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

AUGUST | АВГУСТ VOL. 10 No. 3 2021

60th ANNIVERSARY OF THE FGBI "ARRIAH" POSTGRADUATE SCHOOL

60th Anniversary of Postgraduate School of Federal State-Financed Institution "Federal Centre for Animal Health" p. 178

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

AUGUST VOLUME 10 No. 3 2021
Published since 2012

ВЕТЕРИНАРИЯ СЕГОДНЯ

ЕЖЕКВАРТАЛЬНЫЙ НАУЧНЫЙ ЖУРНАЛ

АВГУСТ ТОМ 10 № 3 2021
Основан в 2012 г.

Журнал «Ветеринария сегодня»
включен в Перечень рецензируемых научных изданий (ВАК):
03.02.02 – Вирусология (ветеринарные науки),
06.02.02 – Ветеринарная микробиология, вирусология, эпизоотология, микология с микотоксикологией и иммунология (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
Coordinating Editor: Julia Migulina
Content editors of FGBI "ARRIAH": Elena Guseva, 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. Registered trademark, certificate No. 514190.

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: August 13, 2021
Issued: August 25, 2021

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
Редактор-координатор: Мигулина Юлия
Редакторы-корректоры ФГБУ "ВНИИЗЖ": Гусева Елена, 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. Зарегистрированный товарный знак, свидетельство № 514190.

Тираж 1150 экземпляров. Цена свободная
Подписку на научный журнал «Ветеринария сегодня» можно оформить через Агентство по подписке «Урал-Пресс»: 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: 13 августа 2021 года
Дата выхода в свет: 25 августа 2021 года

16+

Contents

Содержание

DOI: 10.29326/2304-196X-2021-3-38-178-183

60th Anniversary of Postgraduate School of Federal State-Financed Institution "Federal Centre for Animal Health"

T. V. Zhbanova[1], T. B. Nikeshina[2], N. A. Perevozchikova[3], N. A. Marova[4]
FGBI "Federal Centre for Animal Health" (FGBI "ARRIAH"), Vladimir, Russia
[1] *ORCID 0000-0002-9857-5915, e-mail: zhbanova@arriah.ru*
[2] *ORCID 0000-0002-0959-5915, e-mail: nikeshina@arriah.ru*
[3] *Scopus Author ID 6603578671, e-mail: perevozchikova@arriah.ru*
[4] *e-mail: marova@arriah.ru*

SUMMARY

When the All-Union Foot-and-Mouth Disease Research Institute was founded in 1958, an active search for relevant specialists and researchers was organized with a view to recruiting them as staff members. In the early days of its establishment, the All-Union Foot-and-Mouth Disease Research Institute was mainly stuffed with the graduates of the Moscow Academy of Veterinary Medicine named after K. I. Skryabin and the Kharkov, Leningrad, Vitebsk Institutes of Veterinary Medicine. Later they were joined by the graduates of the University of Gorky, the Kazan and Ivanovo Institutes of Veterinary Medicine, the Vladimir Pedagogical Institute and many others. In 1963, the All-Union Foot-and-Mouth Disease Research Institute began to train research workers through postgraduate training programmes and thesis-based programmes. The leading scientists of the Institute were authorized by a decision of the Higher Attestation Commission of the USSR to provide academic supervision to postgraduate students. A specialized council for thesis defense started its activities in regard to Candidate of Science thesis defense in 1976 and in regard to Doctor of Science thesis defense – in 1996. The specialized council for Candidate of Science thesis defense comprised Candidates and Doctors of Sciences, staff members of the All-Union Foot-and-Mouth Disease Research Institute, as well as Doctors of Sciences from the All-Russian Research Institute of Veterinary Virology and Microbiology (VNIIVViM) (N. I. Arkhipov, I. F. Vishnyakov, V. M. Kolosov, N. A. Lagutkin, Yu. I. Petrov, G. A. Safonov, G. G. Yurkov). In 1996, the specialized council for Candidate of Science thesis defense was enlarged, and the following reputable scientists were invited to participate in its activities as its members: two Doctors of Sciences from the VNIIVViM (Ye. M. Khripunov, M. A. Dymin), four staff members of the VGNKI (K. N. Gruzdev, A. N. Panin, V. I. Ulasov, K. V. Shumilov), the staff members of the Veterinary Department of the Ministry of Agriculture of the Russian Federation (O. I. Sukharev) and the Peoples' Friendship University of Russia (V. V. Makarov). The paper provides brief information on the training of scientific personnel for research and production laboratories of the institution, postgraduate school activities, thesis-based programmes, the council for Doctor of Science and Candidate of Science thesis defense.

Keywords: review, All-Union Foot-and-Mouth Disease Research Institute, FGBI "ARRIAH", postgraduate school, thesis council

For citation: Zhbanova T. V., Nikeshina T. B., Perevozchikova N. A., Marova N. A. 60th Anniversary of Postgraduate School of Federal State-Financed Institution "Federal Centre for Animal Health". *Veterinary Science Today*. 2021; 10 (3): 178–183. DOI: 10.29326/2304-196X-2021-3-38-178-183.

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

For correspondence: Tatyana V. Zhbanova, Candidate of Science (Biology), Head of Scientific Personnel Sector, Head of Department of Education and Scientific and Methodical Work, FGBI "ARRIAH", 600901, Russia, Vladimir, Yur'evets, *e-mail: zhbanova@arriah.ru*.

УДК 619:378.126

Аспирантуре федерального государственного бюджетного учреждения «Федеральный центр охраны здоровья животных» – 60 лет

Т. В. Жбанова[1], Т. Б. Никешина[2], Н. А. Перевозчикова[3], Н. А. Марова[4]
ФГБУ «Федеральный центр охраны здоровья животных» (ФГБУ «ВНИИЗЖ»), г. Владимир, Россия
[1] *ORCID 0000-0002-9857-5915, e-mail: zhbanova@arriah.ru*
[2] *ORCID 0000-0002-0959-5915, e-mail: nikeshina@arriah.ru*
[3] *Scopus Author ID 6603578671, e-mail: perevozchikova@arriah.ru*
[4] *e-mail: marova@arriah.ru*

РЕЗЮМЕ

При создании в 1958 г. Всесоюзного научно-исследовательского ящурного института (ВНИЯИ) был организован активный поиск с целью привлечения в штат организации профильных специалистов и научных сотрудников. На заре своего становления ВНИЯИ комплектовался главным образом за счет выпускников Московской ветеринарной академии имени К. И. Скрябина, а также из ветеринарных институтов Харькова, Ленинграда, Витебска. Позже во ВНИЯИ прибыли выпускники Горьковского университета, Казанского и Ивановского ветеринарных институтов, Владимирского педагогического института и многих других. В 1963 г. во ВНИЯИ начали подготовку научных сотрудников через аспирантуру и соискательство. Ведущим ученым организации решением Высшей аттестационной комиссии СССР было разрешено научное руководство аспирантами. С 1976 г. начал работу специализированный совет по защите кандидатских диссертаций, а с 1996 г. – диссертаций на соискание ученой степени доктора наук. В состав кандидатского специализированного совета вошли доктора и кандидаты наук, сотрудники ВНИЯИ, а также доктора наук из покровского ВНИИВВиМ (Н. И. Архипов, И. Ф. Вишняков, В. М. Колосов, Н. А. Лагуткин, Ю. И. Петров, Г. А. Сафонов, Г. Г. Юрков). В 1996 г. диссертационный совет был расширен и для работы в его составе были приглашены авторитетные ученые: доктора наук из ВНИИВВиМ (Е. М. Хрипунов, М. А. Дымин), сотрудники ВГНКИ (К. Н. Груздев, А. Н. Панин, В. И. Уласов, К. В. Шумилов), сотрудники Департамента ветеринарии МСХ РФ (О. И. Сухарев) и Российского университета дружбы народов (В. В. Макаров). В данной статье представлены краткие сведения о подготовке научных кадров для научных и производственных лабораторий института, о работе аспирантуры, системе соискательства, диссертационном совете по защите докторских и кандидатских диссертаций.

Ключевые слова: обзор, ВНИЯИ, ФГБУ «ВНИИЗЖ», аспирантура, диссертационный совет

Для цитирования: Жбанова Т. В., Никешина Т. Б., Перевозчикова Н. А., Марова Н. А. Аспирантуре федерального государственного бюджетного учреждения «Федеральный центр охраны здоровья животных» – 60 лет. *Ветеринария сегодня.* 2021; 10 (3): 178–183. DOI: 10.29326/2304-196X-2021-3-38-178-183.

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

Для корреспонденции: Жбанова Татьяна Валентиновна, кандидат биологических наук, заведующий сектором научных кадров отдела образования и научно-методической работы ФГБУ «ВНИИЗЖ», 600901, Россия, г. Владимир, мкр. Юрьевец, *e-mail: zhbanova@arriah.ru.*

The history of the FGBI "ARRIAH" Postgraduate School goes back to distant sixties of the 20th century. The All-Union Foot-and-Mouth Disease Research Institute (in future – the FGBI "ARRIAH") was established by Order of V. V. Matskevich, Minister of Agriculture of the USSR No. 233-13 of 20 August 1958 "On measures for enhancing virological research activities". The main and the only task that the institution faced was to study foot-and-mouth disease (FMD) virus structure and features and to develop methods for FMD virus diagnosis and prevention.

Within a few years, a research team was formed, the staff members received training and the research was started. Ye. Merkulov, Deputy Head of the Department of Research Institutions informed by the official letter Vladislav P. Onufriyev, Director of the All-Union Foot-and-Mouth Disease Research Institute, that the Postgraduate School for researchers' training was established at the Institute by Order of the USSR Ministry of Higher and Vocational Education No. 113 of 1 April, 1961 [1–3].

In 1964, the Ministry set up a plan on admission 3 persons to the Postgraduate School for intramural training and recommended to admit students in accordance with the "Regulation on postgraduate schools in the higher educational institutions and research institutions" and Order of the USSR Ministry of Higher and Vocational Education No. 50 of 5 February, 1963 "On common model documents on researchers' and scientific tutors' training".

The first student admitted to the Postgraduate School for intramural training was Tamara Ye. Kalugina whose scientific adviser was Vladislav P. Onufriyev, Candidate of Science (Veterinary Medicine) Director of the Institute. Later, Vladislav P. Onufriyev became a scientific adviser for the first students admitted for intramural training: Viktor N. Koropov and Mariya S. Fomina [1, 4, 5].

In 1964, A. V. Yesionov, E. F. Tokarik, L. N. Sokolov and V. N. Kuznethov were admitted to the Postgraduate School for intramural training [1, 2]. In 1965, four more staff-members were admitted to the Postgraduate School for intramural training: T. A. Satina, N. A. Pronina, M. A. Mulyar and A. A. Kravchenko. The postgraduate students' theses were focused on study of FMD virus features, development of tools and methods for FMD diagnosis and prevention as well as creation of a scientifically justified complex of measures providing sustainable FMD freedom of the country.

Next year (1966) 11 students were admitted to the Postgraduate School: I. A. Pronin, N. S. Maslova, Yu. A. Chernyayev, S. S. Rybakov, Ye. L. Shedrin, N. M. Urvantsev, N. I. Yefimov, V. M. Kravchenko, S. V. Kuznetsova, Yu. T. Kiselev as well as Valeriy M. Zakharov graduated from the Moscow State Veterinary Academy by K. I. Skryabin. Currently, Valeriy M. Zakharov, Doctor of Science (Veterinary Medicine), Professor, Honored Veterinarian of the Russian Federation, is the OIE expert on FMD for Eastern Europe, Central Asia and Transcaucasia and gives lectures to postgraduate students.

In the following years, many more graduates of the Moscow State Veterinary Academy and other specialized universities came to the Postgraduate School. It was they who, with their knowledge, perseverance and curiosity,

Fig. 1. Director of the All-Union Foot-and-Mouth Disease Research Institute V. P. Onufriyev delivering a speech at the coordination meeting on FMD, 1965 (a photo from the FGBI "ARRIAH" archive)

managed to defeat the highly dangerous disease and to free the country territory from foot-and-mouth disease.

In 1964, the first Doctoral Thesis (based on the outcomes of the research performed in the All-Union Research Institute of Virology and Microbiology) was defended by Yevgeny Ye. Nikitin, Head of the Laboratory for Bioproduct Drying, in Pokrov. In 1967, Anatoly M. Rakhmanov, Head of Laboratory for Pathomorphology, defended his Doctoral Thesis in the Leningrad Veterinary Institute; his Thesis was based on the materials prepared during his doctoral studies in the Semipalatinsk Zoo-Veterinary Institute. In 1969, Vladislav P. Onufriyev, Director of the Institute, defended his Doctoral Thesis on FMD immunology in the Kazan Veterinary Institute; in 1970, Vasily L. Uzyumov defended his Doctoral Thesis on FMD virus ultrastructure and features in the Leningrad Veterinary Institute [5].

In the early years of the Postgraduate School history, only Doctors of Science and Professors had the right to be scientific advisers. Candidates of Science could also become scientific advisers, but as an exception, upon authorization given by the USSR Ministry of Higher and Vocational Education. Such authorizations were given to V. P. Onufriyev, Ye. Ye. Nikitin, A. P. Proztyakov, Ye. V. Andreyev, A. I. Sobko and V. L. Uzyumov [4–7].

In later years, the following Candidates of Science were authorized by the Order of the Director of the Institute to become scientific advisers without agreeing by the Higher Attestation Commission (HAC): N. N. Dryagalin, V. M. Khukhorov, V. M. Kravchenko, Ye. A. Krasnobayev, V. K. Muravyev, A. S. Okovyty, V. N. Koropov, V. I. Shorshnev, G. A. Khudyakov, V. G. Andreyev, S. S. Rybakov, N. A. Perevozchikova, V. M. Zakharov, S. K. Starov, T. A. Fomina, V. N. Irza, A. V. Scherbakov, A. V. Borisov, V. V. Borisov, Sh. K. Kulyashbekova, V. A. Mischenko, V. V. Drygin, T. Z. Baibikov, V. I. Diev [5, 8].

A total of 300 students have received training at the Postgraduate School since 1963.

For many years, the following outstanding researchers have worked fruitfully at the Institute and have shared their invaluable experience with early-career researchers: Laureates of the Government Prize in the field of science and technology: Anatoly A. Gusev, Associate Member of the Russian Academy of Agricultural Sciences, Professor [5], Andrey I. Dudnikov, Doctor of Science (Veterinary Medicine), Professor, and Zhorzh A. Shazhko, Doctor of Science (Veterinary Medicine), Professor; as well as Konstantin N. Gruzdev, Doctor of Science (Biology Medicine), Professor, Honored Veterinarian of the Russian Federation; Honored Scientists of the Russian Federation: Vasily L. Uzyumov, Doctor of Science (Veterinary Medicine), Professor, Anatoly M. Rakhmanov, Doctor of Science (Veterinary Medicine), Professor, Yuliy A. Chernyayev, Doctor of Science (Biology), Professor, Alexander N. Burdov, Doctor of Science (Veterinary Medicine), Professor; Honored Veterinarians of the Russian Federation – Taufik Z. Baibikov, Doctor of Science (Veterinary Medicine), Lyudmila A. Globenko, Doctor of Science (Biology), Boris A. Glushko, Candidate of Science (Veterinary Medicine), Yelena V. Guseva, Candidate of Science (Veterinary Medicine), Vladimir V. Dainatovich, Vyacheslav I. Diev, Doctor of Science (Veterinary Medicine), Nickolay N. Dryagalin, Candidate of Science (Veterinary Medicine), Valery M. Zakharov, Doctor of Science (Veterinary Medicine), Vasily N. Kuznetsov, Candidate of Science (Veterinary Medicine), Nina S. Maslova, Candidate of Science (Veterinary Medicine), Ivan A. Pronin, Candidate of Science (Veterinary Medicine), Alexander V. Borisov, Doctor of Science (Veterinary Medicine), Victor N. Gerasimov, Doctor of Science (Veterinary

Medicine); Honored Inventor of the Russian Federation Vladimir A. Mischenko, Doctor of Science (Veterinary Medicine), Professor [1, 7].

Advanced training courses were also carried out as a part of highly qualified staff-members' training. At the onset of the Institute's activities such advanced training was performed mainly in virology at the Institute of Virology named after D. I. Ivanovsky and then at Moscow State Veterinary Academy by K. I. Skryabin. Many Institute staff members received such advanced training that allowed arrangement of qualified virological works at the Institute.

In view of development of molecular biology research in the country, advanced training courses and special training courses in molecular biology were organized at the Moscow State University (Pushino) in 1977. Within the said courses leading researchers in this field, A. S. Spirin (currently, Academician of the Russian Academy of Sciences), T. I. Tikhonenko, Associate Member of the Russian Academy of Agricultural Sciences (currently, Associate Member of the Russian Academy of Sciences), and Ye. D. Sverdlov (currently, Academician of the Russian Academy of Sciences), etc. had delivered lecturers and held practical classes at the Institutions of the USSR Academy of Sciences for nine months of the said year. Sufficiently large number of the Institute stuff members received such advanced training and were granted certificates on molecular biology training, namely N. A. Perevozchikova, V. A. Perevozchikov, S. S. Rybakov, T. A. Satina, G. I. Kozhayeva, G. M. Falina, O. N. Okulova, etc., that enabled establishment of the Molecular Biology Department in the Institute in 1983 [1].

In the following years, many staff-members of the Institute underwent internships in leading laboratories of relevant national and foreign institutions to improve their skills.

Active participation of the Institution specialists in various national and international congresses, conferences, relevant meetings promoted experience and new knowledge gaining (Fig. 1, 2).

In 1976, the Institute was permitted by the Order of the USSR Higher Attestation Commission to accept candidate's theses for defense and conduct their defenses. The USSR Higher Attestation Commission approved the composition of the specialized Board for Candidate's Thesis defense in three specialties: 03.00.06 Virology, 16.00.02 Animal pathology, oncology and morphology, 16.00.03 Veterinary microbiology, virology, epizootiology, mycology and immunology [5, 8]. Professor Vladislav P. Onufriyev was approved as Chairman of the Board [3], Professor Vasily L. Uzyumov – as Vice-Chairman of the Board, Yuliy A. Chernyayev, Candidate of Science (Veterinary Medcine) – as Academic Secretary (Fig. 3). At the Institution, 131 theses were defended at the specialized Board for the Candidate's Thesis defense [2, 5, 7].

In 1996, the Higher Attestation Commission of the Russian Federation approved the Thesis Board that was eligible to accept Doctor Thesis and Candidate Thesis for their defense in the following specialties: 16.00.03 Veterinary microbiology, virology, epizootiology, mycology together with mycotoxicology and immunology and 03.00.06 Virology [6, 7].

Professor Anatoly A. Gusev was approved as Chairman of the Thesis Board and Professor Yuliy A. Chernyayev was approved as Vice-Chairman of the Thesis Board. In 1999, Professor Natalya A. Perevozchikova was approved as Vice-Chairman of the Thesis Board. The Academic Secretaries of the Thesis Board were: Professor Vasily L. Uzyumov, Doctor of Science (Veterinary Medicine), Galina M. Semenova, Candidate of Science (Biology),

Fig. 2. Member of the All-Union Academy of Agricultural Sciences named after V. I. Lenin (VASKhNIL), Hero of Socialist Labor, Professor, Doctor of Science (Veterinary Medicine) I. A. Bakulov at the All-Union Foot-and-Mouth Disease Research Institute, 1977 (a photo from the FGBI "ARRIAH" archive)

Fig. 3. The first meeting of the Thesis Council of the All-Union Foot-and-Mouth Disease Research Institute, 1976 (a photo from the FGBI "ARRIAH" archive)

Vladimir S. Russaleyev, Doctor of Science (Veterinary Medicine), Alexey P. Ponomaryev, Doctor of Science (Biology), Tatyana V. Zhbanova, Candidate of Science (Biology). In different years, Chairmen of the Thesis Board were: Valery M. Zakharov, Doctor of Science (Veterinary Medicine), Vladimir S. Russaleyev, Doctor of Science (Veterinary Medicine), Natalya A. Perevozchikova, Doctor of Science (Biology), Viktor N. Irza, Doctor of Science (Veterinary Medicine) [1, 6].

In different periods, the staff-members of the Institute having Doctor of Science degree worked on scientific projects, provided postgraduates with scientific guidance, many of them were members of the Thesis Board: V. P. Onufriyev, A. A. Syusyukin, Ye. Ye. Nikitin, Ye. V. Andreyev, A. P. Prostyakov, V. L. Uzyumov, A. M. Rahmanov, A. I. Sobko, A. N. Burdov, I. S. Kuchmasov, Yu. A. Chernyayev, V. N. Ivanyuschenkov, A. A. Gusev, A. I. Dudnikov, Zh. A. Shazhko, T. Z. Baibikov, A. V. Bocharnikov, N. A. Ulupov, A. A. Dorogovtsev, V. Ya. Davydov. M. V. Kotova, V. A. Mischenko, A. V. Borisov, V. V. Borisov, V. N. Gerasimov, N. S. Mamkov, V. I. Diev, L. A. Globenko, Sh. K. Kulyashbekova, S. S. Rybakov, A. F. Bondarenko, V. V. Drygin, A. P. Ponomaryev, L. L. Voeykov, A. M. Igonin, G. A. Khudyakov [4, 9].

Leading scientists of the FGBI "ARRIAH" take part in examination and attestation commissions, deliver lectures for postgraduates and veterinary specialists.

In the year of its 45th anniversary the FGBI "ARRIAH" Thesis Board was as follows: Viktor N. Irza (Chairman of the Board), Natalya A. Perevozchikova and Natalya S. Mudrak (Vice-Chairmen), Natalya N. Vlasova, Konstantin N. Gruzdev, Valery M. Zakharov, Natalya Ye. Kamalova, Artyom Ye. Metlin, Valery V. Mikhalishin, Vladimir A. Mischenko, Olga V. Pruntova, Vladimir S. Russaleyev. Seventeen Doctor Theses and 325 Candidate Theses were defended at the said Thesis Board [1, 10].

The FGBI "ARRIAH" leading experts were members of the USSR and RF Higher Attestation Commission: Professor Vasily L. Uzyumov (for 13 years); Professor Anatoly A. Gusev, Associate Member of the Russian Academy of Agricultural Sciences, Director; Professor Natalya A. Perevozchikova; Professor Sergey S. Rybakov; Professor Konstantin N. Gruzdev; Professor Vladimir A. Mischenko; Professor Anatoly M. Rakhmanov; Professor Vladimir S. Russaleyev (in different years).

The following Postgraduate School staff members provided postgraduate degree seekers with assistance in preparation of their Doctor Thesis and Candidate Thesis: Galina M. Semenova, Vasily L. Uzyumov, Valentina N. Guskova, Yelena V. Yelnikova – in 2000–2010, and Tatyana V. Zhbanova, Head of the Postgraduate School, from 2010 up to now. Professor Vladimir V. Makarov, Doctor of Science (Biology), provides great assistance in postgraduate students' training [1].

Doctors of Science from five scientific institutions are also being members of the Thesis Board of the FGBI "Federal Centre for Animal Health" (FGBI "ARRIAH"): Federal Research Center for Virology and Microbiology, Federal State Budgetary Scientific Institution All-Russian Research Institute for Veterinary Sanitation, Hygiene and Ecology, Moscow State University of Food Production, Peoples' Friendship University of Russia, Moscow State Academy of Veterinary Medicine and Biotechnology – MVA by K. I. Skryabin.

In addition to postgraduate training, there is a degree seeker-training programme at the FGBI "ARRIAH". Staff-members having research capabilities and skills can

become degree seekers. The Thesis Board approves their Thesis topics upon the recommendation of the Laboratory Head that are as a rule consistent with the field of the research performed in the Laboratory. The Thesis Board also makes a proposal on the approval of Scientific Adviser and determines the research period [7].

There are currently 890 staff-members including 15 Doctors of Science and 122 Candidates of Science in the FGBI "ARRIAH". The FGBI "ARRIAH" annually announces admission to the Postgraduate School, 8–12 post-graduate students per year. All postgraduate students work in the FGBI "ARRIAH" laboratories having modern facilities enabling research covered by their Thesis.

In 2016, the FGBI "ARRIAH" put in operation an apartment building for early-career scientists where postgraduate students are accommodated.

The Rosselkhoznadzor-subordinated FGBI "Federal Centre for Animal Health" (FGBI "ARRIAH") announces admission to the Postgraduate School for 2021–2026 for training in the following: 36.06.01 Veterinary Medicine and Animal Science according to the scientist specialty *06.02.02 Veterinary microbiology, virology, epizootiology, mycology together with mycotoxicology and immunology* and 06.06.01 Biological sciences according to the scientist specialty, *03.02.02 Virology,* and invites early-career specialists interested in research.

REFERENCES

1. Perevozchikova N. A., Lozovoy D. A., Rakhmanov A. M. Brief outline of the history and core activities of the FGBI "Federal Centre for Animal Health" (in commemoration of the 60th anniversary of the institution). Vladimir: FGBI "ARRIAH"; 2018. 120 p. eLIBRARY ID: 42993098. (in Russian)

2. Rakhmanov A. M., Uzyumov V. L. More on the history of establishment and activities of the All-Union Foot-and-Mouth Disease Research Institute [K istorii organizacii i deyatel'nosti Vsesoyuznogo nauchno-issledovatel'skogo yashchurnogo instituta]. *Veterinary Science Today*. 2013; 1 (4): 5–7. Available at: https://www.elibrary.ru/download/elibrary_22408254_78727395.pdf. (in Russian)

3. Rakhmanov A. M., Dudnikov A. I., Uzyumov V. I. Contribution of V. P. Onufriev to the strengthening and development of the All-Union Foot and Mouth Disease Research Institute (to the 80th birthday anniversary of V. P. Onufriev, 1925–1998). *Proceedings of the Federal Centre for Animal Health.* 2005; 3: 434–445. Available at: https://www.elibrary.ru/download/elibrary_24397212_81549508.pdf. (in Russian)

4. Rakhmanov A. M., Uzyumov V. L. History of the All-Union Foot-and-Mouth Disease Research Institute (1958–1992) and the ARRIAH (1992–2003). Vladimir: FGI "ARRIAH"; 2003. 152 p. (in Russian)

5. Uzyumov V. L., Semyonova G. M. Training of scientific personnel in the FGI "Federal Centre for Animal Health". *Proceedings of the Federal Centre for Animal Health.* 2008; 6: 28–34. Available at: https://www.elibrary.ru/download/elibrary_14933075_99888436.pdf. (in Russian)

6. Zakharov V. M., Perevozchikova N. A. At a turning point [Na krutom perelome]. *Veterinary Science Today*. 2013; 2 (5): 6–12. Available at: https://www.elibrary.ru/download/elibrary_22408261_62929055.pdf. (in Russian)

7. Belik Ye. V., Uzyumov V. L., Rakhmanov A. M., Borisov V. V. History of the FGI "Federal Centre for Animal Health" foundation and development. *Proceedings of the Federal Centre for Animal Health.* 2008; 6: 6–21. Available at: https://www.elibrary.ru/download/elibrary_14933073_52117135.pdf. (in Russian)

8. History of the Federal State-Financed Institution "Federal Centre for Animal Health". Ed. by Ye. V. Belik. Vladimir: IP Zhuravleva; 2008. 304 p. (in Russian)

9. Rakhmanov A. M., Glushko B. A., Uzyumov V. L. In commemoration of the 60th Anniversary of the Great Victory (employees of the institute, who took part in the Great Patriotic War of 1941–1945). *Proceedings of the Federal Centre for Animal Health.* 2005; 3: 430–433. Available at: https://www.elibrary.ru/download/elibrary_24397211_30180184.pdf. (in Russian)

10. Prokhvatilova L. B., Perevozchikova N. A., Rakhmanov A. M. Summary of scientific and manufacturing activities of the FGBI "ARRIAH" in 2013. *Veterinary Science Today*. 2014; 2 (9): 6–11. Available at: https://www.elibrary.ru/download/elibrary_22445529_95206887.pdf. (in Russian)

Received on 02.07.2021
Approved for publication on 30.07.2021

INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Tatyana V. Zhbanova, Candidate of Science (Biology), Head of Scientific Personnel Sector, Department of Education and Scientific and Methodical Work, Academic Secretary of Thesis Council, FGBI "ARRIAH", Vladimir, Russia.

Tatiana B. Nikeshina, Candidate of Science (Biology), Head of Department of Education and Scientific and Methodical Work, FGBI "ARRIAH", Vladimir, Russia.

Nataliya A. Perevozchikova, Doctor of Science (Biology), Professor, FGBI "ARRIAH", Vladimir, Russia.

Nadezhda A. Marova, Science Library staff member, FGBI "ARRIAH", Vladimir, Russia.

Жбанова Татьяна Валентиновна, кандидат биологических наук, заведующий сектором научных кадров отдела образования и научно-методической работы, ученый секретарь диссертационного совета ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Никешина Татьяна Борисовна, кандидат биологических наук, начальник отдела образования и научно-методической работы ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Перевозчикова Наталья Александровна, доктор биологических наук, профессор, ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Марова Надежда Алексеевна, сотрудник научной библиотеки ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

DOI: 10.29326/2304-196X-2021-3-38-184-189

Metabolic diseases in cattle

V. A. Mischenko[1], A. V. Mischenko[2], R. V. Yashin[3], V. A. Yevgrafova[4], T. B. Nikeshina[5]
FGBI "Federal Centre for Animal Health" (FGBI "ARRIAH"), Vladimir, Russia
[1] *ORCID 0000-0003-3751-2168, e-mail: mishenko@arriah.ru*
[2] *ORCID 0000-0002-9752-6337, e-mail: mischenko@arriah.ru*
[3] *ORCID 0000-0002-1385-705X, e-mail: yashin@arriah.ru*
[4] *ORCID 0000-0003-3053-6976, e-mail: evgrafova@arriah.ru*
[5] *ORCID 0000-0002-0959-5915, e-mail: nikeshina@arriah.ru*

SUMMARY
The main trend in the development of dairy farming in the Russian Federation suggests maximising milk yield and reducing milk net cost. The economic effectiveness of industrial dairy farming is largely determined by adequate feeding, as well as effective system of measures to ensure animal health and prevent infectious and non-infectious mass diseases. The main reason for the premature retirement of highly productive cows is based on the factors typical of the intensive technologies used in dairy cattle breeding, which lead to the occurrence of metabolic diseases. It is established that the intensity of metabolism is directly linked to the high productivity of cows. With a highly concentrated, mainly silage-based type of feeding, an imbalance of nutrients is often recorded, in particular as regards the sugar/protein ratio, leading to deep metabolic disorders and the development of immunodeficiency states. Metabolic disorders in highly productive cows occur as a result of unbalanced diets as far as protein, carbohydrates, vitamins and minerals are concerned. Acidosis, ruminitis and hepatosis are recorded in disordered cows and heifers. The service period exceeds 100 days in 70–75% of cows. Hepatosis and immunodeficiency states are often found in calves born to cows with signs of deep metabolic disorders. Metabolic disorders often remain unnoticed and become apparent only when pronounced pathological changes occur resulting in decreased productivity and ability to reproduce resistant young animals, as well as culling of animals. Metabolic diseases were recorded in 30–70% of cows examined in large dairy farms. The average lifetime productivity of high-yielding cows is (2.1 ± 0.15) lactations in Russia. The results of epidemiological investigations and laboratory testing of sera samples showed that emulsion inactivated vaccines administered to immunodeficient cattle induce higher titres of virus-specific antibodies than those in animals vaccinated with adsorbed vaccines.

Keywords: review, cattle, metabolic diseases, metabolic disorders, fiber, acidosis, liver dystrophy, biogeochemical zones, metabolic immunodeficiency, hypomicroelementoses, rumen, high-concentration feeding, emulsion inactivated antiviral vaccines, biogeochemical provinces

Acknowledgements: The study was funded by the FGBI "ARRIAH" within the framework of "Veterinary Welfare" research work.

For citation: Mischenko V. A., Mischenko A. V., Yashin R. V., Yevgrafova V. A., Nikeshina T. B. Metabolic diseases in cattle. *Veterinary Science Today*. 2021; 10 (3): 184–189. DOI: 10.29326/2304-196X-2021-3-38-184-189.

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

For correspondence: Vladimir A. Mischenko, Doctor of Science (Veterinary Medicine), Professor, Chief Researcher, Laboratory for Porcine and Horned Livestock Disease Prevention, FGBI "ARRIAH", 600901, Russia, Vladimir, Yur'evets, *e-mail: mishenko@arriah.ru.*

УДК 619:636.22/.28.084:616.391

Метаболические заболевания крупного рогатого скота

В. А. Мищенко[1], А. В. Мищенко[2], Р. В. Яшин[3], В. А. Евграфова[4], Т. Б. Никешина[5]
ФГБУ «Федеральный центр охраны здоровья животных» (ФГБУ «ВНИИЗЖ»), г. Владимир, Россия
[1] *ORCID 0000-0003-3751-2168, e-mail: mishenko@arriah.ru*
[2] *ORCID 0000-0002-9752-6337, e-mail: mischenko@arriah.ru*
[3] *ORCID 0000-0002-1385-705X, e-mail: yashin@arriah.ru*
[4] *ORCID 0000-0003-3053-6976, e-mail: evgrafova@arriah.ru*
[5] *ORCID 0000-0002-0959-5915, e-mail: nikeshina@arriah.ru*

РЕЗЮМЕ
Основная тенденция развития молочного животноводства в Российской Федерации предусматривает увеличение продуктивности коров молочных пород и снижение себестоимости молока. Экономическая эффективность промышленного молочного животноводства во многом определяется полноценным кормлением, эффективной системой мероприятий по обеспечению здоровья животных и профилактике инфекционных и массовых незаразных болезней. Главной причиной преждевременного выбытия высокопродуктивных коров являются факторы, присущие используемым в молочном скотоводстве интенсивным технологиям, приводящим к возникновению метаболических заболеваний. Установлено, что интенсивность обмена веществ имеет прямую

связь с высокой продуктивностью животных. При высококонцентратном, в основном силосно-концентратном, типе кормления часто регистрируется дисбаланс питательных веществ, особенно по сахаро-протеиновому отношению, что приводит к возникновению глубоких нарушений обмена веществ и развитию иммунодефицитных состояний. Метаболические нарушения у высокопродуктивных коров возникают на фоне рационов, несбалансированных по белку, углеводам, витаминам и минеральным веществам. У больных коров и нетелей регистрируются ацидоз, руминит и гепатоз. У 70–75% коров сервис-период превышает 100 дней. У телят, полученных от коров с признаками глубоких нарушений обмена веществ, часто регистрируется гепатоз и иммунодефицитное состояние. Нарушения обмена веществ часто остаются незамеченными и становятся очевидными лишь при ярко выраженных патологических изменениях, которые приводят к снижению продуктивности и способности воспроизведения резистентного молодняка, выбраковке животных. При обследовании коров в крупных молочных животноводческих хозяйствах метаболические заболевания были зарегистрированы у 30–70% животных. В России средняя продолжительность хозяйственного использования высокопродуктивных коров составляет (2,1 ± 0,15) лактаций. Как показали результаты эпизоотологических расследований и данные лабораторных исследований проб сывороток крови, при иммунизации крупного рогатого скота с иммунодефицитным состоянием эмульсионные инактивированные вакцины индуцируют в организме образование вирусспецифических антител в более высоких титрах, чем у животных, привитых сорбированными препаратами.

Ключевые слова: обзор, крупный рогатый скот, метаболические заболевания, нарушения обмена веществ, клетчатка, ацидоз, дистрофия печени, биогеохимические зоны, метаболический иммунодефицит, гипомикроэлементозы, рубец, высококонцентратное кормление, эмульсионные инактивированные противовирусные вакцины, биогеохимические провинции

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

Для цитирования: Мищенко В. А., Мищенко А. В., Яшин Р. В., Евграфова В. А., Никешина Т. Б. Метаболические заболевания крупного рогатого скота. *Ветеринария сегодня*. 2021; 10 (3): 184–189. DOI: 10.29326/2304-196X-2021-3-38-184-189.

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

Для корреспонденции: Мищенко Владимир Александрович, доктор ветеринарных наук, профессор, главный научный сотрудник лаборатории профилактики болезней свиней и рогатого скота ФГБУ «ВНИИЗЖ», 600901, Россия, г. Владимир, мкр. Юрьевец, *e-mail: mishenko@arriah.ru*.

The profitability of industrial dairy farming is ensured by three main factors: genetic potential, well-balanced feeding and freedom from infectious (including transboundary) and non-infectious mass diseases. The genetic potential of highly productive cows can be realized due to intensity of metabolic processes and rigorous neurohumoral regulation. The problem of metabolic disorders is particularly significant in modern industrial livestock production and resulting from one-sided selectional breeding aimed at productivity only [1–4]. Feeding diets are balanced in terms of all nutrients, the sugar-protein ratio and digestible protein content (100–110 g per feed unit) are strictly observed [3, 5–8]. Livestock performance improvement is one of the main factors contributing to a decrease in resistance level and resulting in a higher susceptibility of animals to infections.

The evolutionarily developed process of bovine digestion is aimed at digesting a large amount of fiber-based coarse feed needed for reproduction of cellulolytic bacteria that play an essential role in ruminal digestion [5, 9, 10]. To obtain high milk yields, a high-concentrate type of cow feeding is used. In many farms, the amount of coarse feed fed to cattle is 2.1–3.0 times lower than recommended. As a rule, there is a low content of easily digestible carbohydrates in such diets, leading to the sugar/protein imbalance [4–6, 11]. The poor-quality concentrated feed, even in large quantities, does not ensure synthesis of sufficient amounts of glucose.

In case of concentrate feeding, grain starch is utilized by the ruminal amylolytic bacteria for the synthesis of volatile fatty acids, including the basic lactic acid. At optimal ratios, lactic acid is processed by the rumen microflora into propionic acid, which serves as the main source for the synthesis of glucose and glycogen in the liver. With an excess of protein in the diet and a lack of carbohydrates, a large quantity of ammonia is produced in the rumen inhibiting the propionic acid synthesis [7, 8].

Acidification of the rumen contents (pH 5.5) leads to a significant excess of volatile fatty acids in blood and occurrence of metabolic acidosis, being the main pathogenetic mechanism of liver and kidney dystrophy, as well as other pathological disorders [4, 8, 10–22]. The transition of animals to concentrate feed leads to a shift in the rumen microflora [4, 5, 15, 23]. Feeding excessive amounts of concentrates inhibits the activity of ruminal microorganisms, which subsequently leads to liver fatty infiltration [1, 11, 24]. Rumen acidosis increases the reproduction of amylolytic and lactic acid bacteria, resulting in the suppression of the growth of propionic acid and cellulolytic microorganisms [12, 13, 25]. The highly concentrated type of feeding used, nutrient imbalance, stress, hypodynamia, lack of insolation are the basis for deep metabolic disorders and development of immunodeficiency states [3, 9, 11–13, 17, 21, 26].

The intensity of metabolism is directly linked to livestock productivity. Thus, a deficit of nutrient and plastic substances is continuously recorded in highly productive cows, which is compensated by the decomposition of substances in the organism. The energy demand of cows increases threefold during pregnancy and milk biosynthesis

in the first 2–3 weeks after calving [3, 4, 27]. These cows are able to transform the diet metabolic energy and feed nutrients into milk with a high coefficient, the costs of producing a unit of milk are low, the animals show high intensity metabolism, which leads to a decrease in their immunobiological status even with minor violations in feeding and maintenance. These animals have significantly reduced the ability to adapt to changing environmental conditions and protect themselves from various environmental effects [1, 6, 11, 13, 17, 23, 28, 29]. Therefore, the health of highly productive animals directly depends on the amount of trace elements within the body [4].

Metabolic disorders are the main pathogenetic mechanism of rumen metabolic acidosis and metabolic immunodeficiency in high-yielding cows that experience energy stress [12, 14, 17–21, 29, 30]. It is believed that one of the reasons for the development of metabolic acidosis is a lack of sugars in cows' diets. It was found that metabolic disorders in pregnant cows adversely affect the fetus in-utero development and the quality of colostrum. Dystrophy of the liver, kidneys, spleen and lymph nodes was observed in calves born to cows with signs of metabolic disorders [25, 31]. The observed increased activity of the enzymes most specific for liver cells (aspartate aminotransferase and alanine aminotransferase) occurred due to predominant catabolic processes in this organ as a result of dystrophic changes in its tissue [1, 7, 9–11, 24]. The results of an epizootological study of livestock farms indicate that metabolic disorders are constantly recorded in highly productive cows. The peak of such disorders occurs in animals in the first months after calving, and they vary for different breeds [9–12].

Unbalanced feeding most often affects cows of high-yielding breeds (Holstein-Friesian, etc.), that have an accelerated metabolism and a fine neurohumoral system. The reason for the fact that highly productive cattle are more likely to suffer from metabolic disorders than animals with average productivity is related to biological factors and depends on the rapid conversion of food energy into milk. This mechanism of milk synthesis requires high-quality feed, proper maintenance conditions and constant zootechnical control. The abundance of concentrates causes ruminal digestive disorders (hyperkeratosis and mucosis), liver dystrophy and loss of ovarian functions, as well as obesity and decreased productivity [3, 22, 24].

Newly calved heifers who are in demand of energy for milk synthesis and continuing growth are at risk. If there is a lack of easily digestible carbohydrates, the level of volatile fatty acids elevates in the rumen, while the concentration of butyric acid increases and the content of acetic and propionic acids decreases. If there is a lack of energy, there is little glucose and propionic acid in the animal's blood, which leads to ketogenic processes and hypoglycemia [27], which is often diagnosed with a predominantly concentrated type of feeding and the introduction of acidic feed into the diet of cows [6, 7, 14–16, 22, 32].

Liver dystrophy in highly productive cows is one of the most dangerous diseases, and, along with chronic microelementosis, it causes death [3, 7, 8, 12, 15, 22, 33]. In case of deficient or decreased supply of vital micro- and macronutrients with feed, chronic complex hypomicroelementosis manifested by a decrease in all types of productivity and leading to the development of secondary immunodeficiencies occurs in animals [12, 22, 33]. Animals with the deficiency or excess of such elements as cobalt, copper, zinc, calcium, demonstrate slowed rumination, loss or perversion of appetite, thickening of joints. If there is a lack of alkaline elements (calcium, sodium, magnesium, etc.) and an excessive content of acidic elements (chlorine, phosphorus, sulfur, etc.) in the feed, the acid-base balance of the blood shifts towards acidosis, which reduces the reserve alkalinity of the blood and general resistance [11, 16, 33].

The researchers of the Altai State Agrarian University studied characteristics of clinical and biochemical manifestations of metabolic disorders in highly productive cows in the biogeochemical province of their region. It was found that the soils in this area are deficient in iodine, cobalt, manganese, copper, zinc and molybdenum. The results of the conducted studies showed that metabolic diseases were detected in 30% of the examined animals, while metabolic disorders were recorded not only in cows, but also in calves born to them [33]. These deviations are especially acute in biochemical provinces, where there is a significant imbalance in the content of macro-and microelements in the 'soil–plant (feed)' chain [29, 33]. According to the results of long-term clinical observations and biochemical studies conducted in the farms of the Leningrad Oblast, metabolic disorders (metabolic diseases) are registered in 62% of cows with a milk yield of 25–35 kg per day in the first 2–3 months of lactation after calving [34].

The deficient content of mineral substances (copper, zinc, cobalt) in agricultural soils is the primary link of etiopathogenesis in the biogeocenotic chain and serves a leading factor in the development of mineral metabolism disorders in livestock animals. Microelementoses of alimentary origin are registered in the final link of the biogeocenosis chain at the 'mother – offspring' level, which leads to the development of an immunosuppressive state and secondary immunodeficiency [2, 4].

During epidemiological investigations conducted in specialized livestock farms for industrial milk production in different Subjects of the Russian Federation, it was found that the main cause of death of newborn calves was diarrhea, most often induced by rotavirus, coronavirus and bovine viral diarrhea virus. There were cases of two or three of these pathogens circulating in the same livestock population. It was documentarily confirmed that cows in late stage of pregnancy and heifers were vaccinated against these diseases in the surveyed farms. It is known that during the intrauterine fetus development there is no passive (transplacental) maternal antibody transfer, so the calf is born being unprotected from pathogens, and when it gets into a new environment, it does not have cellular and humoral specific protection. The only means of protecting a newborn calf is colostrum obtained from the immune dam [23, 32].

Metabolic disorders and immunodeficiencies in cows are one of the main reasons for the low effectiveness of vaccination for infectious disease prevention, which leads to premature retirement of animals [22, 32]. For determination of the reasons for the low effectiveness of vaccines used in the field, blood samples were taken from vaccinated cows and heifers 10–15 days prior to calving, and from 2–5-day-old calves born from immunized animals. Along with this, colostrum samples were taken on day 1–3 after calving.

The obtained study results served as the basis for the development of new means of specific prevention of viral diseases in immunodeficient cattle. Inactivated emulsion mono-, bi- and polyvalent vaccines against rotavirus and coronavirus infections, viral diarrhea, parainfluenza-3 and infectious bovine rhinotracheitis were developed at the FGBI "ARRIAH" for the immunization of animals with signs of metabolic immunodeficiency.

A number of production tests conducted in large industrial dairy complexes in 16 Subjects of the Russian Federation showed that the developed emulsion inactivated vaccines induce the formation of rotavirus-, coronavirus-, and viral diarrhea virus – specific antibodies in highly productive cows with signs of metabolic immunodeficiency. Antibodies to these pathogens were detected in cow colostrum at the titers of (11.3 ± 0.8–12.6 ± 0.9) $\log_2$. Antibodies to rotavirus, coronavirus and viral diarrhea causative agent at the titers of (8.5 ± 0.6–9.7 ± 0.8) $\log_2$ were detected in the sera of 3–7-day-old calves who, in a timely manner, received colostrum from cows immunized with an emulsion vaccine [32]. Emulsion vaccines are suitable for vaccination of newborn calves with colostral immunity. The use of emulsion inactivated vaccines against parainfluenza-3, infectious rhinotracheitis, coronavirus infection and bovine viral diarrhea in different combinations allowed to reduce the incidence rate in young animals and retirement level by 30–50% and 15–25%, respectively. The results of epidemiological investigations and laboratory testing of sera samples were the basis for the recommendation to use emulsion vaccines for immunization of immunodeficient cattle [9].

CONCLUSION

The results of epizootological investigations conducted for large-scale livestock farms specializing in milk production indicate that the average service life of highly productive cows with a milk yield of more than 6 thousand kg of milk does not comprise more than three lactations. An excess of concentrated feed, along with a shortage of sugar and coarse feed, leads to digestive and metabolic disorders and accumulation of toxic substances [3]. Intensive metabolic disorders constitute the main pathogenetic mechanism of metabolic diseases that lead to acidosis, liver dystrophy and metabolic immunodeficiency [3, 9, 10, 12, 13, 34]. Pregnant cows with impaired metabolic processes give birth to calves with dystrophy of liver, kidneys, spleen and lymph nodes. Diseases caused by opportunistic microorganisms are often identified in animals with signs of metabolic immunodeficiency. It has been shown that emulsion inactivated vaccines induce virus-specific antibodies in highly productive cows with signs of metabolic disorders in higher titers than those in animals vaccinated with adsorbed vaccines, which can be explained by the peculiarities of immunogenesis when using these vaccines.

REFERENCES

1. Zhukov I. V., Ushkova A. A. Analysis of biochemical status of cattle imported breeding. *Proceedings of the Voronezh State University of Engineering Technologies*. 2014; 4: 118–121. DOI: 10.20914/2310-1202-2014-4-118-121. (in Russian)

2. Kochnev N. N. Influence of technological factors on biochemical status in dairy cows. *Siberian Herald of Agricultural Science*. 2012; 2 (225): 39–45. eLIBRARY ID: 17729501. (in Russian)

3. Mischenko V. A., Turnaev S. N. Challenges of ensuring cow health in industrial animal husbandry: realities, causes and proposed solutions [Problemy obespecheniya zdorov'ya korov v promyshlennom zhivotnovodstve: realii, prichiny ih vyzyvayushchie i predlagaemye resheniya]. *Problems and Prospects of Scientific-Innovative Support of the Agro-Industrial Complex of Regions: Proceedings of the International Scientific and Practical Conference* (*September 8–9, 2020*). Kursk: FSBSI "Kursk Federal Agricultural Research Center"; 2020; 28–34. DOI: 10.18411/isbn978-5-907167-82-7. (in Russian)

4. Konvai V. D., Zabolotnykh M. V. Metabolic disorders in high yielding cows. *Vestnik Omsk SAU*. 2017; 3 (27): 130–136. eLIBRARY ID: 30467978. (in Russian)

5. Petryankin F. P., Lavrentyev A. Yu., Sherne V. S. The influence of feeding on immune status of organism of animals (scientific review). *Veterinarija sel'skohozjajstvennyh zhivotnyh*. 2018; 3: 41–46. eLIBRARY ID: 36759628. (in Russian)

6. Evglevsky Al. A., Turnaev S. N., Tarasov V. Yu., Lebedev A. F., Shvets O. M., Evglevskaya E. P. The health of highly productive cows in livestock industry and practical solution of the problem. *Vestnik of the Kursk State Agricultural Academy.* 2017; 4: 26–30. eLIBRARY ID: 29232144. (in Russian)

7. Mihailova I. I., Evglevskii Al. A., Evglevskaya E. P., Leschenko T. R., Mihailova O. N., Evglevskaya T. A. Prevention of metabolic acidosis in cows with silage-concentrate feeding. *Russian Veterinary Journal. Productive Animals*. 2017; 4: 5–7. eLIBRARY ID: 29188050. (in Russian)

8. Turnaev S. N., Evglevsky Al. A. The reasons for the disposal of highly productive cows on dairy complexes Kursk region: status, problems, solutions. *Vestnik of the Kursk State Agricultural Academy.* 2014; 9: 67–69. eLIBRARY ID: 22978642. (in Russian)

9. Shevkoplyas V. N., Chernykh O. Yu., Terekhov V. I., Limarenko A. A., Mischenko V. A. Measures to prevent retirement of highly productive cows in agricultural establishments of the Krasnodar Krai [Meropriyatiya po preduprezhdeniyu vybytiya vysokoproduktivnyh korov v sel'skohozyajstvennyh predpriyatiyah Krasnodarskogo kraya]: Methodological Recommendations. Krasnodar; 2010. 55 p. (in Russian)

10. Konvay V. D., Zaynchkovsky V. I., Skachkov D. V., Orzhekhovsky S. A. Mechanisms of development of metabolic violations at highly productive cows. *Vestnik Omsk SAU*. 2013; 1 (9): 59–62. eLIBRARY ID: 22507279. (in Russian)

11. Mishchenko V. A., Mishchenko A. V., Dumova V. V., Ermilov I. V., Yakubenko E. V., Chernykh O. Yu. Dysbolic analysis of high milk yield cows. *Veterinaria Kubani*. 2012; 6: 15–17. eLIBRARY ID: 18425045. (in Russian)

12. Evglevsky A. A., Skhira V. N., Evglevskaya E. P., Vanina N. V., Mikhailova I. I., Suleimanova T. A., Pereverzeva J. A. Metabolic acidosis of high-yielding cows: causes, consequences, prophylaxes. *Veterinariya*. 2017; 5: 45–48. eLIBRARY ID: 29155246. (in Russian)

13. Mischenko V. A., Mischenko A. V., Gladilin G. V. Metabolic immunodeficiency in high yielding cattle [Metabolicheskij immunodeficit u vysokoproduktivnogo krupnogo

rogatogo skota]. *Problems and Prospects of Scientific-Innovative Support of the Agro-Industrial Complex of Regions: Proceedings of the International Scientific Conference* (September 11–13, 2019). Kursk: FSBSI "Kursk Federal Agricultural Research Center"; 2019; 614–618. Available at: https://www.elibrary.ru/download/elibrary_41155736_17371499.pdf. (in Russian)

14. Ryzhkova G. F., Evglevsky A. A., Evglevskaya E. P., Minenkov N. A. The redistribution of electrolytes between the erythrocytes and plasma of cows blood at the violation of acid-base balance (acidosis of the rumen). *Vestnik of the Kursk State Agricultural Academy.* 2018; 4: 136–139. eLIBRARY ID: 35017281. (in Russian)

15. Batrakov A. Ia., Vasiliev R. M., Donskaya T. K., Vasilieva S. V. The indices of metabolism in highly productive cows. *Veterinariya*. 2012; 6: 49–52. eLIBRARY ID: 17869654. (in Russian)

16. Chernykh O. Yu., Koshchaev A. G., Lysenko A. A., Lagutin D. V., Krivonos R. A., Kaloshkina I. M., Mischenko A. V. Problem of biological safety of flocks of cattle of dairy breeds in the Russian Federation. *Veterinaria Kubani*. 2018; 4: 4–7. eLIBRARY ID: 35611327. (in Russian)

17. Derezina T. N., Ushakova T. M. Biogeochemical aspects of etiopatogenetion characteristics of microelementoses in cattle in the system "mother–offspring". *Materials of the International Scientific and Practical Conference Dedicated to the 100th Anniversary of the Honored Scientist of the RSFSR, Doctor of Science* (*Veterinary Medicine*), *Professor Andrey A. Kabysh* (*May 19, 2017*). Troitsk: South Ural State Agrarian University; 2017; 114–120. Available at: https://sursau.ru/upload/iblock/654/konf_100kabysh.pdf. (in Russian)

18. Evglevskij A. A., Eryzhenskaya N. F., Skira V. N., Evglevskaya E. P., Ryzhkova G. F., Mikhailova I. I. Energy deficiency of the newly calved cows: problems and solutions. *Vestnik of the Russian Agricultural Sciences*. 2017; 4: 61–63. eLIBRARY ID: 30770207. (in Russian)

19. Evglevskaya E. P., Evglevsky A. A. Innovative developments for the prevention of economically important diseases of highly productive animals in industrial farming. *Vestnik of the Kursk State Agricultural Academy.* 2019; 1: 53–57. eLIBRARY ID: 37107175. (in Russian)

20. Mikhailova I. I., Evglevskaya E. P., Mikhailova O. N., Mikhaleva T. I., Leshchenko T. R. Pathobiochemical changes in the metabolic status of high yielding cows. *Veterinarnaya patologiya*. 2016; 1 (55): 75–80. eLIBRARY ID: 26210931. (in Russian)

21. Evglevsky A. A., Evglevskaya E. P., Mikhaleva T. I., Mikhailova O. N. Immunometabolic activity of the drug based on succinic acid and levamizole [Immunometabolicheskaya aktivnost' preparata na osnove yantarnoj kisloty i levamizola]. *Vestnik of the Kursk State Agricultural Academy.* 2013; 1: 64–65. eLIBRARY ID: 20777355. (in Russian)

22. Mishchenko V. A., Mishchenko A. V., Chernykh O. Yu. Liver pathology problem in high yielding cows. *Veterinaria Kubani*. 2014; 2: 11–12. eLIBRARY ID: 21545869. (in Russian)

23. Golodyaeva M. S., Batracov A. Ya., Videnin V. N., Yashin A. V. The effect of feeding ration on the bio-chemical status and morbidity of heifers and highly productive cows. *International Bulletin of Veterinary Medicine.* 2019; 3: 86–91. eLIBRARY ID: 41138493. (in Russian)

24. Alyokhin Yu. N. Illnesses of a liver at cows with high production of milk (diagnostics, prophylaxis and therapy). *Veterinariya*. 2011; 6: 3–7. eLIBRARY ID: 16443801. (in Russian)

25. Barinov N. D., Kalyuzhniy I. I. Dependence of the immune system on energy metabolism in calves during the colostral period. *Materials of the International Scientific and Practical Conference Dedicated to the 100th Anniversary of the Honored Scientist of the RSFSR, Doctor of Science* (*Veterinary Medicine*), *Professor Andrey A. Kabysh* (*May 19, 2017*). Troitsk: South Ural State Agrarian University; 2017; 21–27. Available at: https://sursau.ru/upload/iblock/654/konf_100kabysh.pdf. (in Russian)

26. Nechaev A. V., Minyuk L. A., Grishina D. Y. Prevention of metabolic diseases of highly-productive cows. *Vestnik Ulyanovsk of State Agricultural Academy.* 2017; 2 (38): 143–147. DOI: 10.18286/1816-4501-2017-2-143-147. (in Russian)

27. Vanina N. V., Evglevskaya E. P., Yerizhenskaya N. F., Evglevsky A. A. Deficiency of energy in high-productive cows – problems and practical solutions. *Integration of Science and Agricultural Production* [*Integraciya nauki i sel'skohozyajstvennogo proizvodstva*]: *Proceedings of the International Scientific and Practical Conference* (*February 16–17, 2017*). Kursk: Kursk State Agricultural Academy; 2017; 299–304. eLIBRARY ID: 29249956. (in Russian)

28. Derezina T. N., Ushakova T. M. Dynamics of parameters of non-specific cramps-santoshi have cattle in the system "mother-offspring" against the background of deficit of vital trace elements. *Materials of the International Scientific and Practical Conference Dedicated to the 100th Anniversary of the Honored Scientist of the RSFSR, Doctor of Science* (*Veterinary Medicine*), *Professor Andrey A. Kabysh* (*May 19, 2017*). Troitsk: South Ural State Agrarian University; 2017; 120–127. Available at: https://sursau.ru/upload/iblock/654/konf_100kabysh.pdf. (in Russian)

29. Mischenko A. V., Mischenko V. A. Factors, which influence the development of post vaccination immunity in livestock. *Veterinariya*. 2020; 11: 3–6. DOI: 10.30896/0042-4846.2020.23.11.03-06. (in Russian)

30. Il E. N., Zabolotnykh M. V. Identification of material exchange disorders high-product cows. *Vestnik of the Kursk State Agricultural Academy.* 2019; 2: 83–89. eLIBRARY ID: 37209181. (in Russian)

31. Leybova V. B., Shapiev I. Sh., Turlova J. V. Biochemical composition of blood in early postpartum and their relationship to reproduction in black and white cows with different milk production. *Dairy and Beef Cattle Farming*. 2014; 6: 32–34. eLIBRARY ID: 22134097. (in Russian)

32. Mischenko V. A., Mischenko A. V., Yashin R. V., Gladilin G. V. Aspects of animal vaccination: known and unknown [Problema vakcinoprofilaktiki zhivotnyh: izvestnoe i neizvestnoe]. *Problems and Prospects of Scientific-Innovative Support of the Agro-Industrial Complex of Regions: Proceedings of the International Scientific and Practical Conference* (*September 8–9, 2020*). Kursk: FSBSI "Kursk Federal Agricultural Research Center"; 2020; 203–208. DOI: 10.18411/isbn978-5-907167-82-7. (in Russian)

33. Trebukhov A. V. The features of metabolic disorders in highly productive cows in the biogeochemical province of the Altai Region. *Bulletin of Altai State Agricultural University*. 2018; 8 (166): 95–99. eLIBRARY ID: 36496985. (in Russian)

34. Batrakov A. Ya., Yashin A. V., Donskaya T. K., Vinnikova S. V. Metabolic processes in high-productive cows their

prophylaxis. *Materials of the International Scientific and Practical Conference Dedicated to the 100th Anniversary of the Honored Scientist of the RSFSR, Doctor of Science* (*Veterinary Medicine*), *Professor Andrey A. Kabysh* (*May 19, 2017*). Troitsk: South Ural State Agrarian University; 2017; 28–34. Available at: https://sursau.ru/upload/iblock/654/konf_100kabysh.pdf. (in Russian)

Received on 09.06.2021
Approved for publication on 19.07.2021

INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Vladimir A. Mischenko, Doctor of Science (Veterinary Medicine), Professor, Chief Researcher, Laboratory for Porcine and Horned Livestock Disease Prevention, FGBI "ARRIAH", Vladimir, Russia.

Alexey V. Mischenko, Doctor of Science (Veterinary Medicine), Senior Researcher, Information and Analysis Centre, FGBI "ARRIAH", Vladimir, Russia.

Roman V. Yashin, Candidate of Science (Biology), Head of Laboratory for Porcine and Horned Livestock Disease Prevention, FGBI "ARRIAH", Vladimir, Russia.

Valeria A. Yevgrafova, Candidate of Science (Veterinary Medicine), Head of Sector, Laboratory for Porcine and Horned Livestock Disease Prevention, FGBI "ARRIAH", Vladimir, Russia.

Tatiana B. Nikeshina, Candidate of Science (Biology), Head of Department of Education and Scientific and Methodical Work, FGBI "ARRIAH", Vladimir, Russia.

Мищенко Владимир Александрович, доктор ветеринарных наук, профессор, главный научный сотрудник лаборатории профилактики болезней свиней и рогатого скота ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Мищенко Алексей Владимирович, доктор ветеринарных наук, старший научный сотрудник информационно-аналитического центра ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Яшин Роман Владимирович, кандидат биологических наук, заведующий лабораторией профилактики болезней свиней и рогатого скота ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Евграфова Валерия Андреевна, кандидат ветеринарных наук, заведующий сектором лаборатории профилактики болезней свиней и рогатого скота ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Никешина Татьяна Борисовна, кандидат биологических наук, начальник отдела образования и научно-методической работы ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

DOI: 10.29326/2304-196X-2021-3-38-190-196

Role of acute respiratory diseases in pathogenesis of distal limb infections in cattle

A. D. Alekseev[1], O. G. Petrova[2], M. I. Barashkin[3], I. M. Milshtein[4], V. D. Moskvin[5]
FSBEI HE "Urals State Agrarian University" (FSBEI HE Ural SAU), Ekaterinburg, Russia
[1] *ORCID 0000-0002-0418-4498, e-mail: alekseev.urgau@mail.ru*
[2] *ORCID 0000-0003-3105-1751, e-mail: super.kafedra2013@yandex.ru*
[3] *ORCID 0000-0002-8865-3027, e-mail: dekanatvet@yandex.com*
[4] *ORCID 0000-0001-6293-718X, e-mail: 4u4@bk.ru*
[5] *ORCID 0000-0002-0450-6987, e-mail: vsloth@mail.ru*

SUMMARY
According to current concepts, ruminal and metabolic acidosis occur due to feeding cattle mainly with preserved acidic feeds such as silage and haylage. However, errors in feeding are not the only etiological factor leading to acidosis. In some cases, metabolic acidosis in cattle can develop along with respiratory infection caused by viral and bacterial agents. The main pathological processes resulting from acute respiratory diseases of cattle are bronchitises, tracheites and pneumonias. When the respiratory tract is affected in cattle, hypoxia occurs, causing intoxication and, thus, leading to ruminal acidosis. As a result, vