city, Hubei Province, China [1]. The novel coronavirus was isolated from the respiratory epithelial cells and identified as the disease agent [2]. In 2020, the majority of the countries reported the disease cases in humans, and the World Health Organization (WHO) announced COVID-19 pandemic [3, 4].

In patients COVID-19 is demonstrated with such pronounced clinical signs as fever, dry cough, dyspnea, rhinorrhea, acute tonsillitis, interstitial infiltrate in lungs [5].

Bat coronavirus (*Rhinolophus sinicus*) is considered to be the ancestor of SARS-CoV-2. The virus was transmitted from bats to humans via intermediate host, which was failed to be established [6–10]. Global spread of SARS-CoV-2 is not limited to human population. There are currently lots of reports of the novel coronavirus detected in animals: minks, dogs, cats, tigers and lions. Experimental infection demonstrated that ferrets and cats are highly susceptible to SARS-CoV-2 and they can transmit the virus from the infected animals to the healthy ones through contacts and air [11–17].

Susceptibility of the animals to SARS-CoV-2 could be explained by the fact that the receptor for the virus entry is angiotensin-converting enzyme 2 (ACE2), which is nearly identical and similar in humans and such animal species as mustelids, felines, suids and simian [16, 18, 19]. There is, therefore, a possibility of SARS-CoV-2 reservoir formation in pet population, and, hence, COVID-19 epidemic surveillance should involve diagnostic and monitoring tests of pets.

The recommended method of COVID-19-specific laboratory diagnosis is SARS-CoV-2 RNA detection using polymerase chain reaction (PCR). This tool is ideal for the primary screening owing to its high sensitivity and specificity [20–23].

Currently a sufficient amount of test-kits for SARS-CoV-2 detection in humans have been developed, but they are not intended for the analysis of biological samples from animals [24–27]. Therefore, development of a reliable, sensitive and specific method for COVID-19 diagnosis in animals is a relevant objective.

The research was aimed at the development of a test-kit for SARS-CoV-2 RNA detection in biological samples from animals using real-time polymerase chain reaction.

MATERIALS AND METHODS

Biological samples. The original SARS-CoV-2 isolate recovered from a human was kindly provided by the Smorodintsev Research Institute of Influenza (Ministry of Health of the Russian Federation). Biological samples collected from humans (nasal and oral swabs) and animals (nasal swabs, lungs) were used in the research. The test objects included both domestic animals (pigs, cattle, sheep, goats) and wild animals (boars, elks, deer, Manchurian wapiti, roe deer, yaks, antelopes, European bison, Caucasian turs, red deer, saiga antelopes, ibexes, musk deer, dzerens). For testing food products swabs were collected from the surfaces of meat products and meat preparations as well as from their package.

Reference strains. Specificity of the method was tested using the viruses belonging to *Coronaviridae* family: SARS-CoV-2, transmissible gastroenteritis virus (TGEV) and porcine epidemic diarrhoea virus (PEDV), porcine respiratory coronavirus (PRCoV), bovine coronavirus (BCoV), avian infectious bronchitis virus (IBV), as well as heterologous viruses: porcine reproductive and respiratory syndrome virus (PRRSV), porcine parvovirus (PPV), porcine circovirus type 2 (PCV-2), Aujeszky's disease virus (ADV), infectious bursal disease virus (IBDV), influenza A virus (IAV), infectious bovine rhinotracheitis virus (BHV-1), parainfluenza-3 virus (bPIV-3).

RNA extraction from 10%-suspension of the biological samples was performed using 6 M guanidinium thiocyanate and glass fiber filters GF/F [28]. Nucleic acid was

handled in the environment controlled according to MU 1.3.2569-09 "Organization of laboratories using nucleic acid amplification methods for the work with materials containing microorganisms I–IV pathogenicity groups".

Polymerase chain reaction. The test procedure is described below. Reaction PCR mixture was formulated, which contained 5 µL of 10× Taq-polymerase buffer, 3 mM Mg^{2+}, 0.2 mM dNTPs, 2 units of Taq-DNA polymerase, 5 pmol of each primer, 5 µL of DNA solution and water added to final volume of 50 µL. The reaction was performed using Mastercycler DNA amplification machine (Eppendorf, Germany). The test procedure included reverse transcription at 42 °C for 15 minutes and 35 amplification cycles at the following temperatures: denaturation at 94 °C for 30 seconds, annealing of primers at 55 °C for 30 seconds and elongation at 72 °C for 40 seconds. The test products were analyzed using 0.001% ethidium bromide 2% agararose gel electrophoresis at 50 mA.

Molecular cloning of SARS-CoV-2 N gene was performed using CloneJET PCR Cloning Kit (Thermo Fisher Scientific, USA) according to the manufacturer's instructions.

Nucleotide sequences. SARS-CoV-2 nucleotide sequences deposited in GenBank database were used in the study. The nucleotide sequences were analyzed using BioEdit software.

Real-time reverse transcription-polymerase chain reaction (real-time RT-PCR). Conditions and modes of the reaction are described below. All reactions were performed using C1000 Touch™ thermocycler and CFX96 detection system (Bio-Rad, USA).

Statistical processing of the data was performed using validation analytical techniques [29, 30]. The following performance properties were determined: sensitivity and specificity, precision under the repeatability and reproducibility conditions.

RESULTS AND DISCUSSION

Design of primers. By the start of the works on the development of the test-kit, the WHO had published several procedures on COVID-19 laboratory diagnosis on its website. As a result of their analysis, primers and probe was selected from the extensive list of oligonucleotides recommended for COVID-19 detection. The selected primers and probe were proposed by the Center for Disease Control and Prevention (CDC, USA):

Primer 1 – GACCCCAAAATCAGCGAAAT;

Primer 2 – TCTGGTTACTGCCAGTTGAATCTG;

Probe – ROX ACCCCGCATTACGTTTGGTGGACC BHQ2.

The chosen oligonucleotides are complimentary to SARS-CoV-2 N gene.

Development and validation of the method for SARS-CoV-2 RNA detection using real-time RT-PCR. Reaction mixture composition, temperature and time of the reaction were specified during the optimization procedure.

The reaction was performed with 25 µL of the mixture containing 0.5 µL (5 pm) of direct and indirect primers, 0.5 µL (5 pm) of the probe, 2.5 µL of the 10× PCR buffer, 4 µL of 25 mM $MgCl_2$, 0.7 µL of 25 mM dNTPs, 0.2 µL (1 unit) of Taq-DNA-polymerase, 0.4 µL (20 units) of M-MLV-reverse transcriptase, 1 µL of nuclease-free water and 5 µL of RNA.

The amplification program included reverse transcription phase at 42 °C for 15 minutes followed by denaturation at 95 °C for 5 minutes and 40 cycles of PCR itself (denaturation at 95 °C for 15 seconds, annealing at 55 °C for 15 seconds and elongation at 60 °C for 20 seconds).

Ct ≤ 35 was taken as the reaction threshold value, when the sample was considered positive.

Specificity of the method was tested using several types of viruses of *Coronaviridae* family (SARS-CoV-2, PRCoV, BCoV, TGEV, PEDV, IBV) and other agents of farm animal diseases (PPV, PCV-2, ADV and PRRSV, IBDV, IAV, BHV-1, bPIV-3), as well as RNA/DNA extracted from porcine, bovine and chicken tissues. The positive reaction was observed only with SARS-CoV-2 RNA, thus being indicative of the specificity of the method (Fig. 1).

The sensitivity was assessed by testing serial 10-fold dilutions of SARS-CoV-2 RNA.

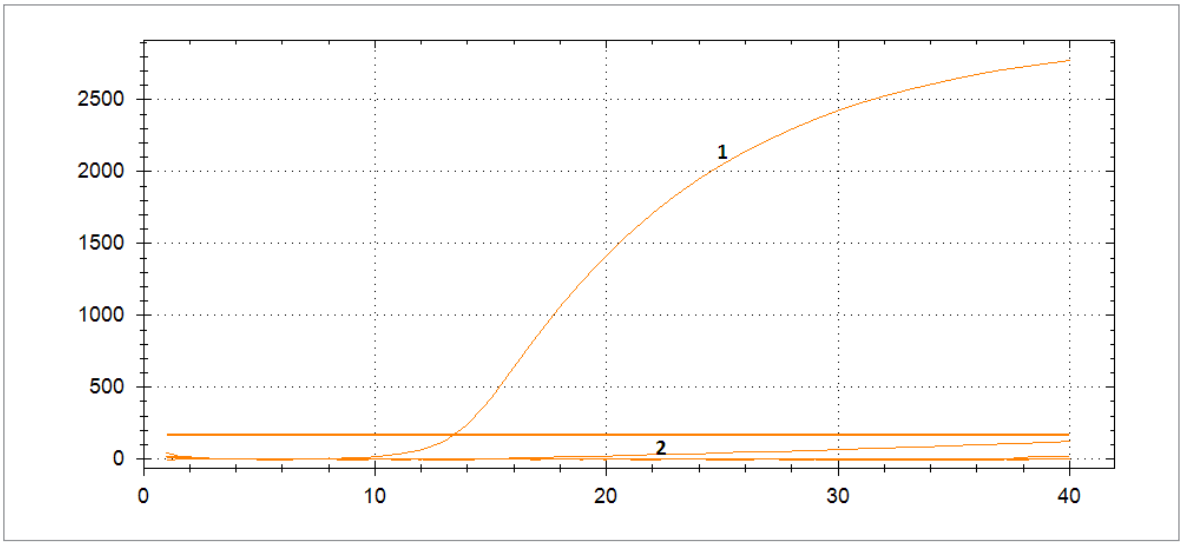

Fig. 1. Detection of SARS-CoV-2 in animal biological samples using real-time PCR:
1 – SARS-CoV-2-containing nasal swab;
2 – SARS-CoV-2-negative sample (TGEV, PEDV, PRCoV, BCoV, IBV, PRRSV, PPV, PCV-2, ADV, IBDV, influenza A virus, BHV-1, bPIV-3, tissue RNA of non-infected pigs, cattle, chickens)

Table 1
Samples used to test sensitivity of the developed test-kit

Sample No.	Description	Status of the sample	Result obtained using the test-kit being validated, *Ct* (interpretation of the result)
1	SARS-CoV-2 RNA sample	positive	14.21 (positive)
2	SARS-CoV-2 RNA sample	positive	14.47 (positive)
3	SARS-CoV-2 RNA sample	positive	16.16 (positive)
4	SARS-CoV-2 RNA sample	positive	18.49 (positive)
5	SARS-CoV-2 RNA sample	positive	21.81 (positive)
6	SARS-CoV-2 RNA sample	positive	19.56 (positive)
7	SARS-CoV-2 RNA sample	positive	23.33 (positive)
8	SARS-CoV-2 RNA sample	positive	24.04 (positive)
9	SARS-CoV-2 RNA sample	positive	22.98 (positive)
10	SARS-CoV-2 RNA sample	positive	23.21 (positive)

The sensitivity was determined as a percentage of positive results obtained in the test-kit being validated to the total number of tests and its was calculated according to the formula:

$$Se = (TP / (TP + FN)) \times 100\%,$$

where TP – true positive result;
FN – false negative result.

All tested SARS-CoV-2 RNA-containing samples demonstrated positive result with the test-kit being validated (Table 1). Therefore, the calculated sensitivity of the validated test-kit amounted to 100%.

Assessment of the test-kit reproducibility. The reproducibility was assessed by testing one positive sample and one negative sample in 10 repeated tests performed under the changed measurement conditions: by the same analyst in parallel tests performed on different days (10 days) and by two different analysts in parallel tests (in 10 repetitions). Precision was assessed by the degree of agreement between the repeated measurements of the same sample. The validated test-kit demonstrated absolute precision, i.e. the positive sample always demonstrated positive result and the negative sample – negative result.

It was therefore established that in its characteristics the real-time RT-PCR is compliant with the requirements to the high quality measurement/test methods and can be used in diagnostic studies.

Following the validation results, methodical guidelines were developed for the detection of SARS-CoV-2 RNA using real-time polymerase chain reaction. These guidelines were further used for COVID-19 diagnosis in animals.

Development of test-kit for SARS-CoV-2 RNA detection using real-time RT-PCR. Production of recombinant plasmid containing SARS-CoV-2 N gene. Any test-kit intended for general use shall meet safety requirements, therefore, a decision was taken to use the recombinant plasmid as positive control.

SARS-CoV-2 N gene region was PCR amplified using coronavirus RNA extracted at the Smorodintsev Research Institute of Influenza (Ministry of Health of the Russian Federation). Primers targeted at the conservative genome region were used for the reaction. Clones containing SARS-CoV-2 N gene region were resulted from the transformation by the ligase mixture of pJET1.2/blunt vector and amplified fragment of the viral gene of the competent *Escherichia coli* JM109 cells. Presence of specific DNA region was checked using nucleotide sequencing.

The recombinant plasmid physical map is shown in Figure 2.

At the next stage, real-time RT-PCR test-kit was developed, which contained specific oligonucleotide primers and fluorescent-labeled oligonucleotide probe. Test conditions were also selected using newly developed diagnosticum contributing to the minimal risk of the test sample contamination and excluding any biased assessments of the results.

For user's convenience the real-time RT-PCR test-kit for detection of SARS-CoV-2 RNA consists of the following components:

No. 1 – RT-PCR mixture;
No. 2 – Taq DNA-polymerase enzyme;
No. 3 – M-MLV reverse transcriptase;
No. 4 – positive control;
No. 5 – negative control.

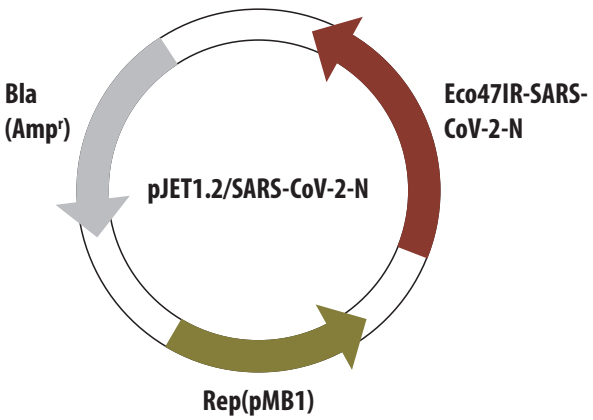

Fig. 2. Physical map of the recombinant plasmid pJET1.2/SARS-CoV-2-N

Table 2
Testing biological samples collected from herbivorous animals for SARS-CoV-2 using "SARS-CoV-2 real-time RT-PCR test-kit"

Animal species	Number of samples tested	Number of positives
pigs	980	0
wild boars	174	0
cattle	220	0
sheep and goats	20	0
wild even-toed ungulates (elks, deer, Manchurian wapiti, roe deer, yaks, antelopes, European bison, Caucasian turs, red deer, saiga antelopes, ibexes, musk deer, dzerens, etc.)	638	0

Fig. 3. "SARS-CoV-2 real-time RT-PCR test-kit" for detection of SARS-CoV-2 RNA in animal biological samples using real-time polymerase chain reaction

Deionized water is used as negative control, and plasmid DNA containing inserted SARS-CoV-2 N gene fragment was used as positive control. Each test-kit comes with the manufacturer's instructions (Fig. 3).

Real-time RT-PCR is performed in one step using programmable amplifier of any model and mixture of PCR reagents and enzymes. RNA can be extracted from the tested samples using any convenient method or commercial test-kit. Negative and positive controls are added to the relative samples.

The results are interpreted according to the presence (or absence) of fluorescence curve intersection with the threshold set at the appropriate level thus corresponding to the presence (or absence) of the cycle threshold value (*Ct*) in the relative column in the table of results. The result is considered positive if the fluorescence accumulation curve for the relative sample has a typical sigmoidal shape and intersects the threshold line.

There are currently lots of reports of SARS-CoV-2 detection in animals (minks, ferrets, cats, dogs, etc.). There is also evidence of the virus detection in herbivorous animals (deer). During the developed method implementation biological samples available in large amounts were used (nasal swabs, esopharyngeal fluids, internal organs, blood) and collected from pigs, cattle, sheep, goats, and wild cloven-hoofed animals. However, no SARS-CoV-2 was detected in these animal species (Table 2). We also failed to detect the viral RNA in the samples of raw meat, meat preparations and in the swabs from their package.

In 2020–2021, the FGBI "ARRIAH" carried out SARS-CoV-2 RNA screening tests of the animal populations in 20 regions of the Russian Federation using the developed test-kit. The published earlier results [11] demonstrated that out of 1,466 tested biological samples from different animal species, only one esopharyngeal swab collected from the domestic cat in Tyumen demonstrated SARS-CoV-2 RNA [11]. The genome of the novel coronavirus was not detected in any of the biological samples collected from the herbivorous animals. Therefore, use of the developed "SARS-CoV-2 real-time RT-PCR test-kit" demonstrated possibility of SARS-CoV-2 detection in pets.

CONCLUSION

The performed studies resulted in the development of the method for SARS-CoV-2 RNA detection using RT-PCR, determination of the validation specifications of the method and preparation of the "SARS-CoV-2 real-time RT-PCR test-kit". The test-kit was used for the analysis of a large number of samples collected from various animal species. The proposed test-kit was put into veterinary practice for the detection of SARS-CoV-2 RNA in animal biological samples within primary and confirmatory diagnosis, for research purposes and monitoring of SARS-CoV-2 spread in animals. The resulted performance specifications of the test-kit comply with the criteria applicable to the high-quality laboratory test methods.

REFERENCES

1. Zhou P., Yang X. L., Wang X. G., Hu B., Zhang L., Zhang W., et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. 2020; 579 (7798): 270–273. DOI: 10.1038/s41586-020-2012-7.

2. Gorbalenya A. E., Baker S. C., Baric R. S., de Groot R. J., Drosten C., Gulyaeva A. A. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nat. Microbiol*. 2020; 5 (4): 536–544. DOI: 10.1038/s41564-020-0695-z.

3. Kiros M., Andualem H., Kiros T., Hailemichael W., Getu S., Geteneh A., et al. COVID-19 pandemic: current knowledge about the role of pets and other animals in disease transmission. *Virol. J*. 2020; 17 (1):143. DOI: 10.1186/s12985-020-01416-9.

4. Ciotti M., Ciccozzi M., Terrinoni A., Jiang W. C., Wang C. B., Bernardini S. The COVID-19 pandemic. *Crit. Rev. Clin. Lab. Sci*. 2020; 57 (6): 365–388. DOI: 10.1080/10408363.2020.1783198.

5. Hsih W. H., Cheng M. Y., Ho M. W., Chou C. H., Lin P. C., Chi C. Y., et al. Featuring COVID-19 cases via screening symptomatic patients with epidemiologic link during flu season in a medical center of central Taiwan. *J. Microbiol. Immunol. Infect*. 2020; 53 (3): 459–466. DOI: 10.1016/j.jmii.2020.03.008.

6. do Vale B., Lopes A. P., Fontes M. D. C., Silvestre M., Cardoso L., Coelho A. C. Bats, pangolins, minks and other animals – villains or victims of SARS-CoV-2? *Vet. Res. Commun*. 2021; 45 (1): 1–19. DOI: 10.1007/s11259-021-09787-2.

7. Cui J., Li F., Shi Z. L. Origin and evolution of pathogenic coronaviruses. *Nat. Rev. Microbiol*. 2019; 17 (3): 181–192. DOI: 10.1038/s41579-018-0118-9.

8. Ning S., Yu B., Wang Y., Wang F. SARS-CoV-2: origin, evolution, and targeting inhibition. *Front. Cell. Infect. Microbiol*. 2021; 11:676451. DOI: 10.3389/fcimb.2021.676451.

9. Wang M. Y., Zhao R., Gao L. J., Gao X. F., Wang D. P., Cao J. M. SARS-CoV-2: structure, biology, and structure-based therapeutics development. *Front. Cell. Infect. Microbiol*. 2020; 10:587269. DOI: 10.3389/fcimb.2020.587269.

10. Farag E. A., Islam M. M., Enan K., El-Hussein A. M., Bansal D., Haroun M. SARS-CoV-2 at the human-animal interphase: A review. *Heliyon*. 2021; 7 (12):e08496. DOI: 10.1016/j.heliyon.2021.e08496.

11. Nikonova Z. B., Ovchinnikova E. V., Scherbakova L. O., Kozlov A. A., Zhestkov P. D., Zinyakov N. G., et al. Vyyavlenie vozbuditelya COVID-19 u zhivotnykh v 2020–2021 gg. = COVID-19 agent detection in animals in 2020–2021. *Molekulyarnaya diagnostika: X Yubileinaya mezhdunarodnaya nauchno-prakticheskaya konferentsiya* (*9–11 noyabrya, 2021 g.*). = *Molecular diagnosis: X anniversary international research-to-practice conference* (*9–11 November 2021*). Moscow: Yulius; 2021; 2: 379–380. eLIBRARY ID: 47983412. (in Russ.)

12. Akimova T. P., Semakina V. P., Mitrofanova M. N., Zhiltsova M. V., Vystavkina E. S., Isakova D. G., et al. SARS-CoV-2 spread in humans and animals. *Veterinary Science Today*. 2021; (2): 88–96. DOI: 10.29326/2304-196X-2021-2-37-88-96.

13. Parolin C., Virtuoso S., Giovanetti M., Angeletti S., Ciccozzi M., Borsetti A. Animal hosts and experimental models of SARS-CoV-2 infection. *Chemotherapy*. 2021; 66 (1–2): 8–16. DOI: 10.1159/000515341.

14. Meekins D. A., Gaudreault N. N., Richt J. A. Natural and experimental SARS-CoV-2 infection in domestic and wild animals. *Viruses*. 2021; 13 (10):1993. DOI: 10.3390/v13101993.

15. Michelitsch A., Wernike K., Ulrich L., Mettenleiter T. C., Beer M. SARS-CoV-2 in animals: From potential hosts to animal models. *Adv. Virus. Res*. 2021; 110: 59–102. DOI: 10.1016/bs.aivir.2021.03.004.

16. Sharun K., Dhama K., Pawde A. M., Gortázar C., Tiwari R., Bonilla-Aldana D. K., et al. SARS-CoV-2 in animals: potential for unknown reservoir hosts and public health implications. *Vet. Q*. 2021; 41 (1): 181–201. DOI: 10.1080/01652176.2021.1921311.

17. Prince T., Smith S. L., Radford A. D., Solomon T., Hughes G. L., Patterson E. I. SARS-CoV-2 infections in animals: reservoirs for reverse zoonosis and models for study. *Viruses*. 2021; 13 (3):494. DOI: 10.3390/v13030494.

18. Maurin M., Fenollar F., Mediannikov O., Davoust B., Devaux C., Raoult D. Current status of putative animal sources of SARS-CoV-2 infection in humans: wildlife, domestic animals and pets. *Microorganisms*. 2021; 9 (4):868. DOI: 10.3390/microorganisms9040868.

19. Hossain M. G., Javed A., Akter S., Saha S. SARS-CoV-2 host diversity: An update of natural infections and experimental evidence. *J. Microbiol. Immunol. Infect*. 2021; 54 (2): 175–181. DOI: 10.1016/j.jmii.2020.06.006.

20. Dhar B. C. Diagnostic assay and technology advancement for detecting SARS-CoV-2 infections causing the COVID-19 pandemic. *Anal. Bioanal. Chem*. 2022; 414 (9): 2903–2934. DOI: 10.1007/s00216-022-03918-7.

21. Kabir M. A., Ahmed R., Iqbal S. M. A., Chowdhury R., Paulmurugan R., Demirci U., Asghar W. Diagnosis for COVID-19: current status and future prospects. *Expert Rev. Mol. Diagn*. 2021; 21 (3): 269–288. DOI: 10.1080/14737159.2021.1894930.

22. Chen Q., He Z., Mao F., Pei H., Cao H., Liu X. Diagnostic technologies for COVID-19: a review. *RSC Adv*. 2020; 10 (58): 35257–35264. DOI: 10.1039/d0ra06445a.

23. Filchakova O., Dossym D., Ilyas A., Kuanysheva T., Abdizhamil A., Bukasov R. Review of COVID-19 testing and diagnostic methods. *Talanta*. 2022; 244:123409. DOI: 10.1016/j.talanta.2022.123409.

24. Corman V. M., Landt O., Kaiser M., Molenkamp R., Meijer A., Chu D. K., et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill*. 2020; 25 (3):2000045. DOI: 10.2807/1560-7917.ES.2020.25.3.2000045.

25. Long C., Xu H., Shen Q., Zhang X., Fan B., Wang C., et al. Diagnosis of the coronavirus disease (COVID-19): rRT-PCR or CT? *Eur. J. Radiol*. 2020; 126:108961. DOI: 10.1016/j.ejrad.2020.108961.

26. Kevadiya B. D., Machhi J., Herskovitz J., Oleynikov M. D., Blomberg W. R., Bajwa N., et al. Diagnostics for SARS-CoV-2 infections. *Nat. Mater*. 2021; 20 (5): 593–605. DOI: 10.1038/s41563-020-00906-z.

27. Smyrlaki I., Ekman M., Lentini A., Rufino de Sousa N., Papanicolaou N., Vondracek M., et al. Massive and rapid COVID-19 testing is feasible by extraction-free SARS-CoV-2 RT-PCR. *Nat. Commun*. 2020; 11 (1):4812. DOI: 10.1038/s41467-020-18611-5.

28. Gribanov O. G., Shcherbakov A. V., Perevozchikova N. A., Gusev A. A. The use of Aerosil A-300 and GF/F (GF/C) filters for purification of DNA fragments, plasmid DNA and RNA. *Biochemistry (Moscow)*. 1996; 61 (6): 1064–1070. Available at: https://biochemistrymoscow.com/ru/archive/1996/61-06-1064/#_pdf. (in Russ.)

29. Nosyrev P., Nosyreva M., Rasskazova T., Korneeva N. Praktikum po GMP. Validatsiya analiticheskikh metodik: teoriya i praktika = GMP Workshop. Validation of analytical methods: theory and practice. *Remedium*. 2003; 10: 69–71; 11: 62–64. (in Russ.)

30. Polyakov I. V., Sokolova N. S. Practical guide on medical statistics. Leningrad: Meditsina; 1975. 151 p. (in Russ.)

Received 28.07.2022
Revised 30.09.2022
Accepted 04.10.2022

INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Anna M. Timina, Candidate of Science (Veterinary Medicine), Senior Researcher, Reference Laboratory for Highly Dangerous Diseases, FGBI "ARRIAH", Vladimir, Russia;
https://orcid.org/0000-0002-0109-3507, e-mail: timina@arriah.ru.

Anastasia S. Yakovleva, Candidate of Science (Biology), Senior Researcher, Reference Laboratory for Highly Dangerous Diseases, FGBI "ARRIAH", Vladimir, Russia;
https://orcid.org/0000-0001-9211-9110,
e-mail: yakovleva@arriah.ru.

Maksim V. Timanov, Leading Veterinarian, Reference Laboratory for Highly Dangerous Diseases, FGBI "ARRIAH", Vladimir, Russia;
https://orcid.org/0000-0001-7496-3043, e-mail: timanov@arriah.ru.

Marina V. Biryuchenkova, Candidate of Science (Biology), Researcher, Reference Laboratory for Highly Dangerous Diseases, FGBI "ARRIAH", Vladimir, Russia;
https://orcid.org/0000-0001-9131-8839,
e-mail: biruchenkova@arriah.ru.

Yelena S. Orlova, Candidate of Science (Biology), Leading Researcher, Reference Laboratory for Highly Dangerous Diseases, FGBI "ARRIAH", Vladimir, Russia;
https://orcid.org/0000-0003-0568-2680,
e-mail: orlova@arriah.ru.

Тимина Анна Михайловна, кандидат ветеринарных наук, старший научный сотрудник референтной лаборатории по особо опасным болезням ФГБУ «ВНИИЗЖ», г. Владимир, Россия;
https://orcid.org/0000-0002-0109-3507, e-mail: timina@arriah.ru.

Яковлева Анастасия Сергеевна, кандидат биологических наук, старший научный сотрудник референтной лаборатории по особо опасным болезням ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *https://orcid.org/0000-0001-9211-9110,*
e-mail: yakovleva@arriah.ru.

Тиманов Максим Викторович, ведущий ветеринарный врач референтной лаборатории по особо опасным болезням ФГБУ «ВНИИЗЖ», г. Владимир, Россия;
https://orcid.org/0000-0001-7496-3043, e-mail: timanov@arriah.ru.

Бирюченкова Марина Вячеславовна, кандидат биологических наук, научный сотрудник референтной лаборатории по особо опасным болезням ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *https://orcid.org/0000-0001-9131-8839,*
e-mail: biruchenkova@arriah.ru.

Орлова Елена Сергеевна, кандидат биологических наук, ведущий научный сотрудник референтной лаборатории по особо опасным болезням ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *https://orcid.org/0000-0003-0568-2680,*
e-mail: orlova@arriah.ru.

DOI: 10.29326/2304-196X-2023-12-1-52-56

Comparative assessment of immunodiffusion and enzyme-linked immunosorbent assay used for bovine leukosis diagnosis

A. R. Mustafayev, M. O. Baratov
Caspian Regional Research Veterinary Institute – Branch of Dagestan Agriculture Science Center, Makhachkala, Republic of Dagestan, Russia

SUMMARY
The implementation of animal health improvement and disease prevention activities with respect to bovine leukosis involves a need for timely detection of cattle infected with bovine leukaemia virus (BLV) on livestock farms. That is why early diagnosis using more sensitive and highly accurate methods is of particular importance. The paper presents the results of cattle serum tests for bovine leukosis with enzyme-linked immunosorbent assay (ELISA) and immunodiffusion (ID), as well as the comparative assessment of their effectiveness. A total of 440 cattle blood samples were subjected to serological testing with immunodiffusion; 37 (8.4%) of them tested positive for bovine leukaemia virus. The cattle blood samples were submitted from the Kumtorkalinsky (127), Karabudakhkentsky (122), Buynaksky (89) Raions, from Makhachkala (56) and Kaspiysk (46). Seropositivity was 17 (13.4%), 8 (6.6%), 5 (5.6%), 4 (7.1%) and 3 (6.5%), respectively. For the comparative assessment of the diagnostic tests, 100 (5 ID-positive and 95 ID-negative) serum samples were taken and tested with ELISA. As a result, specific antibodies against BLV gp51 antigen were detected in 4 ID-negative serum samples. All ID-positive serum samples also tested positive with ELISA. All in all, 9 virus carriers were detected with ELISA, that is 44.4% more than with immunodiffusion. Thus, enzyme-linked immunosorbent assay is characterized by a higher sensitivity, as compared with immunodiffusion, and allows for improved detection of infected animals. However, alongside the advantages, this technique has certain disadvantages, one of which is the high price of the diagnostic test kit for anti-BLV antibody detection and the equipment required.

Keywords: bovine leukaemia virus, specific antibodies, seropositivity, comparative assessment, immunodiffusion, enzyme-linked immunosorbent assay, sensitivity and specificity of method

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

Conflict of interest: The authors declare that there is no conflict of interest.

For correspondence: Arkif R. Mustafayev, Candidate of Science (Veterinary Medicine), Leading Researcher, Laboratory of Infectious Pathology of Farm Animals, Caspian Regional Research Veterinary Institute – Branch of Dagestan Agriculture Science Center, 367000, Russia, Republic of Dagestan, Makhachkala, ul. Dakhadaeva, 88, *e-mail: mustafaev_arkif@mail.ru.*

УДК 619:616.98:578.828.11:616-079.4

Сравнительные аспекты диагностики лейкоза крупного рогатого скота при применении реакции иммунодиффузии и иммуноферментного анализа

А. Р. Мустафаев, М. О. Баратов
Прикаспийский зональный научно-исследовательский ветеринарный институт – филиал ФГБНУ «Федеральный аграрный научный центр Республики Дагестан» (Прикаспийский зональный НИВИ – филиал ФГБНУ «ФАНЦ РД»), г. Махачкала, Республика Дагестан, Россия

РЕЗЮМЕ
При проведении оздоровительно-профилактических мероприятий при лейкозе крупного рогатого скота возникает необходимость своевременного выявления инфицированного вирусом лейкоза поголовья в животноводческих хозяйствах. Поэтому важное значение имеет ранняя диагностика с применением более чувствительных и высокоточных методов. В статье приводятся результаты исследования сывороток крови животных на лейкоз крупного рогатого скота методом иммуноферментного анализа и в реакции иммунодиффузии и изучения их эффективности в сравнительном аспекте. Всего серологическим методом с применением реакции иммунодиффузии было исследовано 440 проб крови крупного рогатого скота, из них 37 (8,4%) оказались сероположительными к вирусу лейкоза. Пробы крови животных были получены из Кумторкалинского (127), Карабудахкентского (122), Буйнакского (89) районов, г. Махачкалы (56) и г. Каспийска (46). Серопозитивность соответственно составила 17 (13,4%), 8 (6,6%), 5 (5,6%), 4 (7,1%) и 3 (6,5%). С целью сравнительного анализа диагностических тестов было отобрано 100 проб сывороток крови: 5 – РИД-положительных и 95 – РИД-отрицательных, которые

исследовали методом иммуноферментного анализа. В результате в четырех РИД-отрицательных пробах выявлены специфические антитела к антигену gp51 вируса лейкоза крупного рогатого скота. При этом все РИД-позитивные пробы сывороток крови методом иммуноферментного анализа также определены как серопозитивные. В общей сложности иммуноферментным анализом было выявлено 9 вирусоносителей, что на 44,4% больше, чем обнаружено с помощью реакции иммунодиффузии. Таким образом, метод иммуноферментного анализа в сравнении с реакцией иммунодиффузии характеризуется более высокой чувствительностью и позволяет дополнительно выявлять инфицированных животных. Однако данный метод кроме преимуществ имеет и ряд недостатков, одним из которых является высокая стоимость диагностического набора для выявления антител к вирусу лейкоза крупного рогатого скота и используемого оборудования.

Ключевые слова: вирус лейкоза крупного рогатого скота, специфические антитела, серопозитивность, сравнительный анализ, реакция иммунодиффузии, иммуноферментный анализ, чувствительность и специфичность метода

Для цитирования: Мустафаев А. Р., Баратов М. О. Сравнительные аспекты диагностики лейкоза крупного рогатого скота при применении реакции иммунодиффузии и иммуноферментного анализа. *Ветеринария сегодня*. 2023; 12 (1): 52–56. DOI: 10.29326/2304-196X-2023-12-1-52-56.

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

Для корреспонденции: Мустафаев Аркиф Рамазанович, кандидат ветеринарных наук, ведущий научный сотрудник лаборатории инфекционной патологии сельскохозяйственных животных, Прикаспийский зональный НИВИ – филиал ФГБНУ «ФАНЦ РД», 367000, Россия, Республика Дагестан, г. Махачкала, ул. Дахадаева, д. 88, *e-mail: mustafaev_arkif@mail.ru.*

INTRODUCTION

Under current conditions of animal husbandry, a continuous control over the movement of cattle latently infected with bovine leukemia virus (BLV) is largely absent. Despite measures taken to combat bovine leukosis caused by this virus, the disease tends to be widely spread worldwide, including in the Russian Federation Subjects [1–5]. The main reasons for this include factors such as late slaughter of BLV infected animals, lack of animal health improvement and disease prevention activities, untimely diagnostic testing, etc. That is why early diagnosis using more sensitive and highly accurate tests and methods is of particular importance [6–11].

At present, immunodiffusion (ID) being a standard and proven method is commonly used for bovine leukosis diagnosis at all the veterinary laboratories, diagnostic units, veterinary clinics of the Republic of Dagestan. The advantage of immunodiffusion over other techniques is that ID testing of one sample is 5–7 times cheaper as compared with similar test systems. Other advantages are that it is easy to perform and does not require any special equipment, thus being easily applicable at the diagnostic units in the distant areas of the Republic. However, immunodiffusion has certain disadvantages such as non-specific reactions, low sensitivity of anti-BLV antigen antibody detection, test reading not earlier than after 48 hours, as demonstrated by multiple studies on bovine leukosis diagnosis [12–14].

Given these drawbacks, animal serum samples should be tested for bovine leukosis using enzyme-linked immunosorbent assay (ELISA); besides, there is a need for the comparative assessment of both techniques for their effectiveness. Many authors provide in their papers the evidence of ELISA being a more sensitive method when it comes to the detection of specific antibodies against BLV antigen as compared with immunodiffusion [15–16]. ELISA can be applied within animal health improvement and disease prevention activities with respect to bovine leukosis, since they necessitate the timely detection of BLV infected cattle on livestock farms [16–20].

In view of the above, the aim of the study was to perform the comparative assessment of ELISA and immunodiffusion test systems used for animal serum tests for detection of specific antibodies against BLV antigen in accordance with methodical guidelines.

MATERIALS AND METHODS

Animal serum samples submitted from different farms of the Karabudakhkentsky, Kumtorkalinsky and Buynaksky Raions, as well as from Makhachkala and Kaspiysk were used as material for diagnostic tests for bovine leukosis with immunodiffusion and ELISA.

Immunodiffusion tests were carried out using the test kit for serological diagnosis of bovine leukosis manufactured by the FKP "Kursk Biofactory – BIOK Company" (Russia). This test system is designed for detection of antibodies against BLV glycoprotein antigen with agar gel precipitation test.

ELISA tests involved the use of the test kit for detection of specific antibodies against BLV gp51 in the individual and pooled samples of cattle blood serum or plasma and milk manufactured by the OOO "Vetbiokhim" (Russia).

All the diagnostic tests were performed in accordance with the "Methodical guidelines for diagnosis of bovine leukosis"[1].

RESULTS AND DISCUSSION

The diagnostic tests of 440 cattle serum samples for bovine leukosis with immunodiffusion were carried out at the Laboratory of Infectious Pathology of Farm

[1] Methodical guidelines for diagnosis of bovine leukosis: approved by the Veterinary Department of the Ministry of Agriculture of the Russian Federation on 23 August 2000 No. 13-7-2/2130. Available at: http://docs.cntd.ru/document/1200118749.

Table
Results of diagnostic tests of animal serum samples submitted from farms of the Republic of Dagestan for bovine leukosis with immunodiffusion and ELISA

Raions and municipalities	Tested with immunodiffusion (ID)			Tested with ELISA		
	number of samples	ID (+)	%	out of ID (–)	out of ID (+)	ELISA (+)
Kumtorkalinsky	127	17	13.4	48	2	3 (+1)
Karabudakhkentsky	122	8	6.6	29	1	3 (+2)
Buynaksky	89	5	5.6	8	2	2
Makhachkala	56	4	7.1	5	0	1 (+1)
Kaspiysk	46	3	6.5	5	0	0
Total	440	37	8.4	100 (95 + 5)		9 (9.0%)

Animals of the Caspian Regional Research Veterinary Institute – Branch of Dagestan Agriculture Science Center in 2022. Of these, 37 samples (8.4% of the test animals) tested positive for bovine leukemia virus. The cattle serum samples were submitted from the following raions and municipalities: the Kumtorkalinsky Raion – 127, the Karabudakhkentsky Raion – 122, the Buynaksky Raion – 89, Makhachkala – 56 and Kaspiysk – 46. The seropositivity in these raions and municipalities was 17 (13.4%), 8 (6.6%), 5 (5.6%), 4 (7.1%), 3 (6.5%), respectively (Table).

At the next stage of the study, 100 cattle serum samples with different serological statuses (based on the immunodiffusion test results) were taken from the 440 samples for ELISA testing: from the Kumtorkalinsky Raion – 50 (48/2), from the Karabudakhkentsky Raion – 30 (29/1), from the Buynaksky Raion – 10 (8/2), from Makhachkala – 5 (5/0) and from Kaspiysk – 5 (5/0). ELISA revealed the presence of specific antibodies against BLV gp51 in 4 samples that had tested negative with immunodiffusion. ELISA tests also detected specific antibodies against BLV antigen in all the ID-positive serum samples.

Thus, a total of 9 virus carriers were detected with ELISA, i.e. 44.4% more than with immunodiffusion (Figure).

The results presented in the Table and the Figure show that ELISA is a more sensitive method. It should be noted that non-specific reactions were observed in some animal serum samples tested with ELISA and the test results for 6 samples were found to be inconclusive. This is probably due to the fact that ELISA is associated with certain limitations; in particular, hemolyzed and contaminated animal serum samples, as well as those subjected to multiple freezing and thawing are not suitable for testing. However, as regards bovine leukosis diagnosis, this technique has certain advantages such as high sensitivity in detection of specific antibodies against BLV gp51, fast availability of test results, the use of a minimal amount of test material (4 µL of serum). The main disadvantages of ELISA include the high price of the diagnostic test kit, as well as the need for availability of a spectrophotometer (reader)

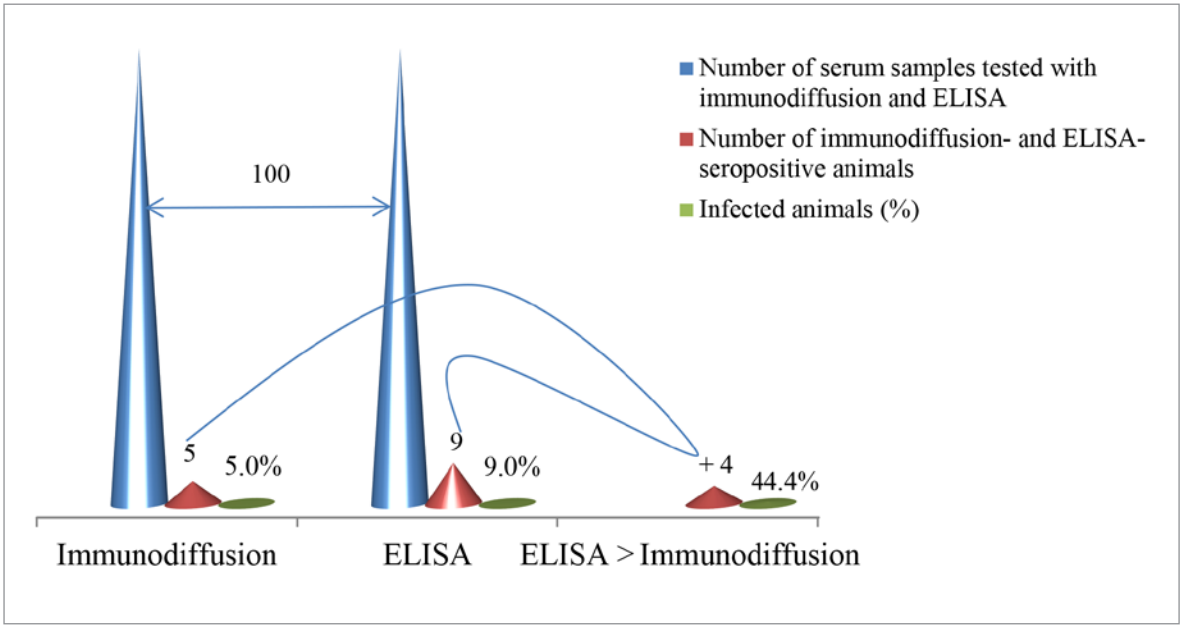

Fig. Comparative assessment of the results of immunodiffusion and ELISA tests of animal serum samples for antibodies against bovine leukemia virus

at the laboratory to be used to measure optical density at a wavelength of 450 nm.

In the light of the above, it can be concluded that ELISA is characterized by a higher sensitivity, as compared with immunodiffusion, and allows for improved detection of BLV infected animals.

CONCLUSION

The comparative assessment of immunodiffusion and ELISA used for bovine leukosis diagnosis shows that ELISA is characterized by higher specificity and sensitivity than immunodiffusion. The ELISA tests of 100 cattle serum samples detected 9 BLV-carriers, whereas only 5 samples had tested positive with immunodiffusion. Thus, the ELISA tests detected about 44.4% more reactors. Such high percentage can be possibly attributed to a small size of the sample of cattle serum samples out of those tested with immunodiffusion. In case of a large-scale ELISA testing for bovine leukosis, the percentage of detected BLV infected animals would probably be lower, ranging between 15 and 30% and thus being consistent with the data provided by other researches in their papers [11, 18].

REFERENCES

1. Gulykin M., Barabanov I., Ivanova L., Stepanova T., Kozireva N., Simonian G., et al. Monitoring of epidemiologic situation with bovine leukemia in production and breeding herds of Russian Federation in 2014–2015. *Veterinaria i kormlenie*. 2016; 4: 5–41. eLIBRARY ID: 26383305. (in Russ.)

2. Mustafayev A. R. Epidemic situation on enzootic bovine leukosis in public and individual farms in the Republic of Dagestan. *Veterinary Science Today*. 2021; (2): 144–150. DOI: 10.29326/2304-196X-2021-2-37-144-150.

3. Mischenko V. A., Petrova O. N., Karaulov A. K., Mischenko A. V. Problema leikoza krupnogo rogatogo skota v Rossiiskoi Federatsii = The problem of bovine leukosis in the Russian Federation. *BIO*. 2018; 3 (210): 26–29; 4 (211): 24–28. eLIBRARY ID: 35562339; eLIBRARY ID: 35562349. (in Russ.)

4. Mustafaev A. R., Gulyukin M. I., Salikhov Yu. S. Monitoring of the spread of the bovine leukemia virus in the Republic of Dagestan in 2018. *Veterinaria i kormlenie*. 2019; 4: 18–20. DOI: 10.30917/ATT-VK-1814-9588-2019-4-5. (in Russ.)

5. Gillet N., Florins A., Boxus M., Burteau C., Nigro A., Vandermeers F., et al. Mechanisms of leukemogenesis induced by bovine leukemia virus: prospects for novel antiretroviral therapies in human. *Retrovirology*. 2007; 4:18. DOI: 10.1186/17424690418.

6. Mustafaev A. R., Budulov N. R., Shikhragimov E. M., Salihov U. S., Gaidarbekova Kh. M. Improving measures against bovine leukemia on the sample of farms of the Dagestan Republic. *Veterinarya*. 2015; 1: 23–26. eLIBRARY ID: 23057434. (in Russ.)

7. Rodríguez S. M., Florins A., Gillet N., de Brogniez A., Sánchez-Alcaraz M. T., Boxus M., et al. Preventive and therapeutic strategies for bovine leukemia virus: lessons for HTLV. *Viruses*. 2011; 3 (7): 1210–1248. DOI: 10.3390/v3071210.

8. Yu C., Wang X., Zhou Y., Wang Y., Zhang X., Zheng Y. Genotyping bovine leukemia virus in dairy cattle of Heilongjiang, northeastern China. *BMC Vet. Res*. 2019; 15 (1):179. DOI: 10.1186/s12917-019-1863-3.

9. Osipova N. A., Agarkova T. A., Dvoeglazov N. G., Khramtsov V. V. Estimation of efficiency of comprehensive anti-leukemia measures in agricultural enterprises. *Siberian Herald of Agricultural Science*. 2019; 49 (5): 73–79. DOI: 10.26898/0370-8799-2019-5-10. (in Russ.)

10. Dvoyeglazov N. G., Agarkova T. A. Some features of sanitation measures against cattle leukosis viral infection using immunodiffusion test and ALISA. *Veterinarian*. 2010; 1: 22–24. eLIBRARY ID: 14477218. (in Russ.)

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

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

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

14. Wu D., Takahashi K., Murakami K., Tani K., Koguchi A., Asahina M., et al. B-1a, B-1b and conventional B cell lymphoma from enzootic bovine leukosis. *Vet. Immunol. Immunopathol*. 1996; 55 (1–3): 63–72. DOI: 10.1016/s0165-2427(96)05631-0.

15. Mustafayev A. R. Bovine leukosis incidence in Republic of Dagestan in 2021. *Veterinary Science Today*. 2022; 11 (3): 216–221. DOI: 10.29326/2304-196X-2022-11-3-216-221.

16. Bajseitov S. T., Novikova N. N., Vlasenko V. S., Krasikov A. P. Comparative evaluation of diagnostic efficiency of immunodiffusion reaction (IDR), ELISA and indirect reaction of immunofluorescence (IIR) for cattle leukemia. *Vestnik of Omsk SAU*. 2020; 1 (37): 97–102. eLIBRARY ID: 42742073. (in Russ.)

17. Dvoeglazov N. G., Khramtsov V. V., Agarkova T. A., Osipova N. A. Comparative analysis of application of ELISA and AGID in diagnosing bovine leukosis. *Siberian Herald of Agricultural Science*. 2015; (1): 89–93. eLIBRARY ID: 23059914. (in Russ.)

18. Donnik I. M., Ponomareva O. I., Krivonos R. A., Lysenko A. A., Koshchaev A. G., Chernykh O. Yu., et al. Elimination of bovine leukemia in industrial production conditions. *Veterinaria Kubani*. 2021; 2: 3–8. DOI: 10.33861/2071-8020-2021-2-3-8. (in Russ.)

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

20. Nekoei S., Hafshejani T. T., Doosti A., Khamesipour F. Molecular detection of bovine leukemia virus in peripheral blood of Iranian cattle, camel and sheep. *Pol. J. Vet. Sci*. 2015; 18 (4): 703–707. DOI: 10.1515/pjvs-2015-0091.

Received 20.12.2022
Revised 12.01.2023
Accepted 20.01.2023

INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Arkif R. Mustafayev, Candidate of Science (Veterinary Medicine), Leading Researcher, Laboratory of Infectious Pathology of Farm Animals, Caspian Regional Research Veterinary Institute – Branch of Dagestan Agriculture Science Center, Makhachkala, Republic of Dagestan, Russia;
http://orcid.org/0000-0002-5142-8360,
e-mail: mustafaev_arkif@mail.ru.

Magomed O. Baratov, Doctor of Science (Veterinary Medicine), Chief Researcher, Head of Laboratory of Infectious Pathology of Farm Animals, Caspian Regional Research Veterinary Institute – Branch of Dagestan Agriculture Science Center, Makhachkala, Republic of Dagestan, Russia;
https://orcid.org/0000-0002-8261-5038,
e-mail: alama500@rambler.ru.

Мустафаев Аркиф Рамазанович, кандидат ветеринарных наук, ведущий научный сотрудник лаборатории инфекционной патологии сельскохозяйственных животных, Прикаспийский зональный НИВИ – филиал ФГБНУ «ФАНЦ РД», г. Махачкала, Республика Дагестан, Россия;
http://orcid.org/0000-0002-5142-8360,
e-mail: mustafaev_arkif@mail.ru.

Баратов Магомед Омарович, доктор ветеринарных наук, главный научный сотрудник, заведующий лабораторией инфекционной патологии сельскохозяйственных животных, Прикаспийский зональный НИВИ – филиал ФГБНУ «ФАНЦ РД», г. Махачкала, Республика Дагестан, Россия;
https://orcid.org/0000-0002-8261-5038,
e-mail: alama500@rambler.ru.

DOI: 10.29326/2304-196X-2023-12-1-57-65

Spatiotemporal analysis of African swine fever spread in wild boar population in Russian Federation, 2007–2022

O. I. Zakharova[1], A. A. Blokhin[1], O. A. Burova[1], I. V. Yashin[1], F. I. Korennoy[2]

[1] Federal Research Center for Virology and Microbiology (FRCVM); Nizhny Novgorod Research Veterinary Institute – Branch of Federal Research Center for Virology and Microbiology (NNRVI – Branch of the FRCVM), Nizhny Novgorod, Russia

[2] FGBI "Federal Centre for Animal Health" (FGBI "ARRIAH"), Vladimir, Russia

SUMMARY

African swine fever is a transboundary disease of all members of *Suidae* family and it causes economic damage to the pig industry and ecology of wild boar as a species. The ASF epidemiology is complex and it is specified by the mechanisms of the agent's transmission in susceptible animal populations. Choice of measures aimed to control and prevent the disease spread in the wild boar population depends mainly on the routes of the disease introduction and stage or phase of the epizootic process. Prevention of the ASFV introduction from an infected region to a free one is the backbone in the infection prevention. Therefore, the research was aimed at the spatiotemporal analysis of African swine fever outbreaks in the wild boar population in the Russian Federation in 2007–2022 and identification of geographical areas that pose risk of new disease epidemics. The analysis was performed using retrospective space-time scan statistics, which does not require data on the wild boar population and which can be used for the assessment of the possibility of new ASF outbreak occurrence upon availability of just data on the reported disease cases and outbreaks. As a result of spatiotemporal cluster analysis, 24 clusters of ASF outbreaks were identified based on the laboratory-confirmed data on the infection in boars found dead, and 22 clusters in hunted wild boars. The analysis results demonstrated spatial heterogeneity of the outbreak cluster distribution in population of wild boars died of the disease and a significant expansion of the passive surveillance geography. Importance and necessity of the enhanced passive surveillance of African swine fever in susceptible animals is demonstrated. The proposed method can be used for regular scanning of a geographic region for the presence of developing zones and areas at risk of re-emerging ASF outbreaks in the wild boar population at different spatial scales.

Keywords: African swine fever, cluster analysis, confidence, wild boar, surveillance, relative risk

Acknowledgement: The study was supported by the Ministry of Education and Science of the Russian Federation in the framework of the State Assignment of the Federal Research Center for Virology and Microbiology FGNM-2021-0003.

For citations: Zakharova O. I., Blokhin A. A., Burova O. A., Yashin I. V., Korennoy F. I. Spatiotemporal analysis of African swine fever spread in wild boar population in Russian Federation, 2007–2022. *Veterinary Science Today*. 2023; 12 (1): 57–65. DOI: 10.29326/2304-196X-2023-12-1-57-65.

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

For correspondence: Olga I. Zakharova, Researcher, Department of Epizootology and Risk Assessment Associated with Animal Health, NNRVI – Branch of the FRCVM, 603950, Russia, Nizhny Novgorod, ul. Veterinarnaya, 3, *e-mail: olenka.zakharova.1976@list.ru.*

УДК 619:616.98:578.842.1:616-036.22:639.111.14(470)

Пространственно-временной анализ распространения африканской чумы свиней в популяции диких кабанов на территории Российской Федерации в 2007–2022 гг.

О. И. Захарова[1], А. А. Блохин[1], О. А. Бурова[1], И. В. Яшин[1], Ф. И. Коренной[2]

[1] Федеральное государственное бюджетное научное учреждение «Федеральный исследовательский центр вирусологии и микробиологии» (ФГБНУ ФИЦВиМ); Нижегородский научно-исследовательский ветеринарный институт – филиал Федерального государственного бюджетного научного учреждения «Федеральный исследовательский центр вирусологии и микробиологии» (ННИВИ – филиал ФГБНУ ФИЦВиМ), г. Нижний Новгород, Россия

[2] ФГБУ «Федеральный центр охраны здоровья животных» (ФГБУ «ВНИИЗЖ»), г. Владимир, Россия

РЕЗЮМЕ

Африканская чума свиней является трансграничной болезнью всех представителей семейства *Suidae*, приносящей экономический ущерб свиноводческой отрасли и экологии кабана как вида. Эпизоотология африканской чумы свиней сложна и определяется механизмами передачи возбудителя в популяциях восприимчивых животных. Выбор мер борьбы и предупреждения распространения заболевания в популяции кабанов зависит в основном от путей заноса, стадии или фазы эпизоотического процесса. Предотвращение заноса вируса африканской чумы свиней из неблагополучного региона в благополучный является основой профилактики инфекции. В связи с этим целью исследований явился пространственно-временной анализ очагов африканской чумы

свиней в популяции кабанов в Российской Федерации в 2007–2022 гг. и обозначение географических территорий, представляющих риск возникновения новых эпизоотий. Анализ проведен с помощью ретроспективной статистики пространственно-временного сканирования, которая не требует данных о численности популяции кабана и которую можно использовать для оценки возможного возникновения новых очагов африканской чумы свиней, когда доступны только данные о зарегистрированных случаях или очагах болезни. При выполнении пространственно-временного кластерного анализа было выявлено 24 кластера очагов африканской чумы свиней, зарегистрированных на основании лабораторно подтвержденных данных об инфицировании кабанов, найденных мертвыми, и 22 кластера – кабанов, добытых на охоте. Результаты проведенного анализа продемонстрировали пространственную неоднородность распределения кластеров очагов инфекции в популяции кабанов, павших от болезни, а также существенное расширение географического охвата территории вследствие применения пассивного мониторинга. Показана важность и необходимость проведения усиленного пассивного мониторинга африканской чумы свиней среди восприимчивых животных. Предлагаемый метод можно использовать для регулярного сканирования географического региона на вероятность формирования зон и территорий риска новых вспышек африканской чумы свиней в популяции дикого кабана на территориях различного пространственного масштаба.

Ключевые слова: африканская чума свиней, кластерный анализ, достоверность, кабан, мониторинг, относительный риск

Благодарность: Работа выполнена при поддержке Минобрнауки РФ в рамках государственного задания ФГБНУ «Федеральный исследовательский центр вирусологии и микробиологии» FGNM-2021-0003.

Для цитирования: Захарова О. И., Блохин А. А., Бурова О. А., Яшин И. В., Коренной Ф. И. Пространственно-временной анализ распространения африканской чумы свиней в популяции диких кабанов на территории Российской Федерации в 2007–2022 гг. *Ветеринария сегодня*. 2023; 12 (1): 57–65. DOI: 10.29326/2304-196X-2023-12-1-57-65.

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

Для корреспонденции: Захарова Ольга Игоревна, научный сотрудник отдела эпизоотологии и оценки риска, связанного со здоровьем животных, ННИВИ – филиал ФГБНУ ФИЦВиМ, 603950, Россия, г. Нижний Новгород, ул. Ветеринарная, д. 3, *e-mail: olenka.zakharova.1976@list.ru.*

INTRODUCTION

African swine fever (ASF) is a viral, contagious disease infecting domestic pigs and wild boars and causing significant damage to the pig industry and ecology of the species at large. The ASF epidemiology is complicated and is determined by the mechanisms of the pathogen transmission in susceptible populations [1–3]. Unlike successful ASF control in domestic pigs, which entails stamping out of the on-farm population, cleaning and disinfection of the contaminated facilities, the disease eradication in wild boars is a challenge [4]. Due to geographical and regional features of wild boar habitats, there is no standard control strategy of the epidemic process, which can be applied for all ASF affected areas [5, 6]. Standard prophylaxis preferably includes measures required to prevent the virus spillover from the affected regions to the disease-free ones [7, 8].

The role of wild boars and density of their population is a disputable factor of ASFV transmission and is still under discussion. However, many scientists and world experts agree that a reduction of the wild boar abundance mitigates the risk of ASFV spillover and transmission both in the hot spots and in the observation zone [7, 9].

Studies on ASF spread in wild boars in the Eastern European countries show that the disease can persist even at a very low level of prevalence and low population density, unless the infected dead animals and their remains are timely eliminated [10–12].

Measures taken to control and prevent ASF spread in wild boars are chosen, mainly, based on the routes of introduction and the stage of the epizootic process. In addition, the territory status is an important factor to be considered when choosing ASF preventive measures. The territory status refers to the situation recorded at the moment of the outbreak registration: disease-free, previously disease-affected, not adjacent to a hot spot – the observation zone. Early detection of the infected wild boars is an important link in the disease control strategy [13].

Methods chosen to detect an increased concentration of the disease outbreaks (clusters) play a significant role in modern epidemiological studies, in public health and preventive veterinary medicine. Their use makes it possible to identify possible etiological and pathogenic reasons behind the epidemics, as well as to choose optimal solutions to eliminate the infections [14].

Time, space and space-time scan statistics [15–21] are widely used now to identify and evaluate clusters of various diseases, including both human and animal infections and non-infectious pathology [18, 19].

Most analytical methods in epidemiology used for early detection of animal diseases are purely time-based. These methods are useful to report outbreaks that simultaneously cover all the areas of the monitored region, but may lag behind in case of local epizooties, which are confined to a certain geographical area. However, purely time-based methods can be used simultaneously for all overlapping parts of the region that differ in size, in order to include all possible disease cases and occurring outbreaks. Nevertheless, this approach causes serious problems, since it includes multiple laboratory tests, which give much more false results than the nominal significance level can show [22, 23].

This work represents retrospective space-time scan statistics, which do not require data on the number of wild boars in areas at ASF risk, and which can be used, if there

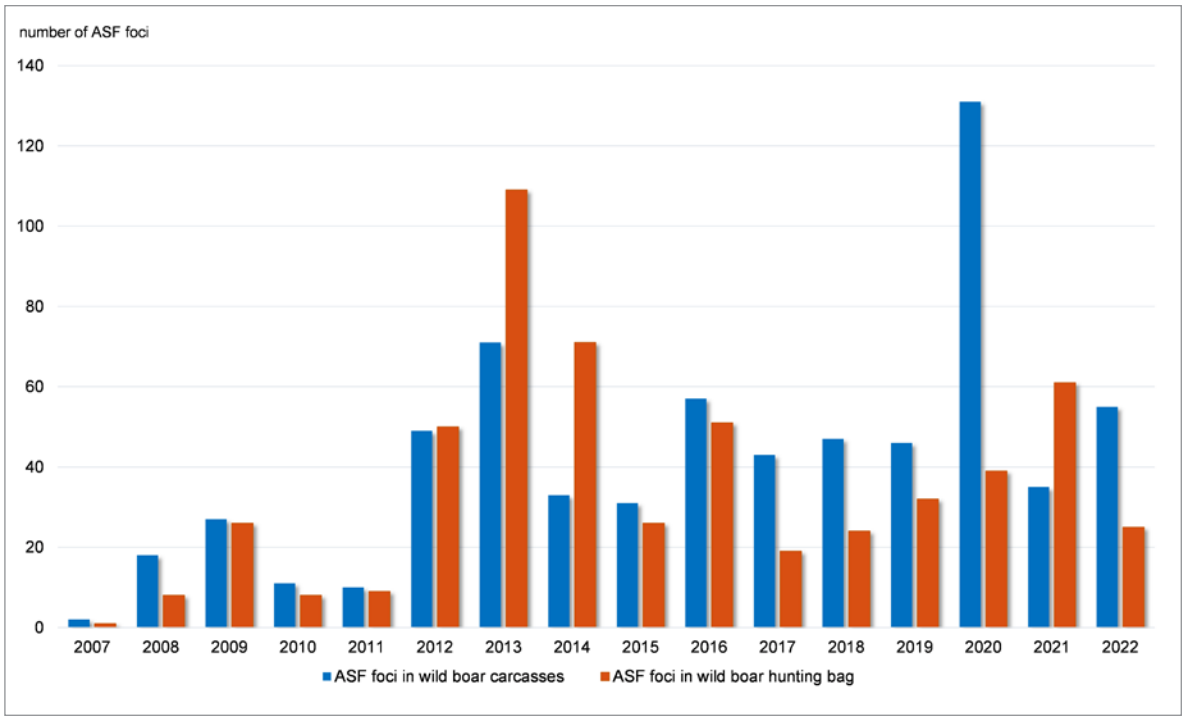

Fig. 1. History of ASF outbreaks reported in the wild boar population (dead animals and remnants thereof – N = 592; hunted animals – N = 562)

is only information on registered dead animals (or their remains) or hunted animals, detected after biomaterial tests. This method can be used to regularly scan geographical regions of various size in order to determine ASF risk zones and territories inhabited by wild boars. For each region, this method considers both potential one-day outbreaks and sporadic outbreaks, so that to spot a rapidly spreading epizooty [20–23]. Therefore, the purpose of the research was to conduct a spatiotemporal analysis of ASF outbreaks and to determine the infection trend in wild boars in the affected subjects of the Russian Federation with the possible identification of geographical territories that pose a risk of local epizooties.

MATERIALS AND METHODS

Our research focused on a retrospective data analysis of ASF outbreaks registered in wild boars. The data were taken from the official reports of the FGBI "Center for Veterinary Medicine" (Moscow)[1]. The term "outbreak" refers to a territory officially notified by the veterinary services of the RF Subject, with given geographical coordinates, where ASFV-infected wild boars are detected. In turn, an individual animal (or a carcass) is considered as a case[2]. Information on the ASF outbreaks registered in wild boars, detected both within passive monitoring and hunting, included the period from 2007 to 2022.

The spatiotemporal analysis was based on the total number of ASF outbreaks in wild boars in the affected Subjects of the Russian Federation. The cluster analysis is based on the Kulldorff's space-time scan statistical method [24]. This method makes it possible to identify clusters of the geographical area under study, where ASF outbreaks (or other studied phenomenon) were grouped more densely than would be expected according to the null hypothesis, which assumes their random distribution. The analysis uses a cylindrical moving scan window where the vertical dimension represents time. As input data, point objects, i.e. ASF outbreaks, are tested, information on the number of cases is given for each outbreak.

The spatiotemporal cluster analysis reveals circular regions (clusters), within which an increased number of ASF outbreaks was detected (as compared with a hypothetical random distribution). Additional characteristics of the clusters are: radius, start and end date, duration, statistical significance (*p*-value), the ratio between the observed number and the expected number of outbreaks within the clusters (ODE). The last characteristic can be considered as a relative risk of outbreak occurrence within the cluster, as compared with the outbreaks registered outside. The SaTScan v8.0 software[3] was used for the cluster analysis, ArcMap 10.8.1 (Esri, USA) was used to visually map the obtained results.

RESULTS

Retrospective analysis of ASF outbreaks in wild boars in the Russian Federation, in 2007–2022. Current ASF outbreaks reported in wild boars in Russia affect small areas, such as hunting farms, with the virus showing a strong tendency to establish itself in the population within a particular area, i.e. to become enzootic. The enzootic process in ASF outbreaks, confirmed by laboratory tests of the material

[1] FGBI "Center for Veterinary Medicine". Epidemic situation. Available at: https://xn----8sbfkcavba6bf4aedue4d.xn--p1ai/o-nas/informatsiya/epizooticheskaya-obstanovka (date of access: 23.01.2023).

[2] WOAH. Terrestrial Animal Health Code. Available at: https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-code-online-access (date of access: 26.01.2023).

[3] SaTScanTM v8.0: Software for the spatial and space-time scan statistics. 2009. Available at: https://www.satscan.org (date of access: 23.01.2023).

Table 1
Characteristics of ASF outbreak clusters in wild boars found dead in the Subjects of the Russian Federation, 2007–2022

Cluster No.	Cluster radius, km	Number of ASF outbreaks observed	Number of ASF outbreaks expected	ODE	Cluster started on	Cluster ended on	Cluster lasted for, days	*P*-value
1	157.88	34	2.9	11.72	02.08.2015	23.07.2016	356	< 0.001
2	82.22	35	3.3	10.60	04.02.2018	25.05.2019	414	< 0.001
3	121.25	45	6.5	6.92	23.02.2020	24.10.2020	244	< 0.001
4	142.14	24	1.36	17.64	04.11.2007	10.10.2009	706	< 0.001
5	158.22	15	0.53	28.30	26.06.2011	04.08.2012	405	< 0.001
6	158.96	32	5.57	5.75	29.04.2012	06.12.2014	951	< 0.001
7	146.71	16	1.02	15.69	16.07.2017	18.11.2017	125	< 0.001
8	68.97	21	2.2	9.55	18.08.2019	22.02.2020	188	< 0.001
9	126.48	11	0.46	23.91	22.11.2009	21.08.2010	272	< 0.001
10	145.87	9	0.23	39.13	26.09.2021	22.01.2022	118	< 0.001
11	40.09	8	0.15	53.33	21.12.2014	28.03.2015	97	< 0.001
12	118.03	12	0.88	13.64	29.07.2018	28.09.2019	426	< 0.001
13	90.69	13	1.17	11.11	25.10.2020	03.04.2021	160	< 0.001
14	88.42	6	0.09	66.67	07.06.2020	25.07.2020	48	< 0.001
15	114.54	12	1.06	11.32	07.07.2013	31.08.2013	55	< 0.001
16	127.17	5	0.05	100.00	17.07.2022	23.07.2022	6	< 0.001
17	99.65	9	0.5	18.00	29.10.2017	07.07.2018	251	< 0.001
18	105.43	8	0.35	22.86	25.10.2020	12.12.2020	48	< 0.001
19	9.86	5	0.06	83.33	29.06.2014	19.07.2014	20	< 0.001
20	79.69	7	0.35	20.00	29.09.2019	28.12.2019	90	< 0.001
21	68.13	5	0.11	45.45	24.11.2013	08.03.2014	104	< 0.001
22	2.11	3	0.02	150.00	07.01.2018	13.01.2018	6	< 0.001
23	21.14	5	0.15	33.33	28.08.2016	10.12.2016	104	< 0.001
24	159.34	12	1.97	6.09	13.09.2020	05.02.2022	510	< 0.001

ODE – observed/expected (this is the ratio of the observed number to the expected number of ASF outbreaks within the cluster, given that the distribution is consistent with the null hypothesis, i.e. the value of the indicator determines the relative risk of new outbreaks within the cluster).

from the dead and hunted boars in the affected RF Subjects, is characterized by sporadic outbreaks in the same geographical areas. History of ASF outbreaks in wild boars is shown in Figure 1.

As we can see, there is a positive trend in infection detection both in dead and hunted animals. This trend may be explained by an increase in the number of the tested animals and by the improvement of veterinary surveillance in the RF Subjects[4]. Despite this, there are differences in the number of ASF outbreaks detected among dead wild boars (51.3% of the total number of the registered ones) and hunted wild boars (48.7%).

Spatiotemporal cluster analysis of ASF outbreaks in wild boars in the RF Subjects (in 2007–2022). Twenty four reliable clusters were identified by the cluster analysis of ASF outbreaks registered in dead wild boars in the affected regions of the Russian Federation (from 2007 to 2022). The peculiar characteristics of the clusters, as shown by the SaTScan software, are given in Table 1.

As Figure 2A shows, ASF clusters were mainly distributed in the center of the European part of Russia, as was demonstrated by the tests of material taken from dead wild boars. These clusters are located in the central and northwestern regions of the European part of the Russian Federation. In the Far East (Fig. 2B), the long-term presence and registration of ASF outbreaks in some parts of the Primorsky Krai and nearby result from a dense population of wild boars (more than 1 animal per 1000 ha)[5].

The spatiotemporal analysis has shown that the following geographical territories in the following clusters demonstrate the greatest probability of ASF registration in the wild boars who pose a relative risk of the disease transmission (Table 1): No. 11 (ODE = 53.33), No. 14 (ODE = 66.67), No. 16 (ODE = 100.00), No. 19 (ODE = 83.33), No. 22 (ODE = 150.00). Clusters No. 2, 4, 6, 12, 24 were characterized by the longest persistence of ASF agent associated with the virus persistence in the environment.

[4] FGBI "Center for Veterinary Medicine". Epidemic situation. Available at: https://xn----8sbfkcavba6bf4aedue4d.xn--p1ai/o-nas/informatsiya/epizooticheskaya-obstanovka (date of access: 23.01.2023).

[5] FNITS Hunt. Hunting resources. Available at: http://www.ohotcontrol.ru/resource (date of access: 23.01.2023).

Fig. 2. Clusters of detected ASF outbreaks reported in the wild boar population (found dead) in the ASF-infected regions of the Russian Federation: A – European part; B – Far East (2007–2022)

The spatiotemporal analysis of ASF outbreaks (from 2007 to 2022) revealed 22 reliable clusters following detection of ASF pathogen or antibodies to the virus in hunted wild boars. Their main characteristics are given in Table 2. Based on the analysis results, it was determined that the greatest probability of new ASF epizooties (relative risk) is observed in the territories of the following clusters: No. 9 (ODE = 66.67), No. 11 (ODE = 71.43), No. 13 (ODE = 35.29), No. 16 (ODE = 50.00), No. 20 (ODE = 150.00), No. 21 (ODE = 57.14). Clusters No. 4, 5, 10, 12, 14, 18 are the longest to preserve ASF pathogen. Within the radius of these clusters, local enzootic territories were formed in the RF Subjects of the Far East, the Volga Region and the Center of the European part of the Russian Federation (Fig. 3A and 3B).

Based on the analysis of ASF clusters in wild boars, a steady spatiotemporal transmission trend is observed: the beginning of epizooties was observed in the North Caucasus and Southern Federal Districts (southern zone) – clusters No. 4, 8, 11. Later, there was a shift of clusters to the central and northern regions of the Russian Federation – clusters No. 5, 10, 15, 17, 19, 22, 23, their territories are characterized by higher animal population density. In recent years, the ASF epizooty has significantly expanded its geographical scope up to the Far East, where a significant number of ASF outbreaks in wild boars were registered from 2019 to 2022.

DISCUSSION

Wild boars play an important role in ASF epizootic cycle as regards the intra-population transmission, and potential transmission to domestic animals. Epizootological surveillance of ASF in wild boars is ensured either by testing all sick or dead animals for the virus genome or for antibodies to the virus (passive monitoring), or by testing all harvested wild boars, i.e. trapped, drive-hunted or shot dead during hunting or with the purpose of the population control (active monitoring) [2].

Information collected by the European Food Safety Agency (EFSA) from the Baltic States and Poland, indicates that passive surveillance increases a likelihood of earlier ASF detection. According to the data provided by many foreign researchers, most primary ASF cases in wild boars were registered during passive observation [25–27].

Research into ASF transmission in Eastern European countries has shown that the disease can persist with very low prevalence among susceptible animals, even if the density of wild boar population is low due to intensive hunting. Since ASF in wild boars has now become enzootic for many European countries, the question still remains unanswered, which type of monitoring (passive or active) is most effective to detect the virus, taking into account low prevalence and low population density. This is especially important, given that most countries, including the Russian Federation, are trying to eradicate ASF through progressive management of pig population aimed at reduction of wild boars [28, 29]. Even with a very low population density, there is a window of uncertainty, when ASF still circulates in animals, but practically is not detected, which complicates any further management, including a possible strategy of the disease eradication [30].

An idea was put forward by scientists from many countries that highly lethal animal infections, such as ASF, are self-limiting, which means that an epizooty rapidly reduces the number of susceptible populations due to the mass mortality [31]. The faster ASF spreads, the faster it reduces the wild boar population. If such

Table 2
Characteristics of spatiotemporal ASF outbreak clusters reported in wild boars hunted in the infected regions of the Russian Federation, 2007–2022

Cluster No.	Cluster radius, km	Number of ASF outbreaks observed	Number of ASF outbreaks expected	ODE	Cluster started on	Cluster ended on	Cluster lasted for, days	*P*-value
1	151.99	79	16.80	4.70	15.09.2013	24.05.2014	251	< 0.001
2	147.60	38	3.65	10.40	16.08.2015	30.07.2016	349	< 0.001
3	153.10	28	2.22	12.61	01.07.2012	23.03.2013	265	< 0.001
4	152.56	24	1.44	16.67	18.11.2007	02.01.2010	776	< 0.001
5	80.57	19	1.44	13.19	05.11.2017	18.05.2019	559	< 0.001
6	158.84	11	0.40	27.50	26.09.2021	30.10.2021	34	< 0.001
7	66.25	12	0.69	17.39	22.10.2017	28.04.2018	188	< 0.001
8	158.69	9	0.37	24.32	07.03.2010	19.02.2011	349	< 0.001
9	24.84	6	0.09	66.67	04.01.2015	07.02.2015	34	< 0.001
10	145.18	11	0.74	14.86	14.11.2021	19.11.2022	370	< 0.001
11	18.83	5	0.07	71.43	09.06.2013	22.06.2013	13	< 0.001
12	158.04	10	0.85	11.76	02.02.2020	20.02.2021	384	< 0.001
13	42.11	6	0.17	35.29	14.07.2019	19.10.2019	97	< 0.001
14	119.05	13	1.67	7.78	30.06.2019	02.01.2021	552	< 0.001
15	18.06	6	0.20	30.00	31.03.2013	20.07.2013	111	< 0.001
16	119.70	5	0.10	50.00	17.01.2021	04.03.2021	76	< 0.001
17	84.63	6	0.21	28.57	17.09.2017	16.12.2017	90	< 0.001
18	141.01	6	0.23	26.09	17.01.2010	12.02.2011	391	< 0.001
19	155.43	6	0.29	20.69	27.10.2019	18.01.2020	83	< 0.001
20	42.10	3	0.02	150.00	24.11.2019	30.11.2019	6	< 0.001
21	144.23	4	0.07	57.14	12.01.2020	07.03.2020	55	< 0.001
22	153.55	5	0.18	27.78	14.08.2016	12.11.2016	90	< 0.001

ODE – observed/expected (this is the ratio of the observed number to the expected number of ASF outbreaks within the cluster, given that the distribution is consistent with the null hypothesis, i.e. the value of the indicator determines the relative risk of new outbreaks within the cluster).

an infected population is simultaneously shot for sanitary or recreational purposes, then the number of wild boars dramatically reduces. Following the decrease in the number of animals, number of interspecies contacts also reduces, and epizooties turn into enzootic outbreaks. Eventually, ASF virus hides due to hunting activities. However, its re-emergence within a few months after lurking in the environment shall be naturally expected. Thus, the epizootological cycle of ASF in wild boars demonstrates that the virus has become enzootic in the affected territories and regularly spills over into disease-free areas [6, 29, 32].

The direct transmission can episodically peak after the breeding season, when the size of the animal population doubles and the growing animals (2–6 months old) explore the habitat, thus, intensifying interspecies contacts; or as a result of animal regrouping or gathering around feedlots [33–35].

Based on the data available in literature and following our own analysis of all the registered outbreaks, it was concluded that the passive monitoring reveals more ASF outbreaks than the active one, and the search for remains of wild boars proportionally increases the probability to detect infected wild boars [34, 36]. Therefore, further spread of ASF in the wild shall be mainly prevented by an active search for dead animals and notification of the relevant veterinary authorities. Such an approach is crucial for understanding epizootological situation at any phase of ASF epizooty, regardless of the wild boar population density [37].

The ASF clusters identified after testing material from hunted wild boars are mainly isolated from each other and look like non-overlapping geographical locations (Fig. 3A and 3B), which means the disease registered outbreaks are evenly distributed, very likely, due to established hunting quotas.

The wild boar hunting quota is set based on the density and total number of animals, in accordance with the approved regulations on permissible withdrawal, and is compared with the one indicated in the application submitted by hunting provider. Therefore, the potential number of wild boars is either kept at a constant level to preserve and regulate the number of animals, or is inflated to meet the interests of hunting providers by increasing the number of the set quotas.

The ASF clusters identified after testing biomaterial from dead animals (Fig. 2A and 2B), generally do not coincide with the clusters identified after testing the hunted

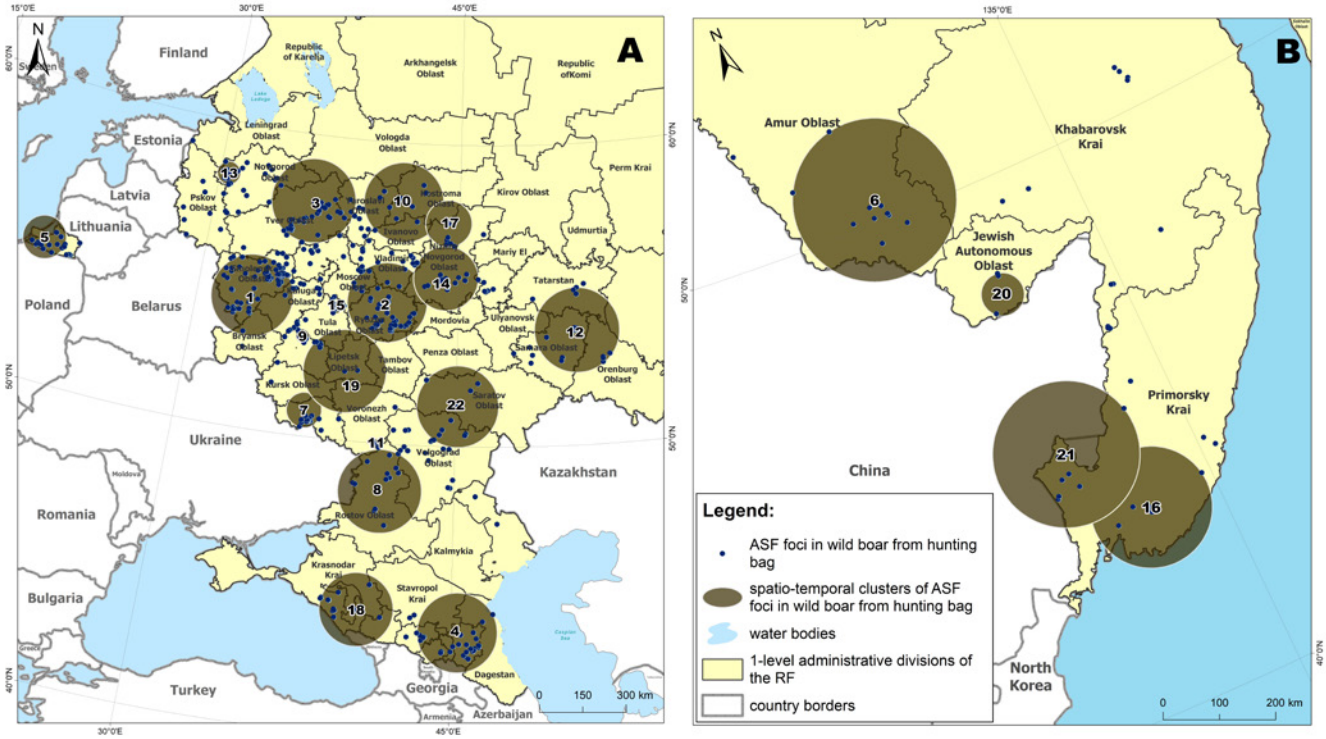

Fig. 3. Clusters of detected ASF outbreaks reported in the wild boar population (hunted animals) in the ASF-infected regions of the Russian Federation: A – European part; B – Far East (2007–2022)

boars, which may suggest ASF virus circulation in the non-hunting territories. It may also mean that not all samples from the shot animals are sent for laboratory tests; dead boars are detected late and are untimely destroyed by owners/tenants of hunting sites without laboratory test results.

As the FGBI "Center for Veterinary Medicine" informs on the number of registered ASF outbreaks, most boars who died from ASF were found in the summer-autumn period. This fact can be apparently associated with active human economic activity, including hunting. The space-time non-coincidence between ASF clusters (both in wild boars dead from the disease or shot), may prove the effectiveness of passive monitoring measures, and makes it possible to identify significantly more infected animals, thus, expanding the ASF detection scope.

The short-term ASF clusters identified in the spatio-temporal analysis and registered after detection of dead animals; suggest that the pathogen currently circulates in the wild boars in the areas of the most affected subjects of the Russian Federation. At the same time, the relative risk of registering new ASF outbreaks in short-term clusters is much higher than that in the long-term ones, presumably associated with such major risk factors of ASF transmission as human economic activity, migration of wild boars, and poor biosafety during hunting.

CONCLUSION

The studies show that measures taken to prevent the disease in wild susceptible animals in the territories that have long been disease-affected shall entail active search, detection and safe disposal of dead boars (or their remains). It is also required to inform potentially interested parties (such as hunters, farmers, veterinarians) about the importance of these activities. Moreover, it is necessary to sustainably reduce the abundance of wild boars. The spatiotemporal cluster analysis revealed local epizooties, helped to study peculiarities of their emergence in the context of dividing ASF outbreaks into groups following tests of biomaterial from the detected dead and hunted wild boars or their remains. Understanding the trends and patterns of ASF transmission in the wild boars, makes it possible to improve the measures taken within the boundaries of the risk zone where the epizooties may occur.

REFERENCES

1. De la Torre A., Bosch J., Sánchez-Vizcaíno J. M., Ito S., Muñoz C., Iglesias I., Martínez-Avilés M. African swine fever survey in a European context. *Pathogens*. 2022; 11 (2):137. DOI: 10.3390/pathogens11020137.

2. EFSA, Baños J. V., Boklund A., Gogin A., Gortázar Ch., Guberti V., et al. Epidemiological analyses of African swine fever in the European Union (September 2020 to August 2021). *EFSA J.* 2022; 20 (5):e07290. DOI: 10.2903/j.efsa.2022.7290.

3. Sauter-Louis C., Conraths F. J., Probst C., Blohm U., Schulz K., Sehl J., et al. African swine fever in wild boar in Europe – a review. *Viruses*. 2021; 13 (9):1717. DOI: 10.3390/v13091717.

4. Dixon L. K., Stahl K., Jori F., Vial L., Pfeiffer D. U. African swine fever epidemiology and control. *Annu. Rev. Anim. Biosci*. 2020; 8: 221–246. DOI: 10.1146/annurev-animal-021419-083741.

5. Pepin K. M., Golnar A. J., Abdo Z., Podgórski T. Ecological drivers of African swine fever virus persistence in wild boar populations: Insight for control. *Ecol. Evol*. 2020; 10 (6): 2846–2859. DOI: 10.1002/ece3.6100.

6. Arzumanyan H., Hakobyan S., Avagyan H., Izmailyan R., Nersisyan N., Karalyan Z. Possibility of long-term survival of African swine fever virus in natural conditions. *Vet. World*. 2021; 14 (4): 854–859. DOI: 10.14202/vetworld.2021.854-859.

7. Bergmann H., Schulz K., Conraths F. J., Sauter-Louis C. A review of environmental risk factors for African swine fever in European wild boar. *Animals (Basel)*. 2021; 11 (9):2692. DOI: 10.3390/ani11092692.

8. Guinat C., Vergne T., Jurado-Diaz C., Sánchez-Vizcaíno J. M., Dixon L., Pfeiffer D. U. Effectiveness and practicality of control strategies for African swine fever: what do we really know? *Vet. Rec*. 2017; 180 (4):97. DOI: 10.1136/vr.103992.

9. Jori F., Bastos A. D. Role of wild suids in the epidemiology of African swine fever. *Ecohealth*. 2009; 6 (2): 296–310. DOI: 10.1007/s10393-009-0248-7.

10. Jori F., Chenais E., Boinas F., Busauskas P., Dhollander S., Fleischmann L., et al. Application of the World Café method to discuss the efficiency of African swine fever control strategies in European wild boar (*Sus scrofa*) populations. *Prev. Vet. Med*. 2020; 185:105178. DOI: 10.1016/j.prevetmed.2020.105178.

11. Probst C., Globig A., Knoll B., Conraths F. J., Depner K. Behaviour of free ranging wild boar towards their dead fellows: potential implications for the transmission of African swine fever. *R. Soc. Open Sci*. 2017; 4 (5):170054. DOI: 10.1098/rsos.170054.

12. Masiulis M., Bušauskas P., Jonušaitis V., Pridotkas G. Potential role of domestic pig carcasses disposed in the forest for the transmission of African swine fever. *Berl. Münch. Tierärztl. Wochenschr*. 2018; 131. DOI: 10.2376/0005-9366-18014.

13. Guberti V., Khomenko S., Masiulis M., Kerba S. African swine fever in wild boar ecology and biosecurity. *FAO Animal Production and Health Manual No. 22*. Rome: FAO, OIE and EC; 2019. 96 p. DOI: 10.4060/CA5987EN.

14. Mushagalusa C. A., Penrith M. L., Etter E. M. C. Spatiotemporal analysis of African swine fever outbreaks on South African smallholder farms, 1993–2018. *J. S. Afr. Vet. Assoc*. 2022; 93 (2): 82–88. DOI: 10.36303/JSAVA.161.

15. Mogano K., Suzuki T., Mohale D., Phahladira B., Ngoepe E., Kamata Y., et al. Spatio-temporal epidemiology of animal and human rabies in northern South Africa between 1998 and 2017. *PLoS Negl. Trop. Dis*. 2022; 16 (7):e0010464. DOI: 10.1371/journal.pntd.0010464.

16. Lu X., Ward M. P. Spatiotemporal analysis of reported classical swine fever outbreaks in China (2005–2018) and the influence of weather. *Transbound. Emerg. Dis*. 2022; 69 (5):e3183–e3195. DOI: 10.1111/tbed.14452.

17. González Gordon L., Porphyre T., Muhanguzi D., Muwonge A., Boden L., Bronsvoort B. M. C. A scoping review of foot-and-mouth disease risk, based on spatial and spatio-temporal analysis of outbreaks in endemic settings. *Transbound. Emerg. Dis*. 2022; 69 (6): 3198–3215. DOI: 10.1111/tbed.14769.

18. Gayawan E., Lima E. E. C. A spatio-temporal analysis of cause-specific mortality in São Paulo State, Brazil. *Cien. Saude Colet*. 2022; 27 (1): 287–298. DOI: 10.1590/1413-81232022271.32472020.

19. Wang Z., Dong W., Yang K. Spatiotemporal analysis and risk assessment model research of diabetes among people over 45 years old in China. *Int. J. Environ. Res. Public Health*. 2022; 19 (16):9861. DOI: 10.3390/ijerph19169861.

20. Kulldorff M. A spatial scan statistic. *Communications in Statistics – Theory and Methods*.1997; 26 (6): 1481–1496. DOI: 10.1080/03610929708831995.

21. Kulldorff M., Heffernan R., Hartman J., Assunção R., Mostashari F. A space-time permutation scan statistic for disease outbreak detection. *PLoS Med*. 2005; 2 (3):e59. DOI: 10.1371/journal.pmed.0020059.

22. Kulldorff M. Prospective time periodic geographical disease surveillance using a scan statistic. *J. R. Stat. Soc. Ser. A*. 2001; 164 (1): 61–72. DOI: 10.1111/1467-985X.00186.

23. Nobre F. F., Stroup D. F. A monitoring system to detect changes in public health surveillance data. *Int. J. Epidemiol*. 1994; 23 (2): 408–418. DOI: 10.1093/ije/23.2.408.

24. SaTScanTM. Software for the spatial and space-time scan statistics. Available at: https://www.satscan.org.

25. EFSA, Boklund A., Cay B., Depner K., Földi Z., Guberti V., et al. Epidemiological analyses of African swine fever in the European Union (November 2017 until November 2018). *EFSA J*. 2018; 16 (11):e05494. DOI: 10.2903/j.efsa.2018.5494.

26. Lange M., Guberti V., Thulke H.-H. Understanding ASF spread and emergency control concepts in wild boar populations using individual-based modelling and spatio-temporal surveillance data. *EFSA supp. publ*. 2018; 15 (11):EN-1521. 46 p. DOI: 10.2903/sp.efsa.2018.EN-1521.

27. Lange M., Reichold A., Thulke H.-H. Modelling advanced knowledge of African swine fever, resulting surveillance patterns at the population level and impact on reliable exit strategy definition. *EFSA supp. publ*. 2021; 18 (3):EN-6429. 61 p. DOI: 10.2903/sp.efsa.2021.EN-6429.

28. Lim J.-S., Vergne T., Pak S.-I., Kim E. Modelling the spatial distribution of ASF-positive wild boar carcasses in South Korea using 2019–2020 national surveillance data. *Animals (Basel)*. 2021; 11 (5):1208. DOI: 10.3390/ani11051208.

29. Gervasi V., Marcon A., Bellini S., Guberti V. Evaluation of the efficiency of active and passive surveillance in the detection of African swine fever in wild boar. *Vet. Sci*. 2019; 7 (1):5. DOI: 10.3390/vetsci7010005.

30. Mazur-Panasiuk N., Żmudzki J., Woźniakowski G. African swine fever virus – persistence in different environmental conditions and the possibility of its indirect transmission. *J. Vet. Res*. 2019; 63 (3): 303–310. DOI: 10.2478/jvetres-2019-0058.

31. Fischer M., Hühr J., Blome S., Conraths F. J., Probst C. Stability of African swine fever virus in carcasses of domestic pigs and wild boar experimentally infected with the ASFV "Estonia 2014" isolate. *Viruses*. 2020; 12 (10):1118. DOI: 10.3390/v12101118.

32. Kramer-Schadt S., Fernández N., Eisinger D., Grimm V., Thulke H.-H. Individual variations in infectiousness explain long-term disease persistence in wildlife populations. *Oikos*. 2009; 118 (2): 199–208. DOI: 10.1111/j.1600-0706.2008.16582.x.

33. O'Neil X., White A., Ruiz-Fons F., Gortázar C. Modelling the transmission and persistence of African swine fever in wild boar in contrasting European scenarios. *Sci. Rep*. 2020; 10 (1):5895. DOI: 10.1038/s41598-020-62736-y.

34. EFSA, Nielsen S. S., Alvarez J., Bicout D. J., Calistri P., Depner K., et al. ASF exit strategy: providing cumulative

evidence of the absence of African swine fever virus circulation in wild boar populations using standard surveillance measures. *EFSA J*. 2021; 19 (3):e06419. DOI: 10.2903/j.efsa.2021.6419.

35. Podgórski T., Śmietanka K. Do wild boar movements drive the spread of African swine fever? *Transbound. Emerg. Dis*. 2018; 65 (6): 1588–1596. DOI: 10.1111/tbed.12910.

36. Zani L., Masiulis M., Bušauskas P., Dietze K., Pridotkas G., Globig A., et al. African swine fever virus survival in buried wild boar carcasses. *Transbound. Emerg. Dis*. 2020; 67 (5): 2086–2092. DOI: 10.1111/tbed.13554.

37. Gervasi V., Guberti V. African swine fever endemic persistence in wild boar populations: key mechanisms explored through modelling. *Transbound. Emerg. Dis*. 2021; 68 (5): 2812–2825. DOI: 10.1111/tbed.14194.

Received 20.12.2022
Revised 23.01.2023
Accepted 16.02.2023

INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Olga I. Zakharova, Researcher, Department of Epizootology and Risk Assessment Associated with Animal Health, NNRVI – Branch of the FRCVM, Nizhny Novgorod, Russia; *https://orcid.org/0000-0002-1408-2989, e-mail: olenka.zakharova.1976@list.ru.*

Andrey A. Blokhin, Candidate of Science (Veterinary Medicine), Leading Researcher, Head of Department of Epizootology and Risk Assessment Associated with Animal Health, NNRVI – Branch of the FRCVM, Nizhny Novgorod, Russia; *https://orcid.org/0000-0001-5161-1184, e-mail: and.bloxin2010@yandex.ru.*

Olga A. Burova, Deputy Head of Department of Epizootology and Risk Assessment Associated with Animal Health, NNRVI – Branch of the FRCVM, Nizhny Novgorod, Russia; *https://orcid.org/0000-0002-5396-0334, e-mail: burovaolga@list.ru.*

Ivan V. Yashin, Candidate of Science (Biology), Leading Researcher, Department of Epizootology and Risk Assessment Associated with Animal Health, NNRVI – Branch of the FRCVM, Nizhny Novgorod, Russia; *https://orcid.org/0000-0001-7359-2041, e-mail: ivanyashin@yandex.ru.*

Fedor I. Korennoy, Candidate of Science (Geography), Senior Researcher, Information and Analysis Centre, FGBI "ARRIAH", Vladimir, Russia; *https://orcid.org/0000-0002-7378-3531, e-mail: korennoy@arriah.ru.*

Захарова Ольга Игоревна, научный сотрудник отдела эпизоотологии и оценки риска, связанного со здоровьем животных, ННИВИ – филиал ФГБНУ ФИЦВиМ, г. Нижний Новгород, Россия; *https://orcid.org/0000-0002-1408-2989, e-mail: olenka.zakharova.1976@list.ru.*

Блохин Андрей Александрович, кандидат ветеринарных наук, ведущий научный сотрудник, руководитель отдела эпизоотологии и оценки риска, связанного со здоровьем животных, ННИВИ – филиал ФГБНУ ФИЦВиМ, г. Нижний Новгород, Россия; *https://orcid.org/0000-0001-5161-1184, e-mail: and.bloxin2010@yandex.ru.*

Бурова Ольга Александровна, заместитель руководителя отдела эпизоотологии и оценки риска, связанного со здоровьем животных, ННИВИ – филиал ФГБНУ ФИЦВиМ, г. Нижний Новгород, Россия; *https://orcid.org/0000-0002-5396-0334, e-mail: burovaolga@list.ru.*

Яшин Иван Вячеславович, кандидат биологических наук, ведущий научный сотрудник отдела эпизоотологии и оценки риска, связанного со здоровьем животных, ННИВИ – филиал ФГБНУ ФИЦВиМ, г. Нижний Новгород, Россия; *https://orcid.org/0000-0001-7359-2041, e-mail: ivanyashin@yandex.ru.*

Коренной Федор Игоревич, кандидат географических наук, старший научный сотрудник информационно-аналитического центра ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *https://orcid.org/0000-0002-7378-3531, e-mail: korennoy@arriah.ru.*

DOI: 10.29326/2304-196X-2023-12-1-66-72

Evaluation of the effectiveness of blood-drop agglutination test for chicken tuberculosis diagnosis

M. O. Baratov
Caspian Regional Research Veterinary Institute – Branch of Dagestan Agriculture Science Center, Makhachkala, Republic of Dagestan, Russia

SUMMARY

Due to the increased role of opportunistic infections, mycobacterioses, parasitocenoses, etc. the detectability of nonspecific reactions to PPD-tuberculin has sharply increased, which makes it difficult to make a diagnosis and brings laboratory test methods to the fore. The aim of the study was to determine practical significance of blood-drop agglutination test in comparison with allergy test, and frequency of avian tuberculosis lesions on internal organs. For comparative assessment of these techniques 4,086 chickens were tested, including 2,000 young chicks aged 6–9 months and 2,086 adult poultry. In order to compare the results of allergy and serological tests, necropsy was performed for reacting chickens, identified using blood-drop agglutination test and demonstrating positive results using both methods. Low effectiveness of the allergy test in comparison with the serological test was established. The blood-drop agglutination test made it possible to additionally identify 311 adult chickens seropositive for tuberculosis in poultry farms. The effectiveness of this serological method in young birds and poor matching of results in comparison with an allergy test have been shown. The necropsy findings confirmed the practical significance of the serological test; generalized tuberculosis process was noted in all birds positively reacting in blood-drop agglutination test. The dependence of internal organ lesions on poultry-keeping conditions was determined in tuberculosis-affected farms in the autumn and spring periods. Internal organ lesions were found in 835 birds out of 1,072 tested poultry. In the autumn period the intestines were affected in most cases (57.2%), lung lesions were found in the least cases (8.2%), and in the spring period tuberculosis lesions were more often detected in the lungs (43.8%), less often in the intestines (35.5%). In the winter period, the morbidity predominantly occurs due to dust infection, and in summer, birds become infected via alimentary route, which explains the results obtained. The identification of a significantly larger number of diseased chickens, both in advanced form and at an early stage, makes it possible to recommend a blood-drop agglutination test for the diagnosis of tuberculosis. The involvement of internal organs directly depends on the poultry keeping system and should be taken into account when veterinary and sanitary measures are performed.

Keywords: tuberculosis, chickens, blood-drop agglutination test, necropsy, poultry farming, diagnosis, PPD-tuberculin, antigen, blood

For citation: Baratov M. O. Evaluation of the effectiveness of blood-drop agglutination test for chicken tuberculosis diagnosis. *Veterinary Science Today*. 2023; 12 (1): 66–72. DOI: 10.29326/2304-196X-2023-12-1-66-72.

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

For correspondence: Magomed O. Baratov, Doctor of Science (Veterinary Medicine), Chief Researcher, Head of Laboratory for Infectious Pathology of Livestock, Caspian Regional Research Veterinary Institute – Branch of Dagestan Agriculture Science Center, 367000, Russia, Republic of Dagestan, Makhachkala, ul. Dakhadaeva, 88, *e-mail: alama500@rambler.ru.*

УДК 619:616.98:579.873.21:636.52/.58:616.076

Оценка эффективности кровяно-капельной реакции агглютинации при диагностике туберкулеза кур

М. О. Баратов
Прикаспийский зональный научно-исследовательский ветеринарный институт – филиал ФГБНУ «Федеральный аграрный научный центр Республики Дагестан» (Прикаспийский зональный НИВИ – филиал ФГБНУ «ФАНЦ РД»), г. Махачкала, Республика Дагестан, Россия

РЕЗЮМЕ

В связи с возросшей ролью оппортунистических инфекций, микобактериозов, паразитоценозов и др. резко увеличилась выявляемость неспецифических реакций на ППД-туберкулин, что затрудняет постановку диагноза и выводит на первый план лабораторные методы исследования. Целью настоящей работы явилось определение практической значимости кровяно-капельной реакции агглютинации в сравнении с аллергической пробой и частоты поражения внутренних органов при туберкулезе птиц. Для проведения сравнительной оценки данных методов было исследовано 4086 кур, из них 2000 гол. составлял молодняк 6–9-месячного возраста и 2086 гол. – взрослая птица. В целях сопоставления результатов аллергических и серологических исследований проводили патолого-анатомическое вскрытие кур из числа реагирующих на аллерген, выявленных с помощью кровяно-капельной реакции агглютинации и положительно реагирующих по обоим методам. Установлена низкая эффективность аллергической пробы в сравнении с серологической реакцией. Кровяно-капельная реакция агглютинации позволила дополнительно выявить в птицеводческих хозяйствах 311 серопозитивных на туберкулез взрослых кур. Показана эффективность данного серологического метода и на молодняке птиц с низким совпадением результатов в сравнении с аллергической пробой. Результаты патолого-анатомического вскрытия подтвердили практическую значимость серологической реакции, во всех случаях у положительно реагирующих в кровяно-капельной реакции агглютинации особей отмечена генерализация туберкулезного процесса. Зависимость пораженности внутренних органов от условий содержания птицы определяли в неблагополучных по туберкулезу хозяйствах в осенний и весенний периоды. Выявили

поражение внутренних органов у 835 гол. из 1072 исследованных особей. В осенний период в большинстве случаев был поражен кишечник (57,2%), в меньшинстве – легкие (8,2%), а в весенний период туберкулезные изменения чаще обнаруживали в легких (43,8%), реже – в кишечнике (35,5%). В зимний период содержания главная причина заболеваемости – пылевая инфекция, в летний же период птицы заражаются алиментарным путем, чем и объясняются полученные результаты. Выявление значительно большего количества больных кур, причем как в запущенной форме, так и на ранней стадии, дает возможность рекомендовать кровяно-капельную реакцию агглютинации для диагностики туберкулеза. Пораженность внутренних органов находится в прямой зависимости от системы содержания птицы и должна учитываться при проведении ветеринарно-санитарных мероприятий.

Ключевые слова: туберкулез, куры, кровяно-капельная реакция агглютинации, патолого-анатомическое исследование, птицеводство, диагностика, ППД-туберкулин, антиген, кровь

Для цитирования: Баратов М. О. Оценка эффективности кровяно-капельной реакции агглютинации при диагностике туберкулеза кур. *Ветеринария сегодня*. 2023; 12 (1): 66–72. DOI: 10.29326/2304-196X-2023-12-1-66-72.

Конфликт интересов: Автор заявляет об отсутствии конфликта интересов.

Для корреспонденции: Баратов Магомед Омарович, доктор ветеринарных наук, главный научный сотрудник, заведующий лабораторией инфекционной патологии сельскохозяйственных животных, Прикаспийский зональный НИВИ – филиал ФГБНУ «ФАНЦ РД», 367000, Россия, Республика Дагестан, г. Махачкала, ул. Дахадаева, д. 88, *e-mail: alama500@rambler.ru.*

INTRODUCTION

Despite the fact that most of the commitments and goals set within animal tuberculosis control programs have been achieved, tuberculosis in both animals and humans currently remains one of the most complex and significant concerns [1–3].

After the change of socio-economic formation, disruption of established links in the livestock management system and a sharp decline in the industry's potential, it became urgent to develop effective methods for control of infectious diseases, in particular avian tuberculosis [4–6].

According to some authors, with the transfer of poultry farming to commercial basis and reduction in poultry raising time period under optimal veterinary and sanitary conditions on complete feed, the problem of avian tuberculosis has become less notable [7, 8].

At the same time, the emerging increase in the number of poultry in private farms, where uncontrolled and unsystematic movement of both poultry and products is typical, causes certain difficulties in carrying out animal health, managerial and anti-tuberculosis measures. Under these conditions, poultry can become a source of constant circulation of mycobacteria in the environment [9].

According to the literature data, in some farms tuberculosis-affected chickens became the source of infection in cattle. In certain cases, milkers who kept chickens in backyard farms were *Mycobacterium avium* vectors [10–17].

In this regard, timely detection of chickens with tuberculosis is of great practical importance for improving the health of farms affected with this disease. The currently practiced diagnostic methods do not allow detecting all birds with tuberculosis [18–20]. This is evidenced by the fact that after the double tuberculinization and isolation of birds reacting to PPD-tuberculin, chickens with a pronounced form of tuberculosis are subsequently detected in disease-free herds according to necropsy findings [20–24].

The issue of improving and finding new, more effective methods of tuberculosis diagnosis in chickens has been of interest to researchers for a long time. So, for this purpose, scientists began to test serological methods based on immunological tests, for example, the agglutination test. In this regard, a tuberculosis antigen was prepared from avian *Mycobacterium avium* culture [25–32].

Some researchers carried out the hemagglutination test using the blood sera of tuberculosis patients [33–36].

In order to find approaches to the development of more advanced methods of avian tuberculosis diagnosis and taking into account the detectability of chickens with a 50% infection rate among non-reactors to tuberculin, the antigen (strain 9 with pronounced antigenic properties) was prepared for blood-drop agglutination test (BDAT) at the All-Union Institute of Experimental Veterinary Medicine (now the Federal Research Center – All-Russia Research Institute of Experimental Veterinary Medicine named after K. I. Scriabin and Ya. R. Kovalenko of RAS).

In 1955 A. V. Prokhorov et al. conducted BDAT of antigen prepared from *Mycobacterium avium* cultures and compared its results with those of a tuberculin test. At the same time, it was found that a higher number of diseased chickens were detected using the serological method rather than when the allergy test was implemented. Thus, out of 23,355 birds tested using the allergen, 756 (3.2%) birds with tuberculosis were identified. Out of 22,599 non-tuberculin reactors, tuberculosis was diagnosed in 2,079 (8.4%) birds when tested with BDAT [23].

Similar results were obtained by other researchers who consider BDAT to be an effective method of avian tuberculosis diagnosis that can be used for identification of diseased chickens both at the initial disease stage and at the stage of systemic infection [37, 38].

Subsequently, in order to detect a mixed infection (pullorum infection and tuberculosis), a complex antigen was manufactured at the Lithuanian Veterinary Research Institute, which is a mixture of the GNKI pullorum antigen and the tuberculosis antigen of the Lithuanian Veterinary Research Institute [39].

In order to isolate the maximum number of infected birds, many researchers recommend conducting a comprehensive study using two methods: BDAT and tuberculinization [38].

Unfortunately, no data on the scientific significance and practical effectiveness of the blood-drop agglutination test in the diagnosis of chicken tuberculosis is available in the literature. Due to the fact that many aspects of this test's performance remain unstudied, there continues to be a multiplicity of concepts about the justifiability and relevance of this test.

It is important to note that due to the increased role of opportunistic infections, mycobacterioses, parasitocenoses, etc., the detectability of nonspecific PPD-tuberculin reactions has sharply increased, which, of course, complicates the diagnosis and brings laboratory test methods to the fore. In this regard, in order to identify chickens affected with tuberculosis, the necessity of serological test methods is justified, though their role, in our view, is often underestimated.

The aim of the study was to compare the effectiveness of the serological test method (BDAT) with the allergy test, as well as to assess lesions in birds' internal organs in correlation with the chicken keeping system.

MATERIALS AND METHODS

BDAT studies in the diagnosis of avian tuberculosis were carried out in farms of the Republic of Dagestan in the period from 2015 to 2021.

A blood-drop agglutination test using whole blood and antigen was used in combination with intradermal allergy diagnostic testing.

One or two drops of antigen (prepared in the laboratory from a day-old meat-peptone agar culture) were pipetted onto a clean slide. Blood was taken from the axillary vein, transferred to the slides and mixed with the antigen. The reaction was considered positive if the mixture became lighter and flakes were formed due to adhesion of microbes to antibodies, and it was considered specific, if agglutination occurred within one minute.

For comparative assessment of BDAT and allergy test 4,086 chickens were tested, including 2,000 6–9 month-old young birds and 2,086 adult birds.

In order to compare the results of allergic, serological and post-mortem tests, 300 chickens were subjected to diagnostic slaughter: 100 birds reacting to allergen, 100 birds identified with BDAT and 100 birds having positive reactions in both diagnostic tests.

To establish the correlation between internal organ lesions and the poultry keeping system, 1,072 birds were slaughtered: 579 – in the autumn, 493 – in the spring.

Allergy tests were carried out in accordance with the "Guidelines for the use of (PPD) tuberculins in mammals and birds"[1] using avian PPD-tuberculin via intradermal inoculation into the wattle at a dose of 0.1 mL. The test result (the formation of swelling at the injection site) was read after 30–36 hours.

During the simultaneous test, two allergens (dry purified tuberculin and dry purified complex allergen from atypical mycobacteria, CAM) were intradermally inoculated into both sides of the chicken wattles at a dose of 0.1 mL: after that differences in severity of reactions to these allergens were determined[2].

All animal handling procedures were performed in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS No. 123).

RESULTS AND DISCUSSION

Comparison of the effectiveness of the BDAT and the allergy test was carried out in farms with the capacity of 2,086 adult poultry considered relatively free from tuberculosis.

The total number of adult poultry in OOO "Buynaksky poultry farm" was 3,678 birds, no PPD-tuberculin reactors were detected there according to the results of scheduled allergy tests over the past 5 years. There were 1,200 animals in the farm SPK "Buglen", positive tuberculin reactors were detected, but the diagnosis was not confirmed later. The diagnosis was not confirmed among adult poultry (768 birds) in the farm "Kazbek" either. No positively reacting birds were found in the flock of 630 birds in the IP "Ruguzh". The test results for adult poultry are shown in Table 1.

It was established that more diseased birds were additionally detected using the BDAT: in OOO "Buynaksky poultry farm" – 3 birds, SPK "Buglen" – 78 birds, KFKh "Kazbek" – 227 birds and IP "Ruguzh" – 3 diseased chickens. The matching of the results of the allergy test and BDAT was noted in 73.90–75.40% of cases.

Testing of young animals of 6–9 months of age was carried out in two farms: on the poultry farms OOO "Karabudakhkentskaya" and at KFKh "Tarki", where over the past 5 years no poultry was subjected to allergy tests. It was possible to identify both seropositive and positively responding to tuberculin birds.

As the results presented in Table 2 show, tuberculosis can be detected with BDAT in birds at an early age. So, 18 birds (1.3%) were detected serologically at the poultry farm OOO «Karabudakhketskaya», and 12 diseased birds (2.0%) were detected in the farm KFKh "Tarki". The matching of BDAT and the allergy diagnostic test results in young animals ranges from 12.5 to 27.8%.

It should be noted that the sensitivity of BDAT at the poultry farm OOO "Karabudakhkentskaya" was higher. Comparing allergy test and serological test results, it can be concluded that a high level of seropositivity indicates the threat of reactivity of the tuberculosis latent form. The high information value of specific antibody detection using BDAT gives grounds to use this test for monitoring in the system of comprehensive infection prevention.

The necropsy results for poultry that reacted to the allergen and were identified using BDAT are shown in Table 3.

During necropsy of positively reacting chickens tested with the BDAT, the tuberculosis systemic process was noted in 13% of the tested birds; lesions of the spleen and intestine were observed in 26% of birds, of liver and intestine – in 14% of tested birds, of liver, intestine and spleen –

[1] Guidelines for the use of (PPD) tuberculins in mammals and birds. Available at: http://www.agrozoo.ru/text/vetprep_html/238.html.

[2] Guidelines for conducting a simultaneous allergy test using tuberculin and a complex allergen from atypical mycobacteria (CAM) in the diagnosis of tuberculosis in animals: approved by the Ministry of Agriculture of the USSR of November 27, 1978. Available at: https://base.garant.ru/70526680.

Table 1
Comparative study of agglutination and allergy test in adult poultry

Name of establishment	Number of tested poultry, birds	Diseased chickens identified with				Matching of BDAT and allergy test results	%
		Allergy test, birds	%	BDAT, birds	%		
OOO "Buynaksky poultry farm"	1,221	–	–	3	0.25	–	–
SPK "Buglen"	400	161	40.25	239	59.75	119	73.90
KFKh "Kazbek"	365	69	18.90	296	81.10	52	75.40
IP "Ruguzh"	100	–	–	3	3	–	–

Table 2
Comparative study of agglutination and allergy test in young poultry

Name of establishment	Number of tested poultry, birds	Diseased chickens identified with				Matching of BDAT and allergy test results	%
		Allergy test, birds	%	BDAT, birds	%		
OOO "Karabudakhkentskaya"	1,400	5	0.36	18	1.30	5	27.80
KFKh "Tarki"	600	16	2.70	12	2.00	2	12.50

in 3% of birds. Tuberculous nodes were detected in the following organs: liver (9%), intestine (7%) and spleen (3%).

During necropsy of chickens identified based only on the results of an intradermal allergic test, systemic tuberculosis was detected in 9% of cases, lesions of the spleen and intestine – in 24% of cases, liver and intestine – in 16% of cases, liver, spleen and intestine – in 1% of cases. As for other organs, lesions were detected in the liver (3%), intestine (4%) and spleen (2%).

Necropsy of chickens with positive reactions detected during diagnostic testing performed by both methods showed the presence of systemic infection in 12%, lesions of the spleen and intestine in 18%, liver and intestine in 15%, liver, spleen and intestine in 2%, liver, intestine and oviduct in 4%, liver, intestine and lymphoid tissues in 1% of the birds subjected to examination. Tuberculous nodes were observed in some organs: liver (6%), intestine (2%) and spleen (1%).

In general, tuberculosis in chickens is accompanied by internal organ lesions typical for this disease. Yellowish-gray or grayish-white tubercles of various shapes and sizes are noted. At the initial stage, some organs are affected with the formation of nodes ranging in size from a poppy seed to a pinhead, clearly visible and separating from healthy tissue.

In advanced cases, there are tuberculous tubercles in several or all internal organs. Sometimes solid knots reaching the size of a hazelnut are formed. Large nodes are often located close to each other, they can merge, forming conglomerates up to 4 cm in size.

Lesions are often observed in several organs, primarily the liver, spleen, and then others, with uniform nodules of the same size.

In general, tuberculosis of chickens is accompanied with patchy lesions in internal organs.

In connection with the above, it seemed reasonable to study correlations between internal organ lesions and

Table 3
Comparison of parameters of internal organ lesions in chickens with tuberculosis

Lesion location	Positive results obtained using		
	BDAT	allergy test	allergy test + BDAT
Systemic form	13	9	12
Spleen and intestine	26	24	18
Liver and intestine	14	16	15
Liver	9	3	6
Intestine	7	4	2
Liver, intestine and spleen	3	1	2
Spleen	3	2	1
Liver, intestine and oviduct	–	–	4
Liver, intestine and lymphoid tissues	–	–	1
There were no macroscopically visible lesions	25	41	39

Table 4
Necropsy findings

Affected internal organs	Infection cases according to the study period			
	autumn		spring	
	number	%	number	%
Liver	83	16	33	10.2
Spleen	27	5.2	18	5.6
Intestine	26	5	2	0.6
Liver and intestine	122	23.4	22	6.8
Systemic form	74	14.6	22	6.8
Liver, spleen and intestine	93	18.5	61	18.9
Intestine and spleen	27	6.4	–	–
Liver, spleen and lungs	27	5.2	32	9.9
Intestine, mesentery and lungs	10	1.9	–	–
Intestine and lungs	8	1.5	–	–
Oviduct	6	1.1	–	–
Spleen and lungs	3	0.5	2	0.6
Liver, spleen and intestine	3	0.5	2	0.6
Muscles	2	0.4	–	–
Liver and lungs	1	0.38	28	14.9
Lungs	2	0.4	14	4.3
Liver, intestine and lungs	–	–	8	2.4
Liver, lungs and oviduct	–	–	12	3.7
Liver and gizzard	–	–	2	0.6
Liver, spleen and oviduct	–	–	10	3
Lungs and kidneys	–	–	2	0.6
Lungs, liver and kidneys	–	–	12	3.7

the poultry keeping system. For that, poultry in tuberculosis-affected farms were tested after winter and summer periods – in spring and autumn. The results are shown in Table 4.

It has been established that lesions of internal organs were detected in 796 birds out of 1,072 birds subjected to diagnostic slaughter at different times. According to the frequency of lesions, the liver, intestine and spleen come first, however many cases of systemic tuberculosis process are also recorded. Along with this, tuberculous lesions are observed in the lungs, oviduct, kidneys, gizzard, cloaca.

As the results of the post-mortem dissection conducted in the autumn period showed, tuberculous nodes were most often found in the intestine (56.2%), less frequently in the lungs (8.9%), and as regards the spring period: in most cases – in the lungs (39.0%) and in the least cases – in the intestine (33.6%). The poultry keeping conditions are likely to be the reason for this. In winter, dust infection seems to prevail, and in summer, birds become infected via alimentary routes.

CONCLUSIONS

1. For avian tuberculosis diagnosis, it is necessary to conduct a comprehensive study with simultaneous performance of a blood-drop agglutination test and a double intradermal allergy test.

2. The blood-drop agglutination test makes it possible to identify a significantly larger number of chickens with tuberculosis both at the systemic infection stage and at an early stage of the disease.

3. The liver, spleen, intestine in birds with tuberculosis are most often affected, and other organs get affected to a lesser extent.

4. The lesions in the intestine and lungs are directly correlated with the poultry keeping conditions. Lesions in the lungs prevail in the winter period, and lesions in the intestine are more common in summer.

REFERENCES

1. Baratov M. O., Huseynova P. S. Actual bovine tuberculosis situation in the Republic of Dagestan. *Veterinary Science Today*. 2022; 11 (3): 222–228. DOI: 10.29326/2304-196X-2022-11-3-222-228.

2. Kassich Yu. Ya., Borzyak A. T., Kochmarsky A. F., et al. Animal Tuberculosis and Measures to Control It. Ed. by Yu. Ya. Kassich. Kiev: Urozhay; 1990. 303 p. (in Russ.)

3. Sadovnikov N. V., Petrova O. G. Tuberkulez u melkikh zhivotnykh i u ptits = Tuberculosis in small animals and birds. *BIO.* 2019; 4 (223): 20–23. (in Russ.)

4. Bessarabov B. F. Poultry diseases. Moscow: Kolos; 2004. 347 p. (in Russ.)

5. Gavrish V. G. Handbook for veterinary physicians. Rostov-on-Don: Feniks, 2004. 576 p. (in Russ.)

6. Bessarabov B. F., Mel'nikova I. I., Sushkova N. K., Sadchikov S. Yu. Avian diseases. Saint Petersburg: Lan'; 2007. 445 p. (in Russ.)

7. Spiridonov A. N., Petrova O. N., Irza V. N., Karaulov A. K., Nikiforov V. V. Epizootic situation on infectious avian diseases based on analysis of data from veterinary reports. *Veterinary Science Today*. 2015; (4): 18–28. Available at: https://veterinary.arriah.ru/jour/article/view/215.

8. Baratov M. O. Animal tuberculosis: monograph. Makhachkala: Dagestan Agriculture Science Center; 2018. 198 p. eLIBRARY ID: 37122407. (in Russ.)

9. Dvorska L., Matlova L., Ayele W. Y., Fischer O. A., Amemori T., Weston R. T., et al. Avian tuberculosis in naturally infected captive water birds of the *Ardeideae* and *Threskiornithidae* families studied by serotyping, IS901 RFLP typing, and virulence for poultry. *Vet. Microbiol*. 2007; 119 (2–4): 366–374. DOI: 10.1016/j.vetmic.2006.09.010.

10. Yarbaev N., Mirzoev D. M., Khasanov N. R. Sistema protivotuberkuleznykh meropriyatii v skotovodstve i ee protivoepizooticheskaya effektivnost' = The system of anti-tuberculosis measures in cattle breeding and its anti-epizootic effectiveness. *Problemy razvitiya sel'skokhozyaistvennoi nauki Respubliki Tadzhikistan: materialy konferentsii, posvyashchennoi 85-letiyu akademika G. A. Alieva = Problematic Issues of Agricultural Science Development in the Republic of Tajikistan: Proceedings of the Conference Dedicated to the 85th Anniversary of Academician G. A. Aliyev.* Dushanbe; 2001; 105–107. (in Russ.)

11. Sangari F. J., Parker A., Bermudez L. E. *Mycobacterium avium* interaction with macrophages and intestinal

epithelial cells. *Front. Biosci*. 1999; 4 (4): 582–588. DOI: 10.2741/sangari.

12. Plattner B. L., Huffman E., Jones D. E., Hostetter J. M. T lymphocyte responses during early enteric *Mycobacterium avium* subspecies *paratuberculosis* infection in cattle. *Vet. Immunol. Immunopathol*. 2014; 157 (1–2): 12–19. DOI: 10.1016/j.vetimm.2013.11.001.

13. Douarre P. E., Cashman W., Buckley J., Coffey A., O'Mahony J. M. Isolation and detection of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) from cattle in Ireland using both traditional culture and molecular based methods. *Gut. Pathog.* 2010; 2 (1):11. DOI: 10.1186/1757-4749-2-11.

14. Zhurnakova M. A., Malygin V. I., Borisenkova A. N., Bolotnikov I. I. Paraallergicheskie reaktsii na tuberkulin u krupnogo rogatogo skota, zarazhennogo ptich'im tipom mikobakterii = Para-allergic reactions to tuberculin in cattle infected with avian mycobacterium. *Veterinariya*. 1964; 3: 23–25. (in Russ.)

15. Tuzova R. V. Eksperimental'noe izuchenie patogennosti vozbuditelya tuberkuleza ptich'ego tipa dlya krupnogo rogatogo skota = Experimental study of the pathogenicity of avian tuberculosis agent for cattle. *Bor'ba s poteryami v zhivotnovodstve.* Minsk; 1963; 20–30. (in Russ.)

16. Tuzova R. V. Tuberculosis in livestock and poultry. Minsk: Uradzhai; 1983. 263 p. (in Russ.)

17. Kokurichev P. I., Rotov V. I. Poultry tuberculosis. Moscow; Leningrad: Sel'khozgiz; 1959. 133 p. (in Russ.)

18. Bakulov I. A., Yurkov G. G., Peskovatskov P. P., Vedernikov V. V. Methodical guidelines for epizootological investigation. Moscow: Kolos; 1982. 16 p. (in Russ.)

19. Naimanov A. Kh. Differentsiatsiya allergicheskikh reaktsii na tuberculin = Differentiation of allergic reactions to tuberculin. *Veterinariya*. 2002; 3: 10–13. eLIBRARY ID: 26691713. (in Russ.)

20. Zhumash A. S., Bazarbaev M. B., Sargaskaev D. T. Chastota proyavleniya nespetsificheskikh tuberkulinovykh reaktsii u zhivotnykh v blagopoluchnykh po tuberkulezu khozyaistvakh = The frequency of manifestation of non-specific tuberculin reactions in animals in tuberculosis-free farms. *Vestnik Kirgizskogo NII zhivotnovodstva, veterinarii i pastbishch im. A. Duisheeva*. Bishkek; 2007; 1: 282–285. (in Russ.)

21. Schmidt V., Köhler H., Heenemann K., Möbius P. Mycobacteriosis in various pet and wild birds from Germany: Pathological findings, coinfections, and characterization of causative mycobacteria. *Microbiol. Spectr*. 2022; 10 (4):e0045222. DOI: 10.1128/spectrum.00452-22.

22. Shivaprasad H. L., Palmieri C. Pathology of mycobacteriosis in birds. *Vet. Clin. North Am. Exot. Anim. Pract*. 2012; 15 (1): 41–55. DOI: 10.1016/j.cvex.2011.11.004.

23. Prokhorov A. B., Fomina A. Ya., Akulov A. V. Opyt primeneniya KKRA dlya diagnostiki tuberkuleza kur = Experience of BDAT for avian tuberculosis diagnosis. *Veterinariya*. 1955; 11: 42–45. (in Russ.)

24. Tell L. A., Woods L., Cromie R. L. Mycobacteriosis in birds. *Rev. Sci. Tech*. 2001; 20 (1): 180–203. DOI: 10.20506/rst.20.1.1273.

25. Pavlas M., Michalská A., Huňady M. Diagnosis of avian tuberculosis-mycobacteriosis by rapid agglutination. *Acta Vet. Brno*. 1993; 62 (1): 63–69. DOI: 10.2754/avb199362010063.

26. Moses H. E., Feldman W. H., Mann E. C. Mycobacterial rapid agglutination antigens and their diagnostic value in tuberculosis of fowl. *Amer. J. Veterin. Res*. 1943; 4 (12): 390–394.

27. Visy L., Dozsa I., Paszlor L. Serological slide agglutination tests using an antigen prepared from decapsulated tuberculosis bacteria on zoo birds. *Proceedings of the V International Symposium on Diseases in Zoo Animals.* 1963; 214–219.

28. Akylbekova K. T., Kasymov T. K., Kasymbekov Zh. B., Kim V. I., Nurgaziev R. Z. Serological diagnosis of animal tuberculosis. *Vestnik «Prostranstvo uchenykh v mire»*. 2018; 4: 8–10. (in Russ.)

29. Cromie R. L., Brown M. J., Forbes N. A., Morgan J., Stanford J. L. A comparison and evaluation of techniques for diagnosis of avian tuberculosis in wildfowl. *Avian Pathol.* 1993; 22 (3): 617–630. DOI: 10.1080/03079459308418948.

30. Hiller K., Schliesser T., Fink G., Dorn P. Zur serologischen Diagnose der Hühnertuberkulose = The serological diagnosis of tuberculosis in chickens. *Berl. Münch. Tierärztl. Wochenschr*. 1967; 80 (11): 212–216. PMID: 5627883. (in German)

31. Hawkey C., Kock R. A., Henderson G. M., Cindery R. N. Haematological changes in domestic fowl (*Gallus gallus*) and cranes (*Gruiformes*) with *Mycobacterium avium* infection. *Avian Pathol.* 1990; 19 (2): 223–234. DOI: 10.1080/03079459008418675.

32. Kwatra M. S., Sharma G. L., Singh G. Passive hemagglutination test in diagnosis of experimental tuberculosis in ducks, compared with other fowl. *Avian Diseases.* 1972; 16 (5): 1035–1041. DOI: 10.2307/1588826.

33. Greuel H., Pothmann F. J., Quitte C., Wilms D. Beitrag zur Hämagglutinationsreaktion auf Tuberkulose. *Beiträge zur Klinik der Tuberkulose*. 1954; 110: 483–491. DOI: 10.1007/BF02148553. (in German)

34. Vior *C.* Untersuchung zur Herstellung eines Antigens für Frischblut bei Geflügeltuberkulose. *Lucr. sti. Inst. Pat. Igiena Anim. București.* 1962; 12: 57–66. (in German)

35. Ivanov M. M. Some problems of tuberculosis control in cattle and specificity of the tuberculin reaction. *Bull. Off. Int. Epizoot.* 1965; 63 (9): 1403–1418. PMID: 5893671.

36. Ivanoff K. Uber Geflügeltuberkulose und ihre Verbreitung in Bulgarien. *Zeitschrift für Infektionskrankheiten, parasitäre Krankheiten und Hygiene der Haustiere.* 1933; 44: 243–249. (in German)

37. Semenchuk K. L., Tribo L. P. O serologicheskoi diagnostike tuberkuleza ptits = Serological diagnosis of avian tuberculosis. *Trudy VIEV*. 1966; 32: 162–165. (in Russ.)

38. Kuznetsov V. A. Sravnitel'naya otsenka krovekapel'noi reaktsii agglyutinatsii i allergicheskoi proby pri diagnostike tuberkuleza kur = Comparative analysis of blood-drop agglutination test and allergic test for chicken tuberculosis diagnosis. *Tezisy dokladov itogovoi nauchnoi konferentsii za 1968 god = Abstracts of presentations at the final scientific conference in 1968.* Vitebsk: Vitebskii veterinarnyi institut; 1969; 33–35. Available at: https://repo.vsavm.by/bitstream/123456789/15421/1/k-1969-1-1-33-35.pdf. (in Russ.)

39. Bal'chyunas I. I. Comparative assessment lifetime diagnosis of chicken tuberculosis and pullorum disease: author's abstract of Doctor of Science thesis (Veterinary Medicine). Kharkiv; 1974. 43 p. (in Russ.)

Received 17.10.2022
Revised 06.12.2022
Accepted 10.01.2023

INFORMATION ABOUT THE AUTHOR / ИНФОРМАЦИЯ ОБ АВТОРЕ

Magomed O. Baratov, Doctor of Science (Veterinary Medicine), Chief Researcher, Head of Laboratory for Infectious Pathology of Livestock, Caspian Regional Research Veterinary Institute – Branch of Dagestan Agriculture Science Center, Makhachkala, Republic of Dagestan, Russia;
https://orcid.org/0000-0002-8261-5038,
e-mail: alama500@rambler.ru.

Баратов Магомед Омарович, доктор ветеринарных наук, главный научный сотрудник, заведующий лабораторией инфекционной патологии сельскохозяйственных животных, Прикаспийский зональный НИВИ – филиал ФГБНУ «ФАНЦ РД», г. Махачкала, Республика Дагестан, Россия;
https://orcid.org/0000-0002-8261-5038,
e-mail: alama500@rambler.ru.

DOI: 10.29326/2304-196X-2023-12-1-73-78

Clinical and morphological features of salmonellosis in cows and calves affected by heat stress

L. I. Drozdova, E. N. Shilova, I. A. Shkuratova, M. V. Ryaposova
Federal State Budgetary Scientific Institution "Ural Federal Agrarian Scientific Research Centre, Ural Branch of the Russian Academy of Sciences" (FSBSI UrFASRC, UrB of RAS), Ekaterinburg, Russia

SUMMARY

Exposure to abnormally high temperatures in the Middle Urals has led to chronic heat stress in cattle. Despite the measures taken to protect the dairy herd from overheating (fans, irrigation with cool water, livestock sunshades), the heat stress undermined the immunity of animals, thus, leading to an excessive rise in *Salmonella* population in the gastrointestinal tract, increased intestinal permeability, and could also be the cause of increased virulence of such serotypes as – *Salmonella choleraesuis*, that are not typical for cattle. The simultaneous presence of two pathogens of *Salmonella enterica* subsp. *enterica* species: i.e. *Salmonella enteritidis* and *Salmonella choleraesuis* – caused a number of clinical and morphological changes in adult cows. In cows, the infection manifested itself by persistent diarrhea with mucus, reduced milk production and intoxication. In calves, the disease was accompanied by septic signs, severe intoxication, hyperthermia (40.5–41.0 °C), severe dehydration (more than 7%) and death. Histopathological examination of bovine parenchymal organs revealed changes typical for salmonellosis pathological process: specific salmonella granulomas were found in parenchymal organs (liver, spleen). Some pathological processes typical for sepsis were found in other parenchymal organs (kidneys, heart). The nature of pathological changes indicates that there is a septic process that spreads both hematogenically and lymphogenically, and the structure of granulomas most likely indicates that such a pathogen as *Salmonela choleraesuis* circulates in cattle.

Keywords: salmonellosis, cattle, heat stress, morphological changes of parenchymal organs, salmonella granuloma

For citation: Drozdova L. I., Shilova E. N., Shkuratova I. A., Ryaposova M. V. Clinical and morphological features of salmonellosis in cows and calves affected by heat stress. *Veterinary Science Today*. 2023; 12 (1): 73–78. DOI: 10.29326/2304-196X-2023-12-1-73-78.

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

For correspondence: Irina A. Shkuratova, Doctor of Science (Veterinary Medicine), Professor, Associate Member of the Russian Academy of Sciences, Director of the Ural Scientific Research Veterinary Institute – Branch of the FSBSI UrFASRC, UrB of RAS, 620142, Russia, Ekaterinburg, ul. Belinsky, 112a, *e-mail: Shkuratova@bk.ru*.

УДК 619:579.842.14:616-022.6:636.2

Клинико-морфологические особенности сальмонеллеза у коров и телят на фоне теплового стресса

Л. И. Дроздова, Е. Н. Шилова, И. А. Шкуратова, М. В. Ряпосова
ФГБНУ «Уральский федеральный аграрный научно-исследовательский центр Уральского отделения Российской академии наук» (ФГБНУ УрФАНИЦ УрО РАН), г. Екатеринбург, Россия

РЕЗЮМЕ

Воздействие аномально высоких температур на Среднем Урале привело к развитию хронического теплового стресса у крупного рогатого скота. Несмотря на меры, которые принимались для защиты молочного стада от перегрева (вентиляторы, орошения прохладной водой, создание затенения дворов), тепловой стресс ослаблял иммунную систему животных, что приводило к чрезмерному увеличению популяции сальмонелл в желудочно-кишечном тракте у животных, нарушению проницаемости кишечной стенки для бактерий, а также мог быть причиной повышения вирулентности для крупного рогатого скота не свойственных им серотипов – *Salmonella choleraesuis*. Одновременное присутствие двух патогенов вида *Salmonella enterica* subsp. *enterica*: *Salmonella enteritidis* и *Salmonella choleraesuis* – вызвало комплекс клинико-морфологических изменений в организме взрослых коров. У коров инфекция проявлялась стойкой слизистой диареей, потерей уровня лактации и развитием интоксикации. У телят заболевание сопровождалось септическими явлениями, выраженной интоксикацией, гипертермией (40,5–41,0 °С), развитием значительного (более 7%) обезвоживания и гибелью. При гистологическом исследовании паренхиматозных органов коров выявлены изменения, характерные для развития патологического процесса, свойственного сальмонеллезу: в паренхиматозных органах (печени, селезенке) обнаружены специфические сальмонеллезные гранулемы. В других паренхиматозных органах (почках, сердце) обнаружен комплекс патологических процессов, характерных для сепсиса. Характер патологических изменений свидетельствует о развитии септического процесса, распространяющегося как гематогенно, так и лимфогенно, причем строение гранулем, скорее всего, указывает на циркуляцию в организме крупного рогатого скота такого возбудителя, как *Salmonella choleraesuis*.

Ключевые слова: сальмонеллез, крупный рогатый скот, тепловой стресс, морфологические изменения паренхиматозных органов, сальмонеллезная гранулема

Благодарности: Работа выполнена при финансовой поддержке Минобрнауки России в рамках Программы фундаментальных научных исследований в Российской Федерации на долгосрочный период (2021–2030 годы) по направлению «4.2.1.5 Разработка технологий прижизненного управления качеством животноводческого сырья для получения высококачественных и безопасных продуктов питания».

Для цитирования: Дроздова Л. И., Шилова Е. Н., Шкуратова И. А., Ряпосова М. В. Клинико-морфологические особенности сальмонеллеза у коров и телят на фоне теплового стресса. *Ветеринария сегодня*. 2023; 12 (1): 73–78. DOI: 10.29326/2304-196X-2023-12-1-73-78.

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

Для корреспонденции: Шкуратова Ирина Алексеевна, доктор ветеринарных наук, профессор, член-корреспондент РАН, директор Уральского научно-исследовательского ветеринарного института – структурного подразделения ФГБНУ УрФАНИЦ УрО РАН, 620142, Россия, г. Екатеринбург, ул. Белинского, д. 112а, *e-mail: Shkuratova@bk.ru.*

INTRODUCTION

Salmonellosis is one of the most severe human toxicoinfections with a pronounced zooanthroponotic nature of transmission [1–4].

Dairy cattle are a permanent salmonellosis reservoir [5–7]. The enzootic outbreaks are permanently caused by bacteria carriers that excrete *Salmonella* in concentrations up to 10^{14} CFU/g per day in faeces and 10^2–10^5 CFU/mL in milk and colostrum [8, 9]. C. L. Holschbach et al. [10] together with A. G. Glotov et al. [11, 12] found that 20% of clinically diseased and 5% of clinically healthy cows periodically or continuously shed the pathogen into the environment. Asymptomatic *Salmonella* carriers who shed bacteria with faeces can be reported among adult cattle at all production stages. The clinical manifestation of the disease is usually associated with calves of one month of age and older [11–15]. However, on some farms salmonellosis clinical signs are registered in cows as well. According to some researchers [16, 17], the disease is seasonal (late summer/autumn), which may be explained by high ambient temperature and heat stress impact. At the same time, *Salmonella* isolates from asymptomatic cattle and from cattle with clinical manifestation usually do not differ in serogroup, serotype or sensitivity to antimicrobial drugs [15].

As encyclopedic data on bovine salmonellosis suggest, it is an infectious disease that affects young cattle from the age of 10 days, characterized by a pathological process in the digestive organs, and, in case of a chronic form, it also affects the pulmonary system and almost all body systems. Granuloma formation in livers revealed by histological examination of organs is the main morphological feature of salmonellosis-affected animals [10–12].

This paper describes a case of salmonellosis on a dairy farm in adult cows and calves, which, in our opinion, is associated with abnormal average monthly temperatures of the spring-summer period. Alongside with that, both clinical and morphological changes typical for salmonellosis were detected.

The scientific novelty of the research is related to the fact that new atypical pathomorphological changes were detected in cattle, i.e. salmonella-caused hepatic granulomas (more similar to granulomas found in pigs with *Salmonella choleraesuis* infection) and thromboendophlebitis, which is also unusual for cattle.

The purpose of this research is to characterize pathomorphological and histological signs in cattle associated with the simultaneous detection of two *Salmonella* species in these animals – *Salmonella enteritidis* and *Salmonella choleraesuis*.

MATERIALS AND METHODS

The research was carried out within Long-term program of basic research in the Russian Federation (2021–2030), scientific project "Developing technologies for lifetime quality management of livestock raw materials to obtain high-quality and safe food" in the Department of veterinary laboratory diagnostics with the testing laboratory of the FSBSI UrFASRC, UrB of RAS, as well as in independent laboratory OOO "Quality Med" (Ekaterinburg).

For microbiological tests, faecal samples were taken from the rectum of each cow with clinical signs of diarrhea, disposable gloves were used. Samples of parenchymal organs (lungs, liver, spleen, lymph nodes) were taken from the dead calves in compliance with the rules on sampling biomaterial for microbiological tests[1]. In total, samples from 12 animals were examined.

The tested biomaterial was inoculated into nutrient media: 5% sheep blood agar (Columbia agar, Bio-Rad, France; defibrinated sheep blood, E&O Laboratories Ltd, UK); yolk-salt agar (nutrient agar for cultivation of microorganisms – GRM-agar, Federal Budgetary Institution of Science "State Research Center for Applied Microbiology and Biotechnology", FBIS SRCAMB, Russia); chromogenic agar (UriSelect4 Agar, Bio-Rad, France); Endo agar (FBIS SRCAMB, Russia); Sabouraud agar with 2% glucose and chloramphenicol (SIFIN diagnostics GmbH, Germany); 500 µL were inoculated into a test tube with 4.5 mL of magnesium medium (FBIS SRCAMB, Russia) and after 24 hours of incubation, they were re-inoculated to bismuth sulfite agar (FBIS SRCAMB, Russia).

Then the inoculated Petri dishes were placed in a thermostat with aerobic conditions at a temperature of (37 ± 1) °C, the dishes with blood agar were incubated in the environment containing 5% CO_2. The inoculations were incubated for 72 hours, growth assessed after 24, 48, 72 hours.

The grown colonies were identified by MALDI-ToF mass spectrometry (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry) using mass

[1] Rules on sampling pathological material, blood, feed and sending them for laboratory tests, as approved by the Veterinary Department of the Ministry of Agriculture of the USSR on June 24, 1971.

spectrometer Vitek® MS (bioMérieux, France). For this purpose, the bacterial mass was applied to a spot on the slide, coated with 1 μL of the matrix (*α*-cyano-3-hydroxycinnamic acid), dried at room temperature, then the mass spectra of ribosomal proteins were read by the device and compared with the database using the Myla software.

To diagnose clostridial infection in the biomaterial using polymerase chain reaction (PCR), "RealBest-Vet DNA *Clostridium difficile/Clostridium perfringens*" test kits were used. A set of reagents "RealBest-Vet DNA *Clostridium difficile* tcdA/tcdB/CDT" (AO Vector-Best, Russia) was used to type *Clostridium* toxin formation. Multiplex test systems made in Russia were used to detect antimicrobial resistance Erm gene in bacteria (A, B, C), blaCIT/blaDHA, blaCTX/blaOXA. Real-time amplification was done using QuantStudio™ 5 equipment (Thermo Fisher Scientific, USA).

In addition, faeces samples from cows and calves with clinical signs of diarrhea were tested for rotavirus and coronavirus antigens, bovine viral diarrhea and *Escherichia coli* enterotoxemic strain (K99). Solid phase enzyme-linked immunosorbent assay (AG-ELISA) was used for the tests, i.e. the kits "IDEXX Rota-Corona-k99 Ag Test", "IDEXX SNAP BVDV Antigen Test" (IDEXX, USA). SUNRISE reader (Tecan Austria GmbH, Austria) was used for reading results.

Parenchymal organs and tissues from adult cows and calves that died of typical signs of salmonellosis were subjected to histological tests. The material for histological test was fixed in 10% formalin (aqueous solution), poured into paraffin, the sections were stained with hematoxylin and eosin according to generally accepted methods and examined under an Olympus microscope (Japan) at various magnifications from 100 to 600.

RESULTS AND DISCUSSION

A salmonellosis outbreak in cattle was first recorded in September 2020 after an abnormally hot spring-summer season. An average monthly temperature of March in the Sverdlovsk Oblast was positive for the first time in the history of weather records, and the annual temperatures set an absolute record, amounting to an abnormal 5.3 °C, which is 0.7 °C higher than the previous record in 2008.

The research was carried out on a dairy farm with 2,500 cows. A large outbreak in cows of the 2nd and 3rd lactation and in first-calf heifers was detected. The cows are kept under loose housing. Newborn calves are grown individually in cages up to 2 months of age.

During the test period, the air temperature in the room where the animals were kept reached 29.5 °C, the humidity was 42%, the air velocity was 0.6 m/s. High air temperature combined with low humidity and lack of ventilation resulted in a heat stress in cows. The animals developed rapid respiratory rate up to (61.60 ± 5.30) breaths per minute, short breathing; with the heart rate reaching (119.80 ± 4.21). According to some researchers [16, 18], prolonged exposure to heat damages the animals' health, impairs their overall resistance, as well as reproductive traits.

The cattle vaccination program on this farm includes the use of a vaccine against a number of acute respiratory viral infections, which contains an inactivated component against viral diarrhea. The vaccination is carried out annually, starting from the age of 30 days, tetravalent inactivated vaccine HIPRABOVIS® 4 (Laboratorios Hipra, S. A., Spain) is used. The animals were not vaccinated against clostridiosis and salmonellosis.

The cows demonstrated clinical signs of salmonellosis, including mucus-filled diarrhea, dehydration of the first degree (less than 5%), and a decrease in productivity by 14%. The number of animals with clinical manifestation of salmonellosis was 23%. No hyperthermia was observed in adult cows.

On the same farm, gastrointestinal disorders were registered in calves, accompanied by septic phenomena, severe intoxication, hyperthermia (40.5–41.0 °C), significant (more than 7%) dehydration and death among the young. The disease developed in 93% of calves in the facilities, at the age of 7–14 days, was characterized by a sharp onset and an acute course. The lethality was 20.8%. 80% of death cases were reported in the first 2 days of the disease, the disease evolved into a subcute form in 20% of calves with a following death reported at the age of 25–30 days.

AG-ELISA was used to exclude the antigen of bovine viral diarrhea virus, which can rival *Salmonella* for its ability to cause diarrhea in adult animals.

Tests of biological material from cows with diarrhea revealed pathogens of the genus *Salmonella*, the species *Salmonella enterica* subsp. *enterica*: *Salmonella enteritidis*, which is a bovine pathogen, and *Salmonella choleraesuis*, which is a non-typical pathogen for this species [5, 10, 19, 20].

Rotavirus (by AG-ELISA) and *Clostridium perfringens* (by PCR) were detected in addition to salmonellosis agent in calves with clinical signs of the disease.

Histological liver analysis (Fig. 1) of both cows and calves revealed a specific morphological sign of salmonella infection – granuloma [21].

Moreover, these granulomas had some distinctive features and differed from the granulomas typical for bovine salmonellosis. The ones we detected were more similar to the granulomas found in pigs infected with the *Salmonella choleraesuis* serotype. Complete lysis of granuloma cells was observed in granulomas of this kind, in addition to that, there were cells necrotized as a result of karyorrhexis and karyopyknosis. Pronounced congestive hyperemia was observed at the same time in hepatic microvasculature with signs of fatty liver degeneration (Fig. 2).

Alongside with it, we detected a rapid hematogenous intra-organ spread of infection with formation

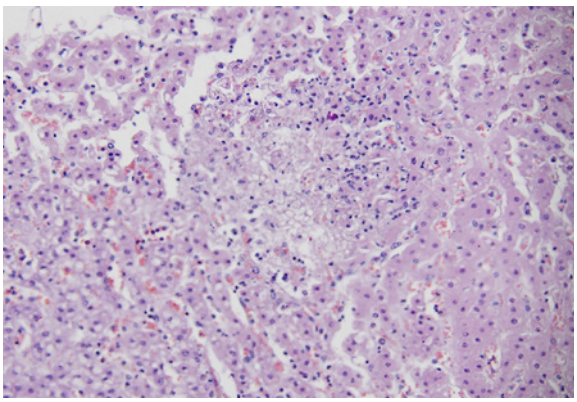

Fig. 1. Granuloma in the liver. Hematoxylin and eosin stain (100× magnification)

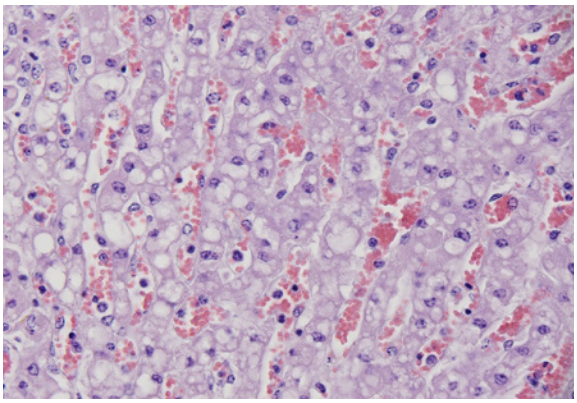

Fig. 2. Congestion in hepatic microvasculature and fatty liver. Hematoxylin and eosin stain (400× magnification)

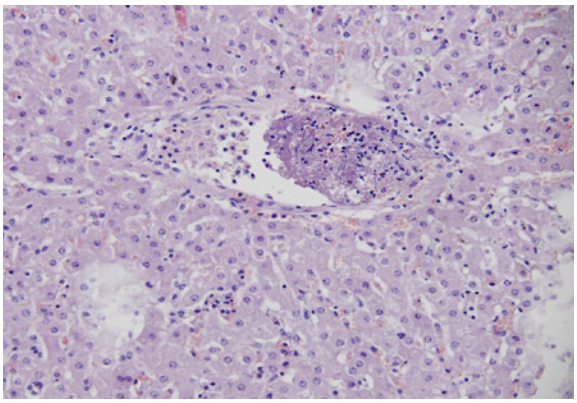

Fig. 3. Thromboendophlebitis in cow's liver (granuloma in the vessel lumen). Hematoxylin and eosin stain (200× magnification)

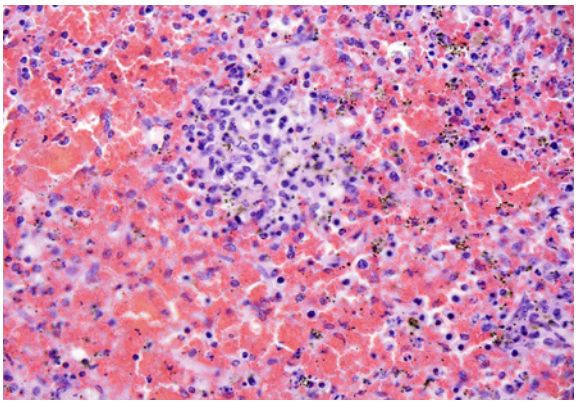

Fig. 4. Granuloma in the spleen without signs of necrosis. Hematoxylin and eosin stain (200× magnification)

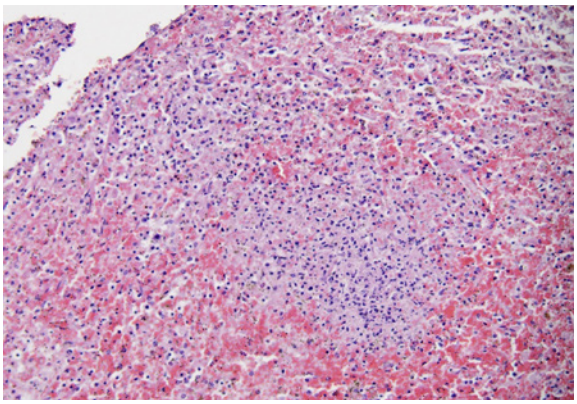

Fig. 5. Necrotizing granuloma in spleen. Hematoxylin and eosin stain (100× magnification)

of thromboendophlebitis in cow liver slides, which is a characteristic feature of porcine salmonellosis and a rather rare phenomenon for cattle (Fig. 3).

An important sign of sepsis is the lesion of the spleen, where specific salmonella granulomas are also found due to hemorrhagic infiltration (Fig. 4).

At the same time, granulomas were found in other parts of the spleen, represented by necrotic masses similar to granulomas in the liver (Fig. 5).

In other parenchymal organs (kidneys, heart) there was a number of pathological sings typical for sepsis: damage to the endothelium, i.e. desquamation in the vessel lumen, fibrinoid necrosis of the vessel walls, loops of glomeruli, leukostasis, the release of single neutrophils through the vessel wall, activation of the coagulation system – single blood clots in capillaries and venules, increased vascular permeability – diapedetic hemorrhages. Granuloma formation was also detected in the lungs, in addition to catarrhal pneumonia with pronounced proliferation of the interstitial stroma with a sharp expansion of the lymphatic vessels lumen and granulation tissue cells in them (Fig. 6, 7).

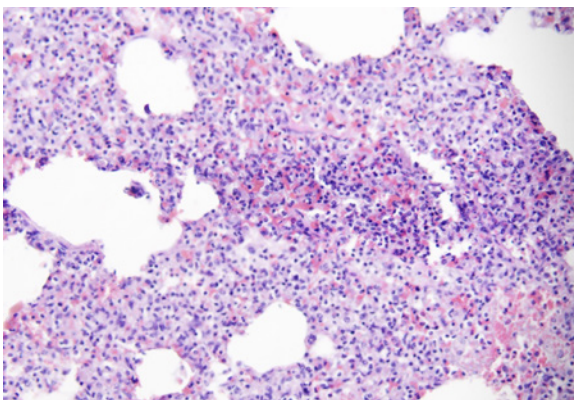

Fig. 6. Granulomatosis caused by catarrhal pneumonia. Hematoxylin and eosin stain (100× magnification)

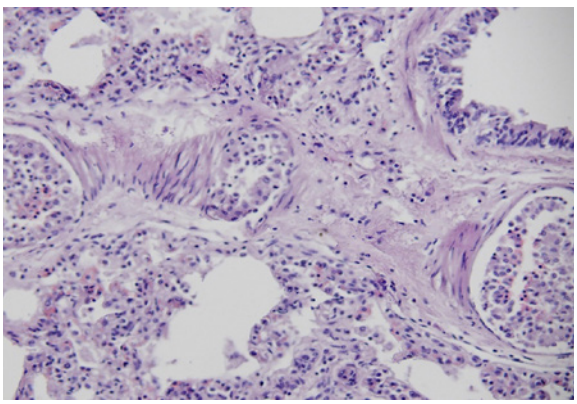

Fig. 7. A sharp expansion of the lymph vessels lumen in the overgrown interstitial lung tissue. Hematoxylin and eosin stain (200× magnification)

CONCLUSION

Exposure to abnormally hot temperatures in the Middle Urals has led to chronic heat stress in cattle. Despite the measures taken to protect the dairy herd from overheating (fans, irrigation with cool water, livestock sunshades), heat stress impaired the animals' immune system, thus leading to an excessive rise in *Salmonella* population in the gastrointestinal tract, increased intestinal permeability, and could also be the cause of increased virulence of such serotypes as – *Salmonella choleraesuis* that are not typical for cattle [16, 17].

The simultaneous presence of two pathogens: i.e. *Salmonella enteritidis* and *Salmonella choleraesuis* – caused a number of clinical and morphological changes in adult cows. The infection manifested itself by persistent diarrhea, reduced milk production and development of intoxication.

Histological examination of the parenchymal organs of cows revealed changes typical for salmonellosis pathological process. The nature of pathological changes suggests that there is a septic process spreading both hematogenically and lymphogenically. The structure of granulomas most likely indicates the circulation of *Salmonella choleraesuis* in cattle.

REFERENCES

1. Baisarova Z. T. Salmonells: classification, antigenic structure and factors of pathogenicity. *Vestnik Meditsinskogo instituta*. 2021; 1 (19): 88–92. DOI: 10.36684/med-2021-19-1-88-92. (in Russ.)

2. Kostenko Iu. G., Khramov M. V., Davleev A. D. The problem and prophylaxis of foodborne salmonellosis. *Veterinariya*. 2012; 4: 9–13. eLIBRARY ID: 17663533. (in Russ.)

3. Mezencev S. V. Salmonellosis – national and import. *Veterinariya.* 2015; 6: 30–32. eLIBRARY ID: 23568338. (in Russ.)

4. Mezentsev S. V. The application of HACCP principles by farm animal slaughter companies. *Bulletin of Altai State Agricultural University.* 2015; 11 (133): 122–126. eLIBRARY ID: 24902454. (in Russ.)

5. Van Kessel J. A., Karns J. S., Wolfgang D. R., Hovingh E., Schukken Y. H. Dynamics of *Salmonella* serotype shifts in an endemically infected dairy herd. *Foodborne Pathog. Dis*. 2012; 9 (4): 319–324. DOI: 10.1089/fpd.2011.1054.

6. Rodriguez-Rivera L. D., Wright E. M., Siler J. D., Elton M., Cummings K. J., Warnick L. D., Wiedmann M. Subtype analysis of *Salmonella* isolated from subclinically infected dairy cattle and dairy farm environments reveals the presence of both human- and bovine-associated subtypes. *Vet. Microbiol*. 2014; 170 (3–4): 307–316. DOI: 10.1016/j.vetmic.2014.02.013.

7. Cummings K. J., Warnick L. D., Elton M., Gröhn Y. T., McDonough P. L., Siler J. D. The effect of clinical outbreaks of salmonellosis on the prevalence of fecal *Salmonella* shedding among dairy cattle in New York. *Foodborne Patchog. Dis*. 2010; 7 (7): 815–823. DOI: 10.1089/fpd.2009.0481.

8. Bezborodova N. A. Modern approach to the problem of clostridiosis in animal husbandry: sampling, laboratory diagnostics, prevention (overview). *Russian Journal "Problems of Veterinary Sanitation, Hygiene and Ecology"*. 2020; 3 (35): 392–402. DOI: 10.36871/vet.san.hyg.ecol.202003016. (in Russ.)

9. Bezborodova N. A., Kozhukhovskaya V. V., Petropavlovsky M. V., Tomskikh O. G. Polymerase chain reaction in the diagnosis of latent, asymptomatic and chronic infectious diseases of cattle. *Issues of Legal Regulation in Veterinary Medicine*. 2019; 4: 30–33. DOI: 10.17238/issn2072-6023.2019.4.30. (in Russ.)

10. Holschbach C. L., Peek S. F. *Salmonella* in dairy cattle. *Vet. Clin. North Am. Food. Anim. Pract*. 2018; 34 (1): 133–154. DOI: 10.1016/j.cvfa.2017.10.005.

11. Glotov A. G., Glotova T. I. Cattle salmonellosis on big dairy farms (Review. P. 1). *Veterinariya*. 2020; 2: 3–7. DOI: 10.30896/0042-4846.2020.23.2.03-07. (in Russ.)

12. Glotov A. G., Glotova T. I. Cattle salmonellosis on big dairy farms (Review. P. 2). *Veterinariya*. 2020; 3: 3–7. DOI: 10.30896/0042-4846.2020.23.3.03-07. (in Russ.)

13. Beliaev L. I., Beliaeva M. M. Epizootic justification correct diagnostics and prevention of infectious animal diseases in factor. *Veterinariya*. 2013; 5: 14–16. eLIBRARY ID: 19033248. (in Russ.)

14. Poryvaeva A. P., Krasnoperov A. S., Tomskikh O. G., Lysova Ya. Yu. Model of estimation of risk of development of complications when dyspepsia in calves. *Agrarian Bulletin of the Urals*. 2019; 1 (180): 31–37. DOI: 10.32417/article_5ca4e6ff8e24c2.87308778. (in Russ.)

15. Subbotin V. V., Loschinin M. N., Sokolova N. A., Kolomytsev S. A. Salmonellosis – the actual problem of Veterinary Medicine. *Veterinaria I kormlenie*. 2013; 4: 59–61. eLIBRARY ID: 20291920. (in Russ.)

16. Rud E. N., Kuzminova E. V., Semenenko M. P., Abramov A. A., Rud N. A. Heat stress problem in dairy farming. *Veterinaria Kubani*. 2020; 3: 10–11. DOI: 10.33861/2071-8020-2020-3-10-11. (in Russ.)

17. Edrington T. S., Ross T. T., Callaway T. R., Martinez C. H., Hume M. E., Genovese K. J., et al. Investigation into the seasonal salmonellosis in lactating dairy cattle. *Epidemiol. Infect*. 2008; 136 (3): 381–390. DOI: 10.1017/S0950268807008680.

18. Buryakov N. P., Buryakova M. A., Aleshin D. E. Heat stress and feeding features of the dairy cattle. *Russian Veterinary Journal. Productive Animals*. 2016; 3: 5–13. eLIBRARY ID: 26538900. (in Russ.)

19. Semina A. N., Abgarian S. R. Identification of *Salmonella enteritidis* and *Salmonella typhimurium* by polymerase chain reaction. *International Journal of Veterinary Medicine*. 2018; (4): 39–43. eLIBRARY ID: 36552499. (in Russ.)

20. Carroll L. M., Buehler A. J., Gaballa A., Siler J. D., Cummings K. J., Cheng R. A., Wiedmann M. Monitoring the microevolution of *Salmonella enterica* in healthy dairy cattle populations at the individual farm level using whole-genome sequencing. *Front. Microbiol*. 2021; 12:763669. DOI: 10.3389/fmicb.2021.

21. Pecoraro H. L., Thompson B., Duhamel G. E. Histopathology case definition of naturally acquired *Salmonella enterica* serovar Dublin infection in young Holstein cattle in the northeastern United States. *J. Vet. Diagn. Invest*. 2017; 29 (6): 860–864. DOI: 10.1177/1040638717712757.

Received 31.10.2022
Revised 08.12.2022
Accepted 20.12.2022

INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Lyudmila I. Drozdova, Doctor of Science (Veterinary Medicine), Professor, Leading Researcher, FSBSI UrFASRC, UrB of RAS, Ekaterinburg, Russia; *https://orcid.org/0000-0001-9689-1781, e-mail: drozdova43@mail.ru.*

Evgenia N. Shilova, Doctor of Science (Veterinary Medicine), Associate Professor, Leading Researcher, FSBSI UrFASRC, UrB of RAS, Ekaterinburg, Russia; *https://orcid.org/0000-0002-9506-6883, e-mail: adelaida.gurgenovna@mail.ru.*

Irina A. Shkuratova, Doctor of Science (Veterinary Medicine), Professor, Associate Member of the Russian Academy of Sciences, Director of the Ural Scientific Research Veterinary Institute – Branch of the FSBSI UrFASRC, UrB of RAS, Ekaterinburg, Russia; *https://orcid.org/0000-0003-0025-3545, e-mail: info@urnivi.ru.*

Marina V. Ryaposova, Doctor of Science (Biology), Associate Professor, Leading Researcher, FSBSI UrFASRC, UrB of RAS, Ekaterinburg, Russia; *https://orcid.org/0000-0002-5699-3924, e-mail: riaposova76@mail.ru.*

Дроздова Людмила Ивановна, доктор ветеринарных наук, профессор, ведущий научный сотрудник ФГБНУ УрФАНИЦ УрО РАН, г. Екатеринбург, Россия; *https://orcid.org/0000-0001-9689-1781, e-mail: drozdova43@mail.ru.*

Шилова Евгения Николаевна, доктор ветеринарных наук, доцент, ведущий научный сотрудник ФГБНУ УрФАНИЦ УрО РАН, г. Екатеринбург, Россия; *https://orcid.org/0000-0002-9506-6883, e-mail: adelaida.gurgenovna@mail.ru.*

Шкуратова Ирина Алексеевна, доктор ветеринарных наук, профессор, член-корреспондент РАН, директор Уральского научно-исследовательского ветеринарного института – структурного подразделения ФГБНУ УрФАНИЦ УрО РАН, г. Екатеринбург, Россия; *https://orcid.org/0000-0003-0025-3545, e-mail: info@urnivi.ru.*

Ряпосова Марина Витальевна, доктор биологических наук, доцент, ведущий научный сотрудник ФГБНУ УрФАНИЦ УрО РАН, г. Екатеринбург, Россия; *https://orcid.org/0000-0002-5699-3924, e-mail: riaposova76@mail.ru.*

DOI: 10.29326/2304-196X-2023-12-1-79-86

Organization of live animal transportation process in the Russian Federation

M. A. Shibayev, I. M. Klinovitskaya, A. V. Belchikhina, A. K. Karaulov
FGBI "Federal Centre for Animal Health" (FGBI "ARRIAH"), Vladimir, Russia

SUMMARY
The paper covers the issues of organization of live animal transportation process in the Russian Federation as one of the factors of epidemiological risk associated with the spread of infectious animal disease pathogens. The legal framework, regulating the organization of live animal transportation using different vehicles, as well as quantitative data on live animal movements, taken from state veterinary information system "VetIS" ("Mercury" and "Cerberus" components) were analyzed. The analysis showed that live farmed animals are moved using all transportation means available, motor vehicles, planes, trains, ships as well as by driving. It was established that the major means of transport used to move live animals in the territory of the Russian Federation are motor vehicles. According to the analysis results 4.49 billion animals, including 4.41 billion poultry, 79.8 million large and small ruminants, pigs, horses, fur animals and bees were moved within the country in 2021. At the same time the number of issued veterinary accompanying documents for movements of cattle, poultry and pigs (i.e. in fact the transportations themselves) is much higher than the number of movements of other species. It was revealed that today only the movement of animals by railway is regulated in one way or another. The paper presents the suggestions to introduce the procedures aimed at improvement of biological safety and animal welfare during transportation. The results of the analysis performed can be used to optimize the control of animal transportation in the territory of the Russian Federation by competent authorities.

Keywords: live animals, animal transportation, animal welfare, veterinary accompanying documents, control, biological safety

Acknowledgements: This work was funded by the FGBI "ARRIAH" within the scope of research activities "Veterinary Welfare". The authors express their gratitude to the staff of the Rosselkhoznadzor IT Centre for some quantitative data provided.

For citation: Shibayev M. A., Klinovitskaya I. M., Belchikhina A. V., Karaulov A. K. Organization of live animal transportation process in the Russian Federation. *Veterinary Science Today.* 2023; 12 (1): 79–86. DOI: 10.29326/2304-196X-2023-12-1-79-86.

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

For correspondence: Mikhail A. Shibayev, Candidate of Science (Veterinary Medicine), Head of Sector, Information and Analysis Centre, FGBI "ARRIAH", 600901, Russia, Vladimir, Yur'evets, *e-mail: shibaev@arriah.ru.*

УДК 636.081.4:619:614.31(470)

Организация процесса перевозки живых животных в Российской Федерации

М. А. Шибаев, И. М. Клиновицкая, А. В. Бельчихина, А. К. Караулов
ФГБУ «Федеральный центр охраны здоровья животных» (ФГБУ «ВНИИЗЖ»), г. Владимир, Россия

РЕЗЮМЕ
Материалы статьи освещают вопросы организации процесса перемещения живых животных в Российской Федерации как одного из важнейших факторов эпизоотологического риска распространения возбудителей инфекционных болезней животных. Проведен анализ нормативно-законодательной базы, регламентирующей вопросы организации перевозки живых животных различными видами транспорта в стране, а также количественных данных о перемещении живых животных из государственной информационной системы в области ветеринарии «ВетИС» (компоненты «Меркурий» и «Цербер»). В рамках выполненного исследования было определено, что перемещение живых сельскохозяйственных животных по территории страны происходит всеми видами доступного транспорта: автомобильным, воздушным, железнодорожным, водным, а также способом перегона. Установлено, что основным способом перемещения живых животных по территории Российской Федерации является автомобильный транспорт. Результаты анализа демонстрируют, что за 2021 г. по территории страны было перемещено 4,49 млрд гол. животных, из которых 4,41 млрд гол. составляет живая птица, порядка 79,8 млн гол. – крупный и мелкий рогатый скот, свиньи, лошади, пушные звери и пчелы. В то же время количество оформленных ветеринарно-сопроводительных документов для целей перемещения крупного рогатого скота, птицы и свиней (т. е., по сути, самих транспортных событий или фактов перевозки животных) значительно превалирует относительно перемещения других видов животных. Выявлено, что на сегодняшний день в той или иной мере законодательно регулируется только перевозка животных железнодорожным транспортом. В работе высказаны предложения по внедрению процедур, направленных на повышение биологической безопасности процесса перевозки животных и обеспечение благополучия животных. Результаты проведенного аналитического исследования могут быть использованы в рамках оптимизации контроля процесса перевозки живых животных по территории Российской Федерации со стороны компетентных органов.

Ключевые слова: живые животные, перевозка животных, благополучие животных, ветеринарно-сопроводительные документы, контроль, биологическая безопасность

Благодарности: Работа выполнена за счет средств ФГБУ «ВНИИЗЖ» в рамках тематики научно-исследовательских работ «Ветеринарное благополучие». Авторы выражают благодарность коллективу информационно-вычислительного центра Россельхознадзора за предоставление некоторых количественных данных.

Для цитирования: Шибаев М. А., Клиновицкая И. М., Бельчихина А. В., Караулов А. К. Организация процесса перевозки живых животных в Российской Федерации. *Ветеринария сегодня*. 2023; 12 (1): 79–86. DOI: 10.29326/2304-196X-2023-12-1-79-86.

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

Для корреспонденции: Шибаев Михаил Александрович, кандидат ветеринарных наук, заведующий сектором информационно-аналитического центра ФГБУ «ВНИИЗЖ», 600901, Россия, г. Владимир, мкр. Юрьевец, *e-mail: shibaev@arriah.ru.*

INTRODUCTION

Thanks to available modern vehicles and developed logistics, animals are transported over long distances in a relatively short time. Transportations can occur frequently during animals' lifetime. Animals that are moved to participate in sports competitions or exhibition events are most frequently moved. On average, three to four transportations occur during the lifetime of food-producing animals [1].

With animals and livestock products, infectious disease agents can be moved over considerable distances, crossing physical and administrative boundaries. Despite the existing mechanisms for movement control over animal consignments, there is a certain probability of animal disease agent spread to disease free territories. The Food and Agriculture Organization of the United Nations (FAO) describes animal transportation as an event "ideal for the spread of diseases". Moreover, this can occur under the influence of various both controlled and uncontrolled factors. For example, the movement of animals with a latent or subclinical infection, in case of biased laboratory results, non-comprehensive clinical examination of animals, violation of quarantine conditions, etc. In addition, the illegal movement of infected or contaminated live animals and livestock products contributing to uncontrolled spread of infectious diseases over considerable distances and in various directions is also of great significance [2–6].

A certain number of transport events happening during the animal life and the fact that each event makes the spread of an infectious disease possible emphasizes the need to ensure appropriate biosafety measures during animal movement [1, 6].

The purpose of this work was to analyze measures to ensure the biological safety of live animal movement across the territory of the Russian Federation and to develop proposals for correction and optimization of this process.

MATERIALS AND METHODS

The theoretical basis of the study was the analysis of the legal framework regulating live animal transportation by various means of transport in the Russian Federation.

The practical basis for the analysis of quantitative data on the movement of live animals across the territory of the Russian Federation in 2021 was the official information from the state veterinary information system "VetIS" (components "Mercury" and "Cerberus").

The quantitative (numerical) data were processed using Microsoft Office Excel software.

The generally accepted methods of data analysis were used: generalization and formalization of information, methods of comparative analysis and descriptive statistics.

RESULTS AND DISCUSSION

Quantitative and species structure of animals moved across the territory of the Russian Federation. The movement of live farmed animals, such as cattle, small ruminants, pigs, horses, poultry (chickens, geese, ducks, turkeys), fur animals (rabbits, foxes, minks, arctic foxes), as well as bees in the territory of the Russian Federation in 2021 was analyzed. Herewith, the categorization of the selected animal species based on their purpose did not matter, whether it is breeding, slaughter or rearing.

The units of measurement in the analysis were animals, with the exception of bees, which were accounted for in bee colonies / bee packages. That is why further in the text the term "animals" will be used taking into account the above statements. The choice of these categories of animals is explained by the results of a preliminary analysis, which showed that exactly these groups of animals provide the absolute majority in the volume of moved live animals, i.e. the influence of other animals on the results is insignificant.

The results of the analysis demonstrate a huge number of animals moved by different means of transport (Fig. 1).

4.49 billion animals were moved during the specified time period among them 98.2% is live poultry (4.41 billion animals). The number of other animals presented in the study was about 79.8 million animals It should also be taken into account that during a certain time the same animals could be moved several times depending on the production cycle of their rearing and slaughter. Since in this case the number of moved animals is described, then, of course, the number of their movements will be

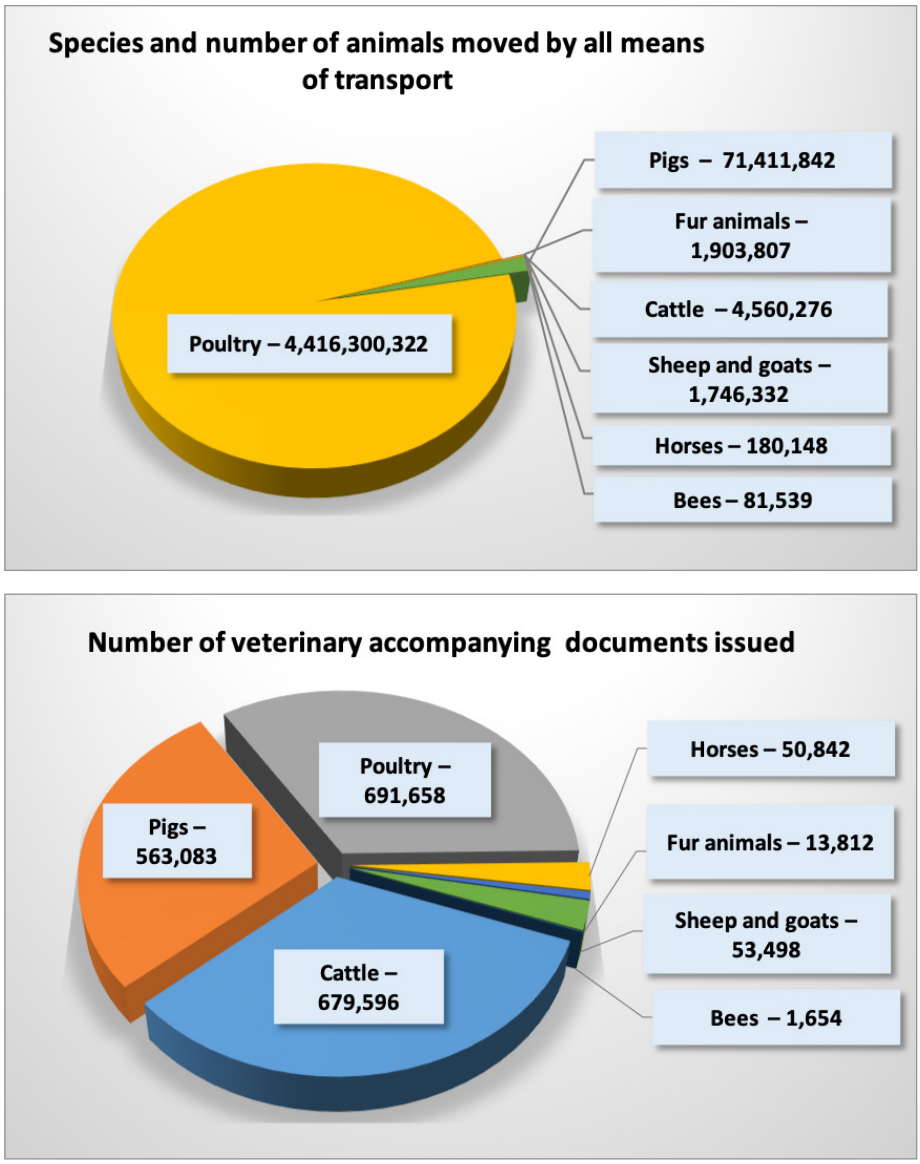

Fig. 1. Number of live animals moved in the territory of the Russian Federation in 2021

disproportionately less, since in most cases animals are moved in consignments, and veterinary and accompanying documents (VADs) are issued for a consignment of animals, and not for a specific animal. The latter is more typical for moving pets, i.e. companion animals, as well as for moving animals for personal use and individuals of special breeding value.

The analysis of the data showed that the largest number of VADs was issued for the movements of cattle, poultry and pigs, which significantly prevails over the number of movements of other animal species by tens and hundreds of times (Fig. 1). This should be taken into account during arrangement and conducting of surveillance over live animal movements across the territory of the Russian Federation, as well as during forecasting of the epidemic situation in a certain territory and in the country as a whole, since the movement of live animals should be considered as a social/economic factor or circumstance that determines the likelihood of epidemic situation complication, i.e. the movement of animals is nothing more than an epidemiological risk factor.

Means of transport used to move live animals in the territory of the Russian Federation. Due to the fact that various animal species are transported by different means of transport, and the ways of moving live animals across the country were analyzed.

The results presented in Figure 2 show that in the majority of cases the animals are moved across the country by road, namely 99.9% of the total number of moved animals. Out of the animals transported by other means of transport, the largest number were moved by driving or using public transport, little more than 6 million animals. As for the number of issued VADs for animal movements by various means of transport, the results of the analysis suggest that the largest number of documents were issued for movements of animals by road (about 2 million VADs) and public transport/driving (about 73 thousand VADs).

To determine the species structure of animals transported across the country by various means of transport, the available quantitative data were analyzed. The results showed that farmed poultry (including day-old chickens)

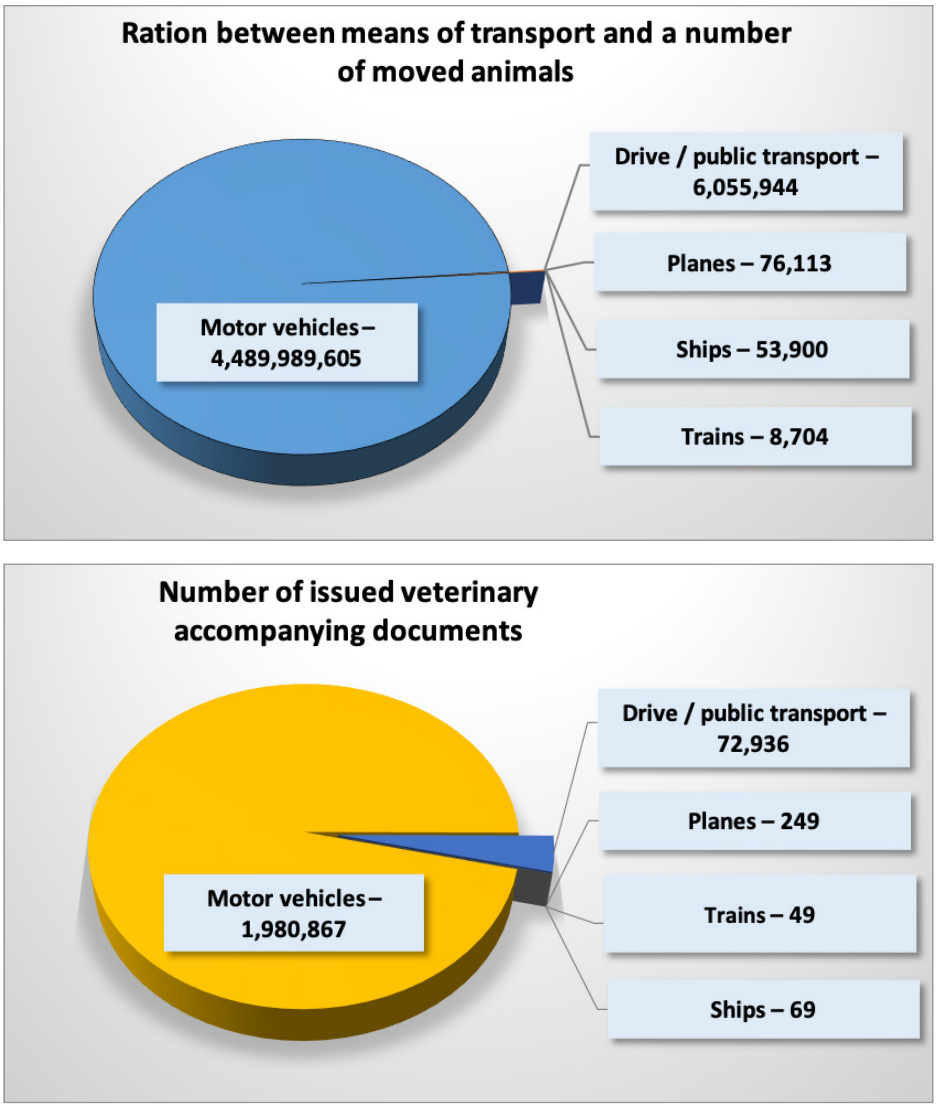

Fig. 2. Means of transport used to move live animals in the territory of the Russian Federation in 2021

is the main category of animals transported by air, rail, road and water transport. Separately, it is worth noting that driving or public transport are used in the vast majority of cases for movement of cattle and pigs. Other animal species are moved using the above-mentioned ways much less frequently. More detailed results of the analysis are presented in Figure 3.

Speaking about the number and ratio of issued VADs for transportation of animals by various means of transport, i.e. in fact, the transportation events themselves or the facts of animal transportation, it should be noted that the quantitative and species distribution does not differ from the one which is shown in Figure 3, with the exception of the length of digits.

Based on the results of the analysis, it can be logically concluded that in order to control the movement of live animals across the territory of the Russian Federation, the greatest resources (organizational, human, material, etc.) should be provided specifically for automobile and, to a certain extent, for public transport, as they are the major means of transport used to move animals, and consequently, they are also the leading factors of the epidemiological risk of spread of animal disease agents. However, the redistribution and concentration of resources in these areas should not have an impact on and weaken the control over the movement of animals by other means of transport. In addition, control over the movement of animals should be organized and adjusted taking into account the data obtained in this work, i.e. taking into account the species structure of animals transported by one or another type of transport.

Legislative aspects of animal movement in the Russian Federation. As shown in the previous section of this work, animals are moved across the territory of the Russian Federation using various types of transport (road, rail, water, air), as well as by driving and using public transport. There is no doubt that animals should be transported in compliance with certain requirements and rules aimed at:

– prevention of the animal disease agent spread, when the moved animal acts as a potential source of such agents;

– protection of the transported animal from the environmental pathogens;

– assurance of animal welfare during transportation, i.e. creation of optimal comfortable conditions for transportation and sticking to "five freedoms" concept: freedom

from hunger and thirst, freedom from discomfort, freedom from pain and injury, freedom from fear and distress, freedom to express normal and natural behavior [7].

A study of the Russian Federation legislation in this area has shown that currently the movement of animals across the country as a whole is regulated by the provisions of the Russian Federation Law dated 14.05.1993 No. 4979-1 "On veterinary medicine" [8]. In particular, article 13 of this law specifies that animals must be transported or driven along the routes agreed with the authorities responsible for federal official veterinary control (supervision) and in compliance with the requirements for the prevention of animal disease occurrence and spread. Indeed, live animals (as well as other regulated goods) are currently moved in the territory of the Russian Federation in accordance with the "Veterinary rules of Russian Federation regionalization" and the "Decision on establishment of infectious animal disease statuses in the Russian Federation regions and movement conditions of goods regulated by state veterinary surveillance" (approved by the Rosselkhoznadzor on 20.01.2017) [9, 10], which establish the regionalization procedure of the Russian Federation territory and the detailed procedure of the regulated goods movement in accordance with the animal disease statuses of the regions. Despite the fact that these documents contain certain requirements for the actual movement of animals, they are of a framework and limited nature. Perhaps a more detailed interpretation of these requirements was not the goal of these documents' development and enforcement. The fact is that at the time of their promulgation, a number of regulatory documents regulating the animal movement were in force in the country.

For example, the basic and fundamental act regulating the transportation of animals by rail is the "Rules of animal transportation by railway" [11]. The analysis of these requirements showed that despite the fact that the rules are not of veterinary nature and are approved by the Russian Federation Ministry of Transport, they contain the minimum necessary provisions for regulating the procedure and conditions of animal transportation by rail, including in veterinary and sanitary aspect.

As for the transportation of animals by air, previously this process was regulated by the "Guidelines on cargo transportation by the USSR domestic airlines" [12]. This manual contained certain requirements for the transportation of animals by aircraft, but since 18.10.2021, this document has become invalid on the territory of the Russian Federation.

Despite the fact that animals are moved across the country, including by water transport, there are no rules governing this process in open sources.

As for the rules for transportation of farm animals by public transport and by driving, there are also no existing regulatory documents in open sources. It is worth noting that the previous "Temporary animal health rules of driving (transportation) of farmed animals to distant pastures" [13] have become invalid on the territory of the Russian Federation since April 2020.

Regarding the requirements for the transportation of animals by road, previously this type of movement was regulated by the "Animal health rules of transportation of animals, poultry, fish, food products and raw materials of animal origin by motor vehicles" (approved by the USSR State Agricultural Committee on 30.01.1986 No. 432-5) [14]. These rules prescribed the animal

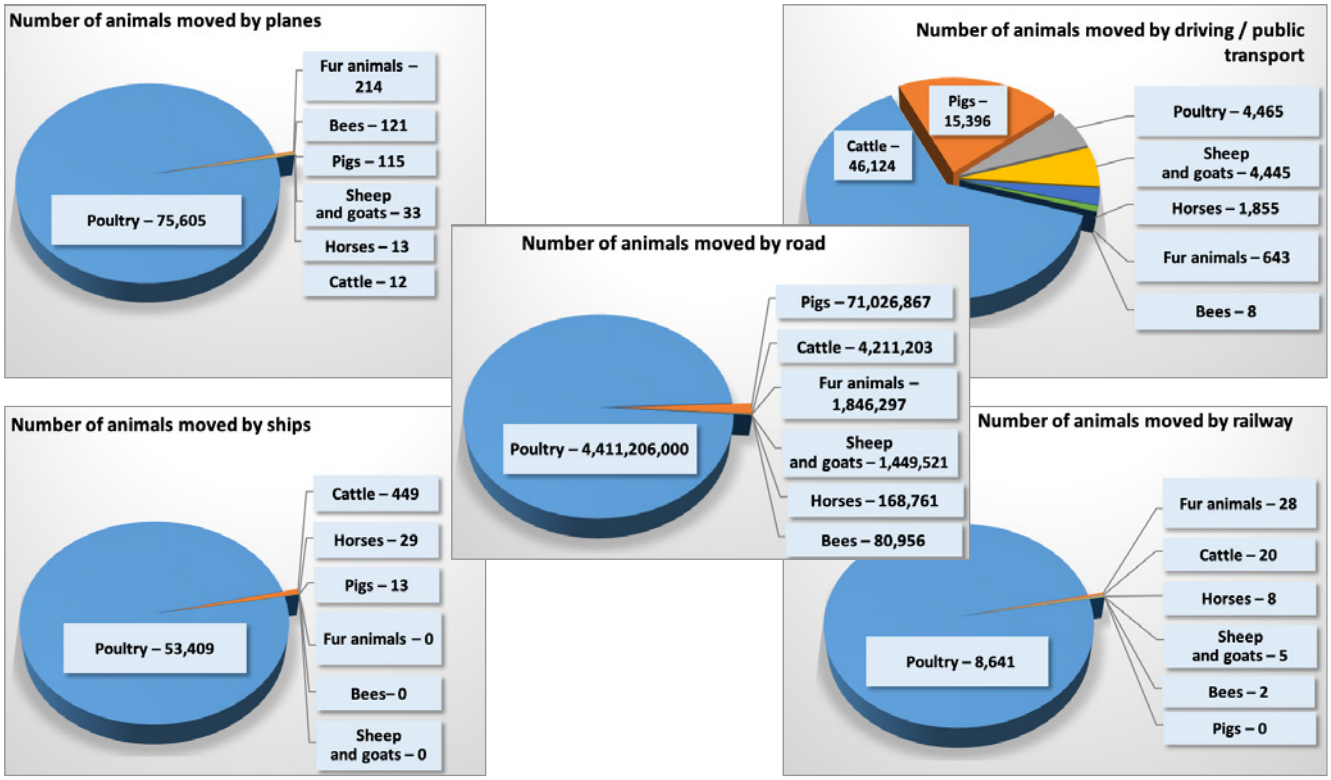

Fig. 3. Species and quantitative distribution of animals moved by different means of transport in the territory of the Russian Federation in 2021

transportation in sufficient detail, taking into account their type, quantity, duration of transportation, etc. However, as in previous cases, the document became invalid on the territory of the Russian Federation in 2020.

In addition, until 2018, the "General rules of transportation by road" [15] were in force, which contained minimum requirements for the transportation of animals and specific requirements for the technical condition of vehicles. However, in 2018, these rules became invalid, and now the current document is the Ordinance of the RF Government No. 2200 dated 21.12.2020 "On approval of rules of cargo transportation by motor vehicles and amendment of paragraph 2.1.1 of the Russian Federation Traffic Code" [16]. At the same time, with regard to the transportation of live animals, this document contains only a single mention that special vehicles are required for animal transportation, as well as an indication that after the transportation of animals and poultry, vehicles and containers must be washed and, if necessary, disinfected. The entire process of animal transportation shall be regulated by legislation. Moreover, the governmental regulation should cover the vehicles themselves. However, the above-mentioned Ordinance of the RF Government does not explain the term "special vehicle for animal transportation" and does not contain requirements for such vehicles. In general, there are currently no existing regulatory documents containing requirements for vehicles for animal transportation. This fact dictates the need for prompt correction of the legislative gap in this area. At the same time, it is possible to complement and amend existing regulations and develop an independent separate document. In any case, government regulation should address such aspects of movement as:

– requirements for moved animals, taking into account their species-age characteristics, physiological state and disease status, including infectious disease status;

– requirements for the vehicle and animal handlers;

– requirements for loading and unloading of animals;

– requirements for transportation conditions (stocking density in the vehicle, feeding, watering and resting of animals, fixation and cages/containers, etc.).

In other words, this document must contain the necessary and sufficient requirements to ensure an appropriate level of biosafety and biosecurity of animals, minimization of zoonotic risks, protection from extreme environmental conditions, as well as the transportation conditions that guarantee the above-mentioned "five freedoms". At the same time, the document should contain both general requirements and take into account the physiological characteristics and behavioral needs of different animal species, including wild and aquatic, as well as fish and insects.

Due to the high importance and relevance of the issue, we will focus separately only on the requirements for the vehicle and animal handlers. In particular, in our opinion and through the lens of foreign experience, the following procedures are proposed to be introduced in the country:

– animal transportation should be licensed by the government and relevant permits should be issued;

– authorized carriers must be added to the appropriate database available to stakeholders;

– authorized carriers must maintain a standard journey log on a mandatory basis;

– the vehicle must be subject to mandatory certification by the competent authority;

– the vehicle must be designed, constructed and operated in such a way as to minimize the risk of injury to animals and ensure their safety;

– the vehicle must be equipped with watering and feeding systems, as well as ventilation and temperature monitoring devices;

– the vehicle must be designed to contain the manure, litter or feed, and to permit thorough cleaning and disinfection after each transportation of animals, i.e. the vehicle must be made from the materials that can withstand repeated cleaning and disinfection;

– the vehicle must necessarily be equipped with recording equipment and a navigation system for displaying and recording data along the route, i.e. vehicles must be designated, but not adapted for the specified purposes;

– drivers and animal handlers should receive appropriate training (confirmed by a relevant certificate) on technical and administrative aspects of the Russian Federation and the Eurasian Economic Union (EAEU) legislation concerning the protection of animals during transportation, and covering at least such themes as animal physiology, practical aspects of animal handling, dealing with emergencies, safety rules, emergency response procedures, etc;

– live animals shall be transported only by authorized carriers using certified vehicles, equipment and appropriately qualified personnel;

– animal transportation routes shall avoid areas/zones under veterinary and sanitary restrictions due to any infectious disease, as well as areas with dense and/or vulnerable animal populations;

– transportation routes shall have approved parking places for animal resting and feeding;

– competent authority shall envisage control measures at any stage of the journey to identify compliances/non-compliances with the current legislation [1, 17–21].

The procedures proposed above should be integrated with the existing state veterinary information system "VetIS" and, in particular, with its components such as "Cerberus" and "Mercury" responsible for movements of goods regulated by the veterinary service, including live animals, since the identification of non-compliances of carriers, vehicles or animal handlers with the established requirements would automatically ban the movement of live animals.

CONCLUSION

The results of the study showed that live farmed animals are moved across the territory of the Russian Federation by all modes of transport: by road, by air, by rail, by water, as well as by driving in various amounts. The species distribution of moved animals shows that agricultural poultry makes up the bulk of transported animals. However, the number of veterinary accompanying documents issued for the movements of cattle, poultry and pigs significantly prevails over the number of movements of other animal species.

In fact, today, only the movement of animals by rail is legally regulated. With regard to the movement of animals by other modes of transport, no existing regulatory documents have been identified. Consequently, the question arises about the completeness

and sufficiency of control over this process in the absence of existing regulations. In particular, this applies to the movement of animals by road, used to transport a huge number of animals in our country, which is not commensurate with the number of animals transported using the others modes, i.e. the main way of moving live animals across the country.

The proposals made in this paper on the regulatory regulation and transportation of animals should serve as a basis for the creation of conditions to ensure the welfare of animals during transportation, protect animals from environmental factors, including animal disease pathogens, and to reduce the risks of pathogens spread in the Russian Federation.

REFERENCES

1. National Biosecurity Standard for Livestock, Poultry and Deadstock Transportation. Available at: https://inspection.canada.ca/animal-health/terrestrial-animals/biosecurity/standards-and-principles/transportation/eng/1519235033628/1519235183948?chap=0#s3c1.

2. FAO. Improved Animal Health for Poverty Reduction and Sustainable Livelihoods: Animal Production and Health Paper 153. Rome: FAO; 2002. 44 p. Available at: https://www.fao.org/3/y3542e/y3542e.pdf.

3. MacDiarmid S. C. The spread of pathogens through international trade. Introduction. *Rev. Sci. Tech*. 2011; 30 (1): 13–29. DOI: 10.20506/rst.30.1.2015.

4. O sostoyanii epizooticheskoi obstanovki v Rossiiskoi Federatsii I predprinimaemykh protivoepizooticheskikh meropriyatiyakh po nedopushcheniyu massovykh zabolevanii sel'skokhozyaistvennykh zhivotnykh: postanovlenie Soveta Federatsii Federal'nogo sobraniya RF ot 28.06.2017 № 207-SF = Epidemiological situation in the Russian Federation and anti-epidemic measures taken to prevent mass diseases in farmed animals: Ordinance of the Federation Council of the RF Federal Assembly No. 207-SF of 28.06.2017. Available at: https://docs.cntd.ru/document/456074703. (in Russ.)

5. Fèvre E. M., Bronsvoort B. M., Hamilton K. A., Cleaveland S. Animal movements and the spread of infectious diseases. *Trends Microbiol*. 2006; 14 (3): 125–131. DOI: 10.1016/j.tim.2006.01.004.

6. Greger M. The long haul: risks associated with livestock transport. *Biosecur. Bioterror.* 2007; 5 (4): 301–311. DOI: 10.1089/bsp.2007.0028.

7. WOAH. Terrestrial Animal Health Code. General provisions. Vol. 1. Paris; 2022. Available at: https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-code-online-access.

8. O veterinarii: Zakon RF ot 14.05.1993 № 4979-1 = On veterinary medicine: RF Law No. 4979-1 of 14.05.1993. Available at: https://www.consultant.ru/document/cons_doc_LAW_4438. (in Russ.)

9. Ob utverzhdenii Veterinarnykh pravil provedeniya regionalizatsii territorii Rossiiskoi Federatsii: prikaz Minsel'khoza Rossii ot 14.12.2015 № 635 = On approval of veterinary rules of Russian Federation regionalization: Order of RF MoA No. 635 of 14.12.2015. Available at: https://fsvps.gov.ru/ru/fsvps/laws/4600.html. (in Russ.)

10. Reshenie ob ustanovlenii statusov regionov Rossiiskoi Federatsii po zaraznym boleznyam zhivotnykh i usloviyakh peremeshcheniya podkontrol'nykh gosvetnadzoru tovarov: utv. Rossel'khoznadzorom 20.01.2017 = Decision on establishment of infectious animal disease statuses in the Russian Federation regions and movement conditions of goods regulated by state veterinary surveillance: approved by Rosselkhoznadzor on 20.01.2017. Available at: https://fsvps.gov.ru/ru/fsvps/laws/4857.html. (in Russ.)

11. Ob utverzhdenii Pravil perevozok zheleznodorozhnym transportom zhivotnykh: prikaz Mintransa Rossii ot 19.10.2020 № 427 = On approval of rules of animal transportation by railway: Order of RF Ministry of Transport No. 427 of 19.10.2020. Available at: http://publication.pravo.gov.ru/Document/View/0001202102150021. (in Russ.)

12. Rukovodstvo po gruzovym perevozkam na vnutrennikh vozdushnykh liniyakh Soyuza SSR (RGP): utv. MGA SSSR 20.08.1984 = Guidelines on cargo transportation by USSR domestic airlines: approved by USSR Ministry of Civil Aviation on 20.08.1984. Available at: https://e-ecolog.ru/docs/rwp3GKzwnyUVDsMcIluCI/full. (in Russ.)

13. Vremennye veterinarno-sanitarnye pravila pri organizatsii peregona (perevozki) sel'skokhozyaistvennykh zhivotnykh na otgonnye pastbishcha: utv. GUV MSKh SSSR 04.09.1975 = Temporary animal health rules of driving (transportation) of farmed animals to distant pastures: approved by Chief Veterinary Department of the RF MoA on 04.09.1975. Available at: https://base.garant.ru/70502260. (in Russ.)

14. Veterinarno-sanitarnye pravila perevozki zhivotnykh, ptitsy, ryby, produktov i syr'ya zhivotnogo proiskhozhdeniya avtomobil'nym transportom: utv. Gosagropromom SSSR 30.01.1986 № 432-5 = Animal health rules of transportation of animals, poultry, fish, food products and raw materials of animal origin by motor vehicles: approved by the USSR State Agriculture Committee No. 432-5 on 30.01.1986. Available at: https://legalacts.ru/doc/veterinarno-sanitarnye-pravila-perevozki-zhivotnykh-ptitsy-ryby-produktov. (in Russ.)

15. Obshchie pravila perevozok gruzov avtomobil'nym transportom: utv. Minavtotransom RSFSR 30.07.1971 = General rules of transportation by road: approved by RSFSR Ministry of Motor Transport on 30.07.1971. Available at: https://www.consultant.ru/document/cons_doc_LAW_14117/05da6926bb6ffcb73fedbdc748099f1bbc9fcd98. (in Russ.)

16. Ob utverzhdenii Pravil perevozok gruzov avtomobil'nym transportom i o vnesenii izmenenii v punkt 2.1.1 Pravil dorozhnogo dvizheniya Rossiiskoi Federatsii: postanovlenie Pravitel'stva RF ot 21.12.2020 № 2200 = On approval of rules of cargo transportation by motor vehicles and amendment of Paragraph 2.1.1 of the Russian Federation Traffic Code: Ordinance of the RF Government No. 2200 of 21.12.2020. Available at: http://www.consultant.ru/document/cons_doc_LAW_371981. (in Russ.)

17. Council Regulation (EC) No. 1/2005 of 22 December 2004 on the protection of animals during transport and related operations and amending Directives 64/432/EEC and 93/119/EC and Regulation (EC) No. 1255/97. Available at: https://eur-lex.europa.eu/legal-content/en/ALL/?uri=CELEX%3A32005R0001.

18. Porta F., Mertin I., Waley D. Live Animal Transport: Time to Change the Rules. Brussels; Eurogroup for Animals;

2021. Available at: https://www.eurogroupforanimals.org/files/eurogroupforanimals/2021-12/2020_01_27_efa_transport_white_paper_0.pdf.

19. Lapworth J. W. Engineering and design of vehicles for long distance road transport of livestock: the example of cattle transport in northern Australia. *Vet. Ital*. 2008; 44 (1): 215–222. PMID: 20405427.

20. Mitchell M. A., Kettlewell P. J. Engineering and design of vehicles for long distance road transport of livestock (ruminants, pigs and poultry). *Vet. Ital*. 2008; 44 (1): 201–213. PMID: 20405426.

21. Fike K., Spire M. F. Transportation of cattle. *Vet. Clin. North Am. Food Anim. Pract*. 2006; 22 (2): 305–320. DOI: 10.1016/j.cvfa.2006.03.012.

Received 01.11.2022
Revised 18.11.2022
Accepted 12.12.2022

INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Mikhail A. Shibayev, Candidate of Science (Veterinary Medicine), Head of Sector, Information and Analysis Centre, FGBI "ARRIAH", Vladimir, Russia; *https://orcid.org/0000-0002-9382-0109, e-mail: shibaev@arriah.ru.*

Irina M. Klinovitskaya, Candidate of Science (Economics), Senior Researcher, Information and Analysis Centre, FGBI "ARRIAH", Vladimir, Russia; *https://orcid.org/0000-0001-5347-8620, e-mail: klinovitskaya@arriah.ru.*

Anastasia V. Belchikhina, Junior Researcher, Information and Analysis Centre, FGBI "ARRIAH", Vladimir, Russia; *https://orcid.org/0000-0003-1442-2469, e-mail: belchihina@arriah.ru.*

Anton K. Karaulov, Candidate of Science (Veterinary Medicine), Head of Information and Analysis Centre, FGBI "ARRIAH", Vladimir, Russia; *https://orcid.org/0000-0002-5731-5762, e-mail: karaulov@arriah.ru.*

Шибаев Михаил Александрович, кандидат ветеринарных наук, заведующий сектором информационно-аналитического центра ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *https://orcid.org/0000-0002-9382-0109, e-mail: shibaev@arriah.ru.*

Клиновицкая Ирина Михайловна, кандидат экономических наук, старший научный сотрудник информационно-аналитического центра ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *https://orcid.org/0000-0001-5347-8620, e-mail: klinovitskaya@arriah.ru.*

Бельчихина Анастасия Владимировна, младший научный сотрудник информационно-аналитического центра ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *https://orcid.org/0000-0003-1442-2469, e-mail: belchihina@arriah.ru.*

Караулов Антон Константинович, кандидат ветеринарных наук, руководитель информационно-аналитического центра ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *https://orcid.org/0000-0002-5731-5762, e-mail: karaulov@arriah.ru.*

DOI: 10.29326/2304-196X-2023-12-1-87-96

Analytical hierarchy process as a tool supporting a decision-making for assessment of the risk of transboundary infectious animal disease introduction to the Russian Federation and previously disease-free territories

N. V. Lebedev[1], A. S. Igolkin[1], K. N. Gruzdev[1], D. G. Spirin[2], M. A. Farukh[2], N. V. Ermilov[2], G. G. Eremin[2]

[1] FGBI "Federal Centre for Animal Health" (FGBI "ARRIAH"), Vladimir, Russia

[2] 48th Central Research Institute of the Ministry of Defense of the Russian Federation, Moscow, Russia

SUMMARY

The livestock industry is increasingly taking its place in the economy of the Russian Federation. Its export potential is actively growing. Already, up to 10% of agricultural products are exported to foreign markets. The demand for food steadily increases during crises, which in turn increases the role of the veterinary service, whose tasks include protecting the country's territory from the introduction of infectious diseases of animals from foreign countries; implementation of measures to prevent and eliminate infectious and other diseases in agricultural, domestic, zoo and other animals, fur-bearing animals, birds, fish and bees, as well as the implementation of plans of the regional veterinary service in the field of animal husbandry. The article assesses the validity of the possibilities and use of modern methods of analyzing and predicting the spread of animal morbidity, identifying cause-and-effect relationships and the extent of the spread of particularly dangerous animal diseases. The authors propose to consider the possibility of using the mathematical method of hierarchy analysis as a scientifically sound decision-making support tool when assessing the risk of introducing trans-border infectious animal diseases into previously prosperous territories of the Russian Federation. This approach can be used in the process of choosing the most appropriate alternative from several risk assessment options. The Hierarchy Analysis Method is a mathematical tool for a qualitative systematic approach to solving decision-making problems. This method was developed by the American scientist Thomas Lewis Saati in 1970, since then it has been actively developing and widely used in practice. The hierarchy analysis method can be used not only to compare objects, but also to solve more complex management and forecasting tasks.

Keywords: transboundary infectious animal diseases, disease-free territory, risk assessment, economic damage, epidemic situation, Analytical Hierarchy Process method, decision-making support

Acknowledgements: The authors express their acknowledgements to Anton K. Karaulov, Candidate of Science (Veterinary Medicine), Head of Information Analysis Centre of the Veterinary Surveillance Department.

For citation: Lebedev N. V., Igolkin A. S., Gruzdev K. N., Spirin D. G., Farukh M. A., Ermilov N. V., Eremin G. G. Analytical hierarchy process as a tool supporting a decision-making for assessment of the risk of transboundary infectious animal disease introduction to the Russian Federation and previously disease-free territories. *Veterinary Science Today*. 2023; 12 (1): 87–96. DOI: 10.29326/2304-196X-2023-12-1-87-96.

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

For correspondence: Mikhail A. Farukh, Senior Researcher of the Research Centre, 48th Central Research Institute of the Ministry of Defense of the Russian Federation, 105005, Russia, Moscow, *e-mail: 48cnii@mil.ru*

УДК 619:616.98:616-036.22:517:338.14

Метод анализа иерархий как инструмент поддержки принятия решений при оценке риска заноса трансграничных инфекционных болезней животных на территорию Российской Федерации и на ранее благополучные территории

Н. В. Лебедев[1], А. С. Иголкин[1], К. Н. Груздев[1], Д. Г. Спирин[2], М. А. Фарух[2], Н. В. Ермилов[2], Г. Г. Еремин[2]

[1] ФГБУ «Федеральный центр охраны здоровья животных» (ФГБУ «ВНИИЗЖ»), г. Владимир, Россия

[2] ФГБУ «48 Центральный научно-исследовательский институт» Минобороны России (48 ЦНИИ МО РФ), г. Москва, Россия

РЕЗЮМЕ

Отрасль животноводства все увереннее занимает свое место в экономике Российской Федерации. Активно растет ее экспортный потенциал. Уже сейчас на внешние рынки экспортируется до 10% производимой продукции сельского хозяйства. Спрос на продовольствие стабильно увеличивается в период

кризисов, что, в свою очередь, повышает роль ветеринарной службы, задачами которой является охрана территории страны от заноса заразных болезней животных из иностранных государств, реализация мероприятий по предупреждению и ликвидации инфекционных и иных болезней сельскохозяйственных, домашних, зоопарковых и других животных, пушных зверей, птиц, рыб и пчел и осуществление региональных планов ветеринарного обслуживания животноводства. В статье дается обоснование и оценка возможности использования современных методов для анализа и прогнозирования распространения заболеваемости животных, выявления причинно-следственных связей, масштаба распространения особо опасных болезней животных. Авторами предлагается к рассмотрению возможность применения математического метода анализа иерархий в качестве научно-обоснованного инструмента поддержки принятия решений при оценке риска заноса трансграничных инфекционных болезней животных на ранее благополучные территории Российской Федерации. Данный подход может быть использован в процессе выбора наиболее актуальной альтернативы из нескольких вариантов оценки риска. Метод анализа иерархий – математический инструмент качественного системного подхода к решению проблем принятия решений. Этот метод разработан американским ученым Томасом Льюисом Саати в 1970-х годах, с тех пор он активно развивается и широко используется на практике. Метод анализа иерархий можно применять не только для сравнения объектов, но и для решения более сложных проблем управления и прогнозирования.

Ключевые слова: трансграничные инфекционные болезни животных, благополучная территория, оценка риска, экономический ущерб, эпизоотическая ситуация, метод анализа иерархий, поддержка принятия решений

Благодарности: Авторы выражают благодарность руководителю информационно-аналитического центра Управления ветнадзора кандидату ветеринарных наук Антону Константиновичу Караулову.

Для цитирования: Лебедев Н. В., Иголкин А. С., Груздев К. Н., Спирин Д. Г., Фарух М. А., Ермилов Н. В., Еремин Г. Г. Метод анализа иерархий как инструмент поддержки принятия решений при оценке риска заноса трансграничных инфекционных болезней животных на территорию Российской Федерации и на ранее благополучные территории. *Ветеринария сегодня*. 2023; 12 (1): 87–96. DOI: 10.29326/2304-196X-2023-12-1-87-96.

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

Для корреспонденции: Фарух Михаил Александрович, старший научный сотрудник отдела научно-исследовательского центра, 48 ЦНИИ МО РФ, 105005, Россия, г. Москва, *e-mail: 48cnii@mil.ru.*

INTRODUCTION

The Russian Federation continues to increase the domestic production of animal products, thus contributing to the enhancement of food security of the country, facilitating the availability of food products to the population, provision of raw material for industries [1]. Though still dependent on external economic factors, the livestock sector confidently begins to occupy an important place in the country's economy. Its export potential is actively growing. Up to 10% of agricultural products are already exported to foreign markets. The demand for food is known to steadily increase during crises. The demand for agricultural products is also influenced by demographic factor. By 2030, 765 million new consumers will appear in the world; of these, 340 million will live in Africa, 126 million – in India, 30 million – in China [2]. The government investment policy has produced certain results – modern livestock and poultry facilities for production of pork, milk, poultry meat and eggs have been built. At the same time, the spread of infectious animal diseases, in particular zoonoses, has escalated due to globalization processes. According to the Veterinary Law of the Russian Federation, the tasks in the veterinary field are as follows: to protect the territory of the country from the introduction of contagious animal diseases from foreign countries, to take measures for the prevention and elimination of contagious and other diseases of agricultural, domestic, zoo and other animals, fur-bearing animals, birds, fish and bees, as well as to implement regional plans on the veterinary services to be provided to animal farming sector. A specific feature of Russia is a long land border with the neighbouring countries infected with transboundary animal diseases [3].

Transboundary animal diseases (TADs) are epidemic diseases, which are highly contagious or transmissible and have the potential for rapid transmission; they can easily spread across borders and reach epidemic proportions. The control/management, including eradication, of transboundary animal diseases require cooperation between several countries [4–6]. As defined by the World Organisation for Animal Health, such diseases include African horse sickness, African swine fever (ASF), bluetongue disease, Newcastle disease, swine vesicular disease, vesicular stomatitis, high pathogenic avian influenza, rabbit haemorrhagic disease, lumpy skin disease, classical swine fever, contagious bovine pleuropneumonia, Rift valley fever, sheep pox and goat pox, rinderpest, peste des petits ruminants, foot-and-mouth disease [7]. These infections result in huge economic losses due to the direct and indirect damage, as well as due to the imposition of trade restrictions.

In particular, an epidemic of classical swine fever in the Netherlands in 1997 led to the destruction of 10 million pigs; the estimated damage was US $2.3 billion [8]. Foot-and-mouth disease was responsible for pig industry collapse in Taiwan in the same year. The disease affected more than 6 thousand farms, 4 million pigs were depopulated, losses amounted to US $560 million [9]. The financial losses associated with a foot-and-mouth outbreak in Great Britain in 2001 amounted to £3.1 billion (US $4.4 billion) only for agricultural sector and food industry [10]. An example of the negative effect

of transboundary animal diseases on the economy is an ASF epidemic on the island of Hispaniola in 1978. Total pig depopulation on the island resulted in a dramatic fall of rural population living standards [11]. The situation recurred in 2021 after ASF introduction at first to the Dominican Republic and then to the Republic of Haiti. To ensure timely disease containment, TADs shall be notifiable to the World Organisation for Animal Health [12].

According to Order of the Ministry of Agriculture of the Russian Federation No. 62 of 9 March 2011, TADs are included in the List of contagious and other animal diseases [13].

While infection transmission was limited to its natural ranges in the past years, there are practically no natural barriers for the spread of TADs, first and foremost zoonotic and anthroponotic ones, under the new conditions of active international trade and logistics flows [14].

Official trade in animals and animal products is regulated by international and national legislation. But it is almost impossible to control the illegal movement of animals in informal trade, which results in transboundary transmission of infections. The most common source of infection is meat products; besides, fomites also play a role. Live animals can be a TAD source in case of their contacts with healthy livestock on shared pastures, especially in the mountain areas [15, 16].

In recent years, emerging infections have been increasingly reported. This not only changes the geography and structure of global epidemiology, but also dictates the need to rethink measures to prevent the introduction and spread of animal diseases and methods for their eradication, as well as to search for scientifically sound approaches to decision-making.

MATERIALS AND METHODS

The studies involved the collection and analysis of information on the epidemic situation, the application of probability and risk assessment methods with respect to infectious animal disease introduction to the Russian Federation and pathogen-free areas of the country, methods and practices of managerial decision-making in the veterinary and other fields of activities. The paper also presents the characteristic of TADs. The following commonly accepted data analysis methods were used: compilation and formalization, a comparative analysis method, methods of descriptive statistics.

RESULTS AND DISCUSSION

Transboundary animal diseases are a global challenge. At the same time, the scientific papers available do not fully reflect the specific epidemiological features of such infections, the patterns of their spread within a country or a group of countries, in particular the risk they represent for pathogen-free areas. The preparedness of the Veterinary Services of the countries and regions for proper and rapid detection, investigation and control of outbreaks is important for prevention of transboundary animal disease introduction via different pathways. The analysis performed showed that many countries, especially the developing ones, do not have adequate veterinary and diagnostic capacities to undertake the necessary actions [15, 17–24]. However, in case of introduction of some highly dangerous diseases, the above-mentioned measures turn out to be ineffective, even when taken in the developed countries. For example, at the time of ASF (genotype II) spread in the European Union and China, no progress was made in the identification of the source of infection in any of the affected countries (Hungary, Romania, Bulgaria and Belgium).

The epidemiological analysis methods employed are often framed by guidelines that are descriptive in nature. Only in the beginning of the twenty-first century, papers appeared, in which state-of-the-art geoinformation research techniques, computer programmes and technologies (for example, ArcGIS) began to be used for the analysis of epidemic spread among livestock [25, 26]. The applied use of the geoinformation system in the veterinary field involves the visualization of data on outbreak occurrence/spread, the generation of dynamic and planimetric maps, the identification of correlation between morbidity trends and economic, climatic, geographical, social and other factors [27]. The system allows for the analysis and prediction of morbidity spread, the establishment of the cause-and-effect relationship between increased morbidity and environmental-geographical risk factors, as well as the identification of geographical factors of localization and the extent of highly dangerous livestock disease spread in order to provide a scientific basis for the targeted monitoring of the epidemic situation, etc. [28].

The phylogenetic analysis of infection agents enables the identification of the most probable virus introduction sources and transmission pathways. For example, the phylogenetic analysis of a British isolate of FMD virus (2001) demonstrated its similarity to a South African one [24, 29]. North American strains of porcine epidemic diarrhea virus were found to be similar to the Chinese ones [30]. The genetic analysis of an ASF genotype II agent from China revealed its probable Eastern European origin [31].

The staff members of the Information Analysis Centre of the FGBI "ARRIAH" undertake great efforts towards justification of possibilities and application of modern analysis methods for animal disease spread prediction. The specialists collect and analyze information on the epidemic situation in the foreign countries, analyze the risk of highly dangerous disease introduction to the Russian Federation, in particular during the import/export of animals and agricultural products; they are also involved in mapping, modelling, data base creation and maintenance.

The ArcGIS add-in facilitating the identification of spatio-temporal regularities has found its practical use in the veterinary field. Being based on the notion of the spatio-temporal cube, it enables the detection of temporal trends of increase/decrease in outbreak concentration on the basis of their positional relationship, the analysis of locations and trends of hot spot occurrence, as well as the analysis of data in each particular location.

The following ArcGIS integral geospatial statistics tools are used for the detection and visualization of spatio-temporal disease spread trends: standard deviational ellipse, standard distance, mean center. The geospatial data analysis allows for the detection of spread trends, the identification of risk factors, epidemic forecasting [28].

The geographical approach helps to create geospatial-referenced data bases on animal diseases. Data visualization through maps provides a visual presentation of the epidemic situation with the possibility of preliminary visual analysis thereof [32].

The "trend-based Poisson random walk" model is used to calculate the prognostic values of the number of ASF outbreaks. Modelling is performed with @Risk software using the Monte Carlo simulation technique with 10,000 iterations. The modelling result is presented as a mean expected number of outbreaks, as well as 95% confidence interval [33].

Bifurcation analysis technique is also applied [14].

The application of descriptive routine and newly developed methods allowed to conclude that the probability of TADs occurrence is influenced by several factors:

– the presence of susceptible animals;
– animal population density;
– provision of appropriate recording and identification of animals;
– geographical environment;
– climatic conditions of a region;
– the presence of infection transmission vectors;
– animal management system and animal production management;
– anthropogenic factor activity;
– animal disease control methods applied;
– the level of the Veterinary Service performance;
– the existence of the normative framework for TADs control;
– the existence and implementation of federal and regional programmes;
– monitoring of infectious animal disease agent circulation;
– overall development level of a region/country;
– the extent of inter-agency collaboration;
– implementation of close control and surveillance over animal and animal product movement in compliance with the Decision on the establishment of statuses of the Russian Federation regions with respect to contagious animal diseases and conditions for the movement of commodities subject to official veterinary surveillance;
– the level and consistency of awareness-raising activities among the general public regarding the threat of contagious animal diseases, in particular transboundary ones, as well as the economic impact of their introduction and spread [4, 5, 34–38].

All the aforementioned and some other mathematical methods of epidemic situation analysis are used by the Veterinary Service to assess the risk of TAD introduction to the previously disease-free regions of the Russian Federation and timely prevent the threat of their spread. The timely implementation of anti-epidemic measures in case of an outbreak is the key factor for prevention of the disease spread across the pathogen-free area.

Risk is defined as the probability of an undesirable outcome. This potential is frequently employed to forecast various situations.

Risk assessment is a scientific method for the calculation, with the highest possible objectivity, of the harmful impact of the identified hazard or risk source on the health of humans, animals or the economy. A risk factor is a biological, chemical or physical agent or actions that may inflict harm to, or have a negative effect on, health/performance.

Risk analysis comprises three independent, but closely related, elements: risk assessment, risk factor management, risk communication.

Methodologically, there are the following risk assessment approaches: qualitative, semi-quantitative and quantitative. A qualitative method is the simplest and lowest cost one, it allows to quickly obtain information in its general form. Such risk analysis method ("decision tree") was suggested in the USA in the late 1950s. The analysis of agent introduction risk performed with a qualitative method in order to assess the probability of the agent spread involves the use of system modelling.

The semi-quantitative risk assessment seeks to rank risk levels (high, medium, low) based on the score estimates generated by a group of experts.

It should be noted that a semi-quantitative method is more informative, but requires accurate data, time and special training [21, 39–45]. Risk assessment can help to identify pathogen introduction pathways and potential impact. But qualitative information on unofficial disease introduction pathways is either absent or can be incomplete, and this makes it difficult to measure actual risks. However, this knowledge is necessary for the development of awareness-raising, prevention and epidemiological surveillance programmes based on actual risks [46].

The above-mentioned methods complement one another and are applied simultaneously during decision-making (risk assessment). A qualitative analysis allows to assess the risk of disease introduction to the previously disease-free area, a quantitative method is used to assess the potential disease spread and associated losses, and a semi-quantitative method is employed to assess the overall risk in such situation [47]. All this can be regarded as information support for managerial decision-making process.

Making a decision is the most critical moment. Decision-making is choosing among multiple alternative courses of action to achieve the target goal as the final stage of managerial process. It is essential that a decision-maker has confidence that the decision-making procedure is correct and desirable. The main stages in the managerial decision-making process include: setting of the goal, assessment of the situation, identification of the problem and making a decision to resolve it [48]. The decision-maker should understand the methods supported by theoretical and practical knowledge on decision-making, in particular in the veterinary field, be competent to identify approaches to maintain disease freedom of relevant areas and prevent the entry of TADs agents, be able to apply theoretical knowledge to analyze disease manifestations, to comprehensively utilize intellectual tools to address the arising practical issues.

Analytical Hierarchy Process (AHP) is a mathematical tool for a qualitative systematic approach to handle challenging problems in decision-making. Rather than prescribing a "correct" decision, AHP helps the decision-maker to interactively find the variant (alternative) that best suits the decision-maker's understanding of the problem and requirements to resolve it. This method was developed by Thomas Lewis Saaty, an American scientist, in 1970s [49, 50]; since then it has been actively refined and widely used in practice. Analytical Hierarchy Process can be used not only to compare objects, but also to address more complicated management and prediction challenges [51].

Hierarchy is a system, the levels of which are arranged and numbered in such a way that:

1) the lowest level contains the ranking alternatives;

2) nodes of levels with greater numbers can dominate only the nodes of levels with lesser numbers.

Thus, the links in the hierarchy define one-direction pathways – from the top to the alternatives via intermediate levels represented by nodes-factors (Fig.).

The main advantage of AHP is its versatility – this method can be used to tackle various tasks such as the analysis of possible scenarios of the situation development, resource allocation, client ranking, as well as HR decision-making.

The main disadvantage of AHP is the need for large amounts of information from experts. This method is best suited for cases when the major portion of data is based on the decision-maker's preferences during the process of selection of the best decision from among multiple alternatives.

In a typical decision-making situation:

– several decision variants are considered;

– a criterion is established, based on which the extent, to which one or another decision is appropriate, is determined;

– circumstances, in which the problem is addressed, as well as reasons that influence making one or another decision, are known.

Goal setting in AHP application: let us assume that there are multiple alternatives (decision variants): $V_1, V_2, \ldots V_k$. For our goal of determining TAD risk, these are high, moderate and low. Each alternative is assessed based on the set of criteria: $C_1, C_2, \ldots C_n$. For example, the following assessment criteria are used for ASF introduction risk analysis: feed, contacts with wild animals, contacts with domestic animals, contacts with blood-sucking insects, anthropogenic interference, transport-associated criterion and housing conditions. It is required to determine the level of risk of the disease introduction to the previously free areas.

Let us consider the steps of AHP application.

Step 1 is the preliminary ranking of criteria, as a result of which they will be ranked in descending order of their importance (significance).

Step 2 is the pair-wise comparison of the criteria according to their importance using a nine-point scale and an appropriate $n \times n$ matrix (table).

The pair-wise comparison system yields the result that can be represented as an inverse symmetric matrix. A matrix element (i, j) is the intensity of the hierarchy element i with respect to the hierarchy element j, which is estimated using a 1–9 intensity scale, where estimates mean the following:

– equal importance – 1;

– moderate dominance – 3;

– significant dominance – 5;

– strong dominance – 7;

– very strong dominance – 9;

– for intermediate values even numbers are used – 2, 4, 6, 8.

The following questions are mainly asked during the pair-wise comparison of the elements A and B:

– which of them is more important or has a greater impact;

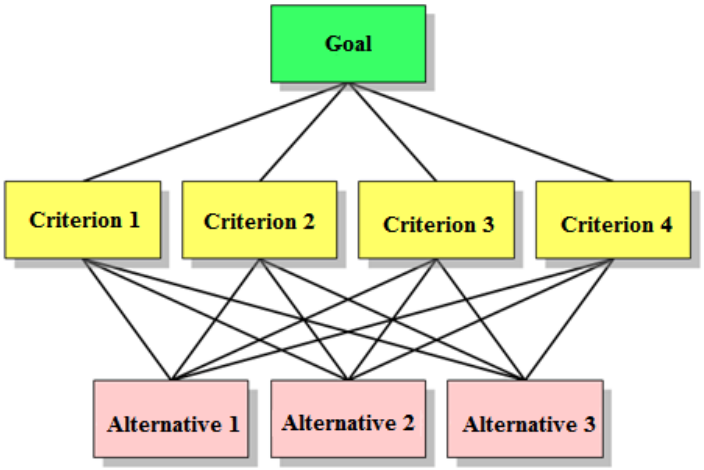

Fig. Simple AHP hierarchy.
To avoid confusion in AHP diagrams, links connecting alternatives and their covering criteria are often omitted or artificially reduced in number

– which of them has a higher probability;

– which of them is more preferable?

Step 3 is the construction of a matrix. If the element i is more important than the element j, a whole number is entered in the C_i:C_j cell corresponding to the row i and column j, and the reciprocal value is entered in the C_j:C_i cell corresponding to the row j and column i (Table 1).

For example, if the weight of the criterion C_1 (feed) is moderately higher than that of C_4 (animal movement), 3 is entered in the C_1:C_4 cell (at the intersection of the first row and the fourth column), and the reciprocal value (1/3) is entered in the C_4:C_1 cell (the fourth row, the first column). If the element j is more important than the element i, a whole number is entered in the C_j:C_i cell, and the reciprocal value is entered in the C_i:C_j cell. If i and j are judged to be equal, 1 (unity) is entered in both cells.

The table is filled in row by row, starting from the most important criterion. At first, whole number estimates are entered, and the corresponding fraction estimates (being the reciprocals of the whole numbers) are entered automatically. The more important a criterion is, the more whole number estimates will be entered in the corresponding

Table 1
Model table for comparison of ASF introduction risk criteria

	C_1	C_2	…	C_n	Geometric means	Normalized priority vector, NPV (formula 3)
C_1						
C_2						
…						
C_n						
Total					formula 2	
λ_{max}	formula 4					
Consistency index, CI	formula 5					
Consistency ratio, CR	formula 6					

row of the matrix, and these estimates will be higher. Since each criterion is equal to itself in importance, the main diagonal of the matrix will always consist of unities. It is obvious that the sum of components is equal to unity. Each component of the normalized priority vector (NPV) represents an importance estimate for the corresponding criterion (for example, the first component represents the importance estimate for the first criterion).

Geometric mean calculation for each row of the matrix:

$$a_1 = \sqrt[n]{\text{product of the 1}^{st}\text{ row elements}};$$

$$a_2 = \sqrt[n]{\text{product of the 2}^{nd}\text{ row elements}};$$

$$\ldots$$

$$a_n = \sqrt[n]{\text{product of the n}^{th}\text{ row elements}}. \quad (1)$$

Calculation of the sum of geometric means:

$$\Sigma a_j = a_1 + a_2 + \ldots + a_n. \quad (2)$$

NPV component calculation:

$$1^{st}\text{ NPV component} = \frac{a_1}{(\Sigma a_j)};$$

$$2^{nd}\text{ NPV component} = \frac{a_2}{(\Sigma a_j)};$$

$$n^{th}\text{ NPV component} = \frac{a_n}{(\Sigma a_j)}. \quad (3)$$

A check for consistency of local priorities by calculating three parameters:

– matrix eigenvalue:

$$\lambda_{max} = \Sigma\text{ of 1}^{st}\text{ column} \times 1^{st}\text{ NPV} + \Sigma\text{ of 2}^{nd}\text{ column} \times \times 2^{nd}\text{ NPV} + \ldots + \Sigma\text{ of n}^{th}\text{ column} \times n^{th}\text{ NPV}; \quad (4)$$

– consistency index:

$$CI = \frac{(\lambda_{max} - n)}{n - 1}; \quad (5)$$

– consistency ratio (%):

$$CR = \frac{CI}{RI}, \quad (6)$$

where RI is random consistency index, which is determined theoretically for a case when estimates in the matrix are presented randomly and depends on the matrix size only, as shown in Table 2 (*step 4*).

Estimates in the matrix are considered to be consistent when CR ≤ 10–15%; otherwise, they should be reconsidered.

Step 5 is the pair-wise comparison of the variants (levels of risk) for each criterion in the same way as the criteria have been compared, and the corresponding tables are filled in.

A check for consistency of local priorities is carried out for each table by calculating three parameters (step 4).

Step 6 is the determination of the global criterion (priority) for each variant (levels of risk):

$$C(V_1) = V_1\text{ for 1}^{st}\text{ crit.} \times 1^{st}\text{ NPV} + V_1\text{ for 2}^{nd}\text{ crit.} \times \times 2^{nd}\text{ NPV} + \ldots + V_1\text{ for n}^{th}\text{ crit.} \times n^{th}\text{ NPV}. \quad (7)$$

$C(V_2)$, $C(V_3)$ … $C(V_k)$ are calculated in a similar way, but V_1 in the expression should be replaced by V_2, V_3 … V_k, respectively. Table 3 is filled in.

Step 7. Determination of the best judgement, for which C value is the highest.

Step 8. The check of the judgement for its consistency:

– calculation of overall consistency index:

$$OCI = CI_1 \times 1^{st}\text{ NPV component} + CI_2 \times \times 2^{nd}\text{ NPV component} + \ldots + CI_n \times n^{th}\text{ NPV component}; \quad (8)$$

– calculation of overall consistency ratio:

$$OCR = \frac{OCI}{ORI}, \quad (9)$$

where ORI is determined according to Table 1 at the level of RI for the matrices of comparison of the variants according to the criteria. The judgement is considered to be consistent when OCR ≤ 10–15%; otherwise, the matrices of comparison of the variants according to the criteria should be revised.

An example is the assessment of risk of TAD introduction to the previously free areas.

Let us assume that it is required to determine the level of risk of TAD introduction to the previously free region 1.

Table 2
Random consistency index

Matrix size	1	2	3	4	5	6	7	8	9	10
RI	0	0	0.58	0.90	1.12	1.24	1.32	1.41	1.45	1.49

Table 3
Model table – calculation of final priorities

	C_1	C_2	…	C_n	Final priorities (formula 7)
	1st NPV component values from Table 2 are indicated	2nd NPV component values from Table 2 are indicated		nth NPV component values from Table 2 are indicated	
V_1					$C(V_1) =$
V_2					$C(V_2) =$
…					…
V_k					$C(V_k) =$
CI	CI_1 value for C_1 is indicated	CI_2 value for C_2 is indicated	…	CI_n value for C_n is indicated	the sum of the column is indicated
OCI	is calculated according to formula 8				
OCR	is calculated according to formula 9				

Table 4
Comparison of transboundary animal disease introduction risk criteria

	C_1	C_2	C_3	C_4	C_5	C_6	C_7	Geometric means	NPV
C_1	1	7	7	7	7	7	7	5.3	0.5
C_2	1/7	1	5	1	7	5	1	1.6	0.15
C_3	1/7	1/5	1	1/5	1/3	1/3	3	0.4	0.04
C_4	1/7	1	5	1	5	3	1	1.4	0.13
C_5	1/7	1/7	3	1/5	1	1/3	1/3	0.4	0.04
C_6	1/7	1/5	3	1/3	3	1	1	0.7	0.07
C_7	1/7	1	1/3	1	3	1	1	0.8	0.07
TOTAL (sum)	1.9	10.5	24.3	10.7	26.3	17.6	14.3	10.6	1
λ_{max}	8.17								
CI	0.19								
CR	0.14								

On 14 January 2021, 12 dead wild boars infected with a TAD agent were detected in the neighbouring region 2 close to the border with the free region.

On 25 February 2021, the TAD agent genome was detected in the sausage brought to the free region 1 from the distant region 3.

On 14 October 2021, it was reported that veterinarians had detected infected animal products in the previously free region 1. The TAD agent genome was detected during laboratory tests of frozen meat delivered to the region 1 from the region 4, to which, judging from documents, the product had been delivered from the region 5.

Here we note three alternatives: V_1 – high risk, V_2 – moderate risk and V_3 – low risk. Each alternative is assessed using the following list of criteria: C_1 – feed, C_2 – anthropogenic interference, C_3 – contacts with wild animals, C_4 – contacts with domestic animals, C_5 – contacts with blood-sucking insects, C_6 – housing conditions, C_7 – transport-associated criterion.

The pair-wise comparison of the criteria according to their importance is carried out using a nine-point scale and a 7 × 7 matrix. The facts given above should also be taken into consideration.

Table 4 is filled in row by row, starting from the most important criterion, using the formulas (2–6). RI value according to Table 2 will be 1.32.

The estimates in the matrix can be considered consistent, since CR = 0.14 falls within CR ≤ 10–15%.

When comparing the variants (levels of risk) with one another, we will consider them to be equally probable.

Then we determine the best judgement, for which the value of each criterion is the highest.

Based on the highest sum of the column for V_1 (31 points), we conclude that the risk of TAD agent introduction to the free region 1 is high.

The calculation of overall consistency ratio according to the formula (9) gives OCR = 0.14% and the judgement can therefore be considered consistent.

CONCLUSION

Given the interconnectedness and dense logistics network among the countries as regards trade in live animals and animal products, the introduction and further spread of highly dangerous diseases, in particular transboundary ones, to the disease-free areas, remain a serious threat to the entire livestock industry at present and will remain such in the near future.

Table 5
Comparison of criteria for transboundary animal disease risk level

	V_1	V_2	V_3
C_1	7	3	1
C_2	7	3	1
C_3	7	3	1
C_4	1	1	1
C_5	1	1	1
C_6	1	1	1
C_7	7	3	1

The potential application of Analytical Hierarchy Process as a decision-making tool for assessment of the risk of transboundary animal disease occurrence and introduction will allow to adequately understand the level of threat and undertake preventive measures in advance. However, when using this methodology, account should be taken of the need for reliable quantitative and qualitative data.

REFERENCES

1. Staroverov V. I., Vartanova M. L. Russia's food security as an important component of the country's demographic policy. *Ekonomicheskie otnosheniya*. 2019; 9 (4): 2851–2862. DOI: 10.18334/eo.9.4.41461. (in Russ.)

2. Vartanova M. L. Global trends in food policy and their impact on demographic development. *Ekonomicheskie otnosheniya*. 2019; 9 (4): 2877–2888. DOI: 10.18334/eo.9.4.41507. (in Russ.)

3. Veterinary Law of the Russian Federation 14.05.1993 No. 4979-1. Available at: https://www.consultant.ru/document/cons_doc_LAW_4438/. (in Russ.)

4. Virology Manual. Viruses and viral infections in humans and animals. Ed. by D. K. Lvov. Moscow: Medical Informational Agency Publishers; 2013. 1200 p. (in Russ.)

5. Animal infectious pathology in 2 vlms. Vol. 1. Ed. by A. Ya. Samuylenko et al. Moscow: Akademkniga; 2006. 910 p. (in Russ.)

6. FAO Expert Consultation on the Emergency Prevention System (EMPRES) for Transboundary Animal and Plant Pests and Diseases (Livestock Diseases Programme) including the Blueprint for Global Rinderpest Eradication and Food and Agriculture Organization of the United Nations. *Prevention and control of transboundary animal diseases: report of the FAO Expert Consultation on the Emergency Prevention System (EMPRES) for Transboundary Animal and Plant Pests and Diseases (Livestock Diseases Programme) including the Blueprint for Global Rinderpest Eradication, Rome, Italy, 24–26 July 1996*. Available at: https://www.fao.org/3/W3737E/W3737E00.htm.

7. Animal Diseases. Available at: https://www.woah.org/en/what-we-do/animal-health-and-welfare/animal-diseases.

8. Meuwissen M. P., Horst S. H., Huirne R. B., Dijkhuizen A. A. A model to estimate the financial consequences of classical swine fever outbreaks: principles and outcomes. *Prev. Vet. Med*. 1991; 42 (3–4): 249–270. DOI: 10.1016/s0167-5877(99)00079-3.

9. Yang P. C., Chu R. M., Chung W. B., Sung H. T. Epidemiological characteristics and financial costs of the 1997 foot-and-mouth disease epidemic in Taiwan. *Vet. Rec*. 1999; 145 (25): 731–734. DOI: 10.1136/vr.145.25.731.

10. Thompson D., Muriel P., Russell D., Osborne P., Bromley A., Rowland M., et al. Economic costs of the foot and mouth disease outbreak in the United Kingdom in 2001. *Rev. Sci. Tech*. 2002; 21 (3): 675–687. DOI: 10.20506/rst.21.3.1353.

11. Zepeda C., Morilla A., Yoon K.-J., Zimmerman J. The social impact of disease control and eradication programs: case studies. *In: Trends in Emerging Viral Infections of Swine*. 2002; 17–20. DOI: 10.1002/9780470376812.ch1c.

12. Makarov V. V., Grubyi V. A., Gruzdev K. N., Sukharev O. I. OIE List and transboundary animal infections: monograph. Vladimir: FGBI "ARRIAH"; 2012. 162 p. (in Russ.)

13. Approval of the List of contagious and other animal diseases: Order of the Ministry of Agriculture of the Russian Federation 09.03.2011 No. 62. Available at: https://base.garant.ru/2174787. (in Russ.)

14. Dudin M. N., Shkodinskiy S. V., Usmanov D. I. Bifurcation analysis of the current state of the Russian economy: the impact of COVID-19 on key development processes. *Ekonomicheskie otnosheniya*. 2022; 12 (2): 155–178. DOI: 10.18334/eo.12.2.114554. (in Russ.)

15. Beltran-Alcrudo D., Falco J. R., Raizman E., Dietze K. Transboundary spread of pig diseases: the role of international trade and travel. *BMC Vet. Res*. 2019; 15:64. DOI: 10.1186/s12917-019-1800-5.

16. Zepeda C., Salman M., Ruppanner R. International trade, animal health and veterinary epidemiology: challenges and opportunities. *Prev. Vet. Med*. 2001; 48 (4): 261–271. DOI: 10.1016/s0167-5877(00)00200-2.

17. Alyokhin A. F., Mischenko V. A. Osobennosti techeniya chumy sredi krupnogo rogatogo skota, vypasayushchegosya na otgonnom pastbishche = Peculiarities of rinderpest course in cattle grazing on distant pastures. *Voprosy veterinarnoi virusologii, mikrobiologii i epizootologii: materialy nauchnoi konferentsii VNIIVViM (Pokrov, 13–18 aprelya 1992 g.). T. 1 = Aspects of Veterinary Virology, Microbiology and Epizootology: Proceedings of the ARRIVV&M Conference (Pokrov, April 13–18, 1992). Vol. 1*. Pokrov; 1992; 151–152. (in Russ.)

18. Bandi Ts. Effectiveness of complex system of anti-epidemic measures against foot-and-mouth disease in Mongolia in 2011–2013: Author's Abstract of Theses for degree of Candidate of Science (Veterinary Medicine). Vladimir; 2013. 25 p. (in Russ.)

19. Belik E. V., Dudnikov S. A., Lyadskiy M. M., Belchihina A. V., Gulyonkin V. M., Karaulov A. K., Dudorova M. V. Analysis of the risk of African swine fever introduction and spread in the Vladimir Oblast: information and analytical review. Vladimir; FGI "ARRIAH". 2009. 97 p. (in Russ.)

20. Bouchemla F., Agoltsov V. A., Popova O. M., Padilo L. P. Assessment of the peste des petits ruminants world epizootic situation and estimate its spreading to Russia. *Vet. World*. 2018; 11 (5): 612–619. DOI: 10.14202/vetworld.2018.612-619.

21. Gulenkin V. M., Karaulov A. K., Lozovoy D. A., Zakharov V. M. Expert risk assessment of FMD introduction to the Russian Federation from infected countries. *Veterinary Science Today*. 2018; (2): 36–41. DOI: 10.29326/2304-196X-2018-2-25-36-41.

22. Karaulov A. K., Gulenkin V. M., Titov M. A., Zakharov V. M., Dudnikova N. S., Dudnikov S. A. Risk analysis of bluetongue (ovine catarrhal fever) introduction with imported cattle to Russia from Western Europe countries. *Russian Veterinary Journal Productive Animals*. 2008; Special Issue (September): 33–35. (in Russ.)

23. Kononov A. V., Karaulov A. K., Gulenkin V. M., Piskunov A. V., Petrova O. N., Sprygin A. V., et al. Prediction for peste des petits ruminants in the Russian Federation for 2019. *In: Predictions for contagious animal diseases in the Russian Federation for 2019*. Vladimir: FGBI "ARRIAH"; 2018; 233–250. (in Russ.)

24. Pharo H. J. Foot-and-mouth disease: an assessment of the risks facing New Zealand. *N. Z. Vet. J.* 2002; 50 (2): 46–55. DOI: 10.1080/00480169.2002.36250.

25. Boev B. V., Makarov V. V. Geo-informatsionnye sistemy i epidemii grippa = Geoinformation systems and influenza epidemics. *Veterinarnaya patologiya*. 2004; 3: 51–59. eLIBRARY ID: 9165685. (in Russ.)

26. Gintsburg A. L., Boev B. V. Komp'yuternoe modelirovanie epidemii = Computer modeling of epidemics. *Science in Russia*. 2005; 5: 52–57. eLIBRARY ID: 17688485. (in Russ.)

27. Belchihina A. V., Dudorova M. V. Razrabotka na baze Arc GIS atlasa epidznachimykh ob"ektov Vladimirskoi oblasti = Development of atlas of epidemiologically-significant facilities of the Vladimir Oblast based on Arc GIS database. *Geoinformatsionnye sistemy v zdravookhranenii RF: dannye, analitika, resheniya: trudy 1-i i 2-i Vserossiiskikh konferentsii* s *mezhdunarodnym uchastiem (26–27 maya, 2011 g. i 24–25 maya 2012 g.) = Geoinformation systems in health care system of the Russian Federation: data, analytics, decisions: proceedings of the 1st and 2nd All-Russia Conferences with international participation (26–27 May, 2011 and 24–25 May, 2012)*. Saint Petersburg: Beresta; 2013; 42–45. (in Russ.)

28. Korennoy F. I. Mathematical and cartographic modeling of the spread of highly dangerous livestock diseases: author's thesis of Candidate of Science (Geography). Moscow; 2019. 22 p. (in Russ.)

29. Amimo J. O., Okoth E., Junga J. O., Ogara W. O., Njahira M. N., Wang Q., et al. Molecular detection and genetic characterization of kobuviruses and astroviruses in asymptomatic local pigs in East Africa. *Arch. Virol*. 2014; 159 (6): 1313–1319. DOI: 10.1007/s00705-013-1942-x.

30. Huang Y. W., Dickerman A. W., Piñeyro P., Li L., Fang L., Kiehne R., et al. Origin, evolution, and genotyping of emergent porcine epidemic diarrhea virus strains in the United States. *mBio*. 2013; 4 (5):e00737-13. DOI: 10.1128/mBio.00737-13.

31. Zhou X., Li N., Luo Y., Liu Y., Miao F., Chen T., et al. Emergence of African swine fever in China, 2018. *Transbound. Emerg. Dis*. 2018; 65 (6): 1482–1484. DOI: 10.1111/tbed.12989.

32. Korennoy F. I., Petrova O. N., Karaulov A. K. Use of the GIS geo-spatial analysis tools for identification of spatio-temporal patterns of zoonotic infection spread. Available at: https://snipchi.ru/updoc/2017/Prezentazii/Коренной%20Ф.%20И.%20«Применение%20инструментов%20геопространственного%20анализа.pdf. (in Russ.)

33. Petrova O. N., Korennoy F. I., Karaulov A. K., Shevtsov A. A., Gulenkin V. M. Prediction for African swine fever in the Russian Federation for 2020 (final version). Available at: https://fsvps.gov.ru/fsvps-docs/ru/iac/asf/publications/asf_prognoz2020.pdf. (in Russ.)

34. Mischenko V. A., Pavlov D. K., Dumova V. V., Nikeshina T. B., Getmanskiy O. I., Kononov A. V., Lisitsyn V. V. Ekologicheskie osobennosti zabolevanii pishchevaritel'noi sistemy novorozhdennykh telyat = Ecological peculiarities of gastro-intestinal diseases in neonatal claves. *Veterinarnaya patologiya*. 2005; 3: 34–38. eLIBRARY ID: 9167885. (in Russ.)

35. Gulenkin V. M., Korennoy F. I., Karaulov A. K., Dudnikov S. A. Cartographical analysis of African swine fever outbreaks in the territory of the Russian Federation and computer modeling of the basic reproduction ratio. *Prev. Vet. Med*. 2011; 102 (3): 167–174. DOI: 10.1016/j.prevetmed.2011.07.004.

36. Korennoy F. I., Gulenkin V. M., Gogin A. E., Vergne T., Karaulov A. K. Estimating the basic reproductive number for African swine fever using the Ukrainian historical epidemic of 1977. *Transbound. Emerg. Dis*. 2017; 64 (6): 1858–1866. DOI: 10.1111/tbed.12583.

37. Korennoy F. I., Gulenkin V. M., Malone J. B., Mores C. N., Dudnikov S. A., Stevenson M. A. Spatio-temporal modeling of the African swine fever epidemic in the Russian Federation, 2007–2012. *Spat. Spatiotemporal. Epidemiol*. 2014; 11: 135–141. DOI: 10.1016/j.sste.2014.04.002.

38. Vergne T., Korennoy F., Combelles L., Gogin A., Pfeiffer D. U. Modelling African swine fever presence and reported abundance in the Russian Federation using national surveillance data from 2007 to 2014. *Spat. Spatiotemporal Epidemiol*. 2016; 19: 70–77. DOI: 10.1016/j.sste.2016.06.002.

39. Dudnikov S. A. Quantitative epidemiology: basics of applied epidemiology and biostatistics. Vladimir: Demiurg; 2005. 459 p. (in Russ.)

40. Miller J., Burton K., Fund J., Self A. Process review for development of quantitative risk analyses for transboundary animal disease to pathogen-free territories. *BioResearch Open Access*. 2017; 6 (1): 133–140. DOI: 10.1089/biores.2016.0046.

41. Oganesyan A. S., Baskakova N. Ye., Karaulov A. K. Methodological Guidelines for semi-quantitative assessment of the risk associated with import (entry/movements) of live animals and products of animal origin: methodical material. Vladimir: FGBI "ARRIAH"; 2016. 22 p. (in Russ.)

42. Cherkassky B. L. Risk in epidemiology. Moscow: Prakticheskaya meditsina; 2007. 476 p. (in Russ.)

43. Shevtsov A. A., Karaulov A. K., Dudnikov S. A., Titov M. A., Usov A. V., Korennoy F. I., Bardina N. S. Analysis of risk of African swine fever introduction to the Russian Federation from Transcaucasia (situation as of June 2008). Vladimir: FGI "ARRIAH"; 2008. 72 p. (in Russ.)

44. Amanatin A., Sudarnika E., Lukman D. W., Wibawan I. W. T. Risk assessment on rabies entry through hunting dog movement with semi-quantitative approach to Sumatera Island, Indonesia. *J. Adv. Vet. Anim. Res*. 2019; 6 (2): 148–157. DOI: 10.5455/javar.2019.f325.

45. Ezell B. Homeland Security Risk Modeling. *In: Handbook of Real-World Applications in Modeling and Simulation. Ed. by J. A. Sokolowski, C. M. Banks*. John Wiley & Sons, Inc.; 2012; 129–164. DOI: 10.1002/9781118241042.ch4.

46. Terebova S. V., Koltun G. G., Podvalova V. V., Korotkova I. P. Risk analysis of the spread of african swine fever in Primorsky krai. *Agrarian bulletin of Primorye*. 2020; 1 (17): 13–19. eLIBRARY ID: 42918095. (in Russ.)

47. Gulenkin V. M., Korennoy F. I., Karaulov A. K. Methodical recommendations for quantitative (score) assessment of the expert group opinions on animal infectious disease epidemiology, animal and fish product safety: approved by FGBI "ARRIAH" 21.09.2017 No. 41-17. Vladimir; 2017. 18 p. (in Russ.)

48. Gudkov P. A. Comparative analysis methods: study guide. Ed. by A. M. Bershadskiy. Penza: Penza State University; 2008. 81 p. (in Russ.)

49. Saaty T. L. The Analytic Hierarchy Process: Planning, Priority Setting, Resource Allocation (Decision Making Series). New York: McGraw-Hill; 1980. 287 p.

50. Saaty T. L. Decision Making with Dependence and Feedback: The Analytic Network Process. Pittsburgh: Rws Publications; 1996. 370 p.

51. Romanov V. N. Systemic analysis for engineers. Saint Petersburg: NWSCTU; 2006. 186 p. (in Russ.)

Received 22.11.2022
Revised 12.12.2022
Accepted 26.01.2023

INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Nikita V. Lebedev, Candidate of Science (Veterinary Medicine), Adviser to the Director, FGBI "ARRIAH", Vladimir, Russia; *https://orcid.org/0000-0002-3600-2409, e-mail: lebn@yandex.ru.*

Лебедев Никита Викторович, кандидат ветеринарных наук, советник директора ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *https://orcid.org/0000-0002-3600-2409, e-mail: lebn@yandex.ru.*

Alexey S. Igolkin, Candidate of Science (Veterinary Medicine), Head of Reference Laboratory for African swine fever, FGBI "ARRIAH", Vladimir, Russia; *https://orcid.org/0000-0002-5438-8026, e-mail: igolkin_as@arriah.ru.*

Konstantin N. Gruzdev, Doctor of Science (Biology), Professor, Chief Researcher, Information and Analysis Centre, FGBI "ARRIAH", Vladimir, Russia; *https://orcid.org/0000-0003-3159-1969, e-mail: gruzdev@arriah.ru.*

Dmitry G. Spirin, Candidate of Science (Chemical), Head of Department, Research Centre, 48th Central Research Institute of the Ministry of Defense of the Russian Federation, Moscow, Russia; *e-mail: 48cnii@mil.ru.*

Mikhail A. Farukh, Senior Researcher, Department of the Research Centre, 48th Central Research Institute of the Ministry of Defense of the Russian Federation, Moscow, Russia; *e-mail: 48cnii@mil.ru.*

Nikolay V. Ermilov, Junior Researcher, Department of the Research Centre, 48th Central Research Institute of the Ministry of Defense of the Russian Federation, Moscow, Russia; *e-mail: 48cnii@mil.ru.*

Gennady G. Eremin, Candidate of Science (Medicine), Deputy Head of the Research Centre, 48th Central Research Institute of the Ministry of Defense of the Russian Federation, Moscow, Russia; *AuthorID: 710033, e-mail: 48cnii@mil.ru*.

Иголкин Алексей Сергеевич, кандидат ветеринарных наук, заведующий референтной лабораторией по африканской чуме свиней ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *https://orcid.org/0000-0002-5438-8026, e-mail: igolkin_as@arriah.ru.*

Груздев Константин Николаевич, доктор биологических наук, профессор, главный научный сотрудник информационно-аналитического центра ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *https://orcid.org/0000-0003-3159-1969, e-mail: gruzdev@arriah.ru.*

Спирин Дмитрий Геннадьевич, кандидат химических наук, начальник отдела научно-исследовательского центра 48 ЦНИИ МО РФ, г. Москва, Россия; *e-mail: 48cnii@mil.ru.*

Фарух Михаил Александрович, старший научный сотрудник отдела научно-исследовательского центра 48 ЦНИИ МО РФ, г. Москва, Россия; *e-mail: 48cnii@mil.ru.*

Ермилов Николай Владимирович, младший научный сотрудник отдела научно-исследовательского центра 48 ЦНИИ МО РФ, г. Москва, Россия; *e-mail: 48cnii@mil.ru.*

Еремин Геннадий Геннадьевич, кандидат медицинских наук, заместитель начальника научно-исследовательского центра 48 ЦНИИ МО РФ, г. Москва, Россия; *AuthorID: 710033, e-mail: 48cnii@mil.ru.*

VETERINARY SCIENCE TODAY JOURNAL INVITES AUTHORS TO SUBMIT THEIR PAPERS FOR PUBLICATION

- The journal was founded by the FGBI "Federal Centre for Animal Health" (FGBI "ARRIAH") in 2012.
- Papers are published in two languages: Russian and English.
- The journal's focal areas include outputs of academic and experimental studies in veterinary medicine and veterinary microbiology, veterinary science development trends, discussion of currently important issues of animal disease monitoring and epidemiology.
- The journal is distributed throughout Russia, as well as in the largest research centres of the world.
- We publish articles authored by distinguished scientists and early career researchers, practitioners, staff members of veterinary institutions with a view of sharing experiences, maintaining consistent compliance with veterinary safety requirements and holding new scholarly discussions.

KEY FOCUSES OF THE JOURNAL

– Insights into main veterinary science development trends.

– Analysis of a wide range of state-of-the-art technologies in animal disease monitoring and epidemiology, presentation of outputs of academic and experimental studies in this field.

– Discussion of currently important issues of veterinary medicine.

PLEASE CONTACT OUR EDITOR OFFICE FOR DETAILED CONDITIONS OF PUBLICATION

Address: 600901, Russia, Vladimir, Yur'evets

Telephones: +7 (4922) 26-15-12, 26-17-65, 26-19-88, ext. 22-27

Contact person: Tatyana B. Nikeshina,
e-mail: nikeshina@arriah.ru

http://veterinary.arriah.ru/jour/index

GENERAL REQUIREMENTS FOR SUBMITTED PAPERS

Papers in two languages – Russian and English – that comprise results of own scientific studies, being up to 6–8 pages (up to 10 pages for reviews) but at least 5 pages (single-spaced, size 12) are accepted for publication. Optimal paper size: 3,000–6,000 words.

Submission of a manuscript to the editors office implies that the author's consent for his/her manuscript use both in paper and electronic formats. Authors are responsible for completeness and reliability of the literature cited in their papers as well as for publication of the borrowed materials without reference to their source. The materials forwarded to the editor office should be accompanied with the letter of the author's organization (the model is available on the site).

STRUCTURE OF PRESENTED PAPER

1. **UDC**
2. **Title of paper**
3. **Full names of authors,** authors' workplaces, city, country, ORCID ID, e-mails.
4. **Summary** (brief and accurate outline of the paper containing data and conclusions of described works): 200–250 words, not more than 2,000 characters.
5. **Key words** (5–6 words, phrases), more precisely representing the paper specificity.
6. **Acknowledgements** (if any information on acknowledgements to people and sponsoring organizations).
7. **For citing**
8. **Conflict of interest**
9. **For correspondence** (full name, scientific title, position, address, e-mail).
10. **Introduction**
11. **Materials and Methods**
12. **Results and Discussion**
13. **Conclusion**
14. **References.** *Vancouver style* – sequentially numbered list of references in the order of their citing (appearing in the text).
15. **Information about the authors** (full name, scientific title, position, city, country).
16. Only illustrated materials (photos, pictures) of good contrast with resolution of at least 300 dots per inch (300 dpi) are accepted for publication, originals are attached to the papers as separate files in .tif or .jpg format (figures not compliant to the requirements will be excluded from the papers as their appropriate typographic reproduction is impossible).

The paper should be presented as follows: Microsoft Word, font Times New Roman, font size – 12,
line spacing – 1, margins – 2 cm, first line indent – 1 cm, text alignment – justified text.

Figures, tables, diagrams, graphs, etc. shall be numerated, have indicated sources and fit to page printing. Table name should be given above the table; figure/graph name – under the figure/graph.

Paper originals and copies are not returned. Authors shall guarantee that submitted materials have not been published before. Compliance with all above-said requirements of the editorial board is essential for paper acceptance for publication in Veterinary Science Today journal.

Фото: Александр Плонский

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

ФГБУ «ФЕДЕРАЛЬНЫЙ ЦЕНТР ОХРАНЫ ЗДОРОВЬЯ ЖИВОТНЫХ»

WOAH REFERENCE LABORATORY FOR FOOT AND MOUTH DISEASE

РЕФЕРЕНТНАЯ ЛАБОРАТОРИЯ ВОЗЖ ПО ЯЩУРУ

WOAH REFERENCE LABORATORY FOR AVIAN INFLUENZA

РЕФЕРЕНТНАЯ ЛАБОРАТОРИЯ ВОЗЖ ПО ГРИППУ ПТИЦ

WOAH REFERENCE LABORATORY FOR NEWCASTLE DISEASE

РЕФЕРЕНТНАЯ ЛАБОРАТОРИЯ ВОЗЖ ПО БОЛЕЗНИ НЬЮКАСЛА

WOAH COLLABORATING CENTRE FOR DIAGNOSIS AND CONTROL OF VIRAL ANIMAL DISEASES IN EASTERN EUROPE, CENTRAL ASIA AND TRANSCAUCASIA

ЦЕНТР ВОЗЖ ПО СОТРУДНИЧЕСТВУ В ОБЛАСТИ ДИАГНОСТИКИ И КОНТРОЛЯ ВИРУСНЫХ БОЛЕЗНЕЙ ЖИВОТНЫХ ДЛЯ СТРАН ВОСТОЧНОЙ ЕВРОПЫ, ЦЕНТРАЛЬНОЙ АЗИИ И ЗАКАВКАЗЬЯ

FAO REFERENCE CENTRE FOR FOOT-AND-MOUTH DISEASE

РЕФЕРЕНТНЫЙ ЦЕНТР ФАО ПО ЯЩУРУ

FAO REFERENCE CENTRE FOR ZOONOTIC CORONAVIRUSES

РЕФЕРЕНТНЫЙ ЦЕНТР ФАО ПО ЗООНОЗНЫМ КОРОНАВИРУСАМ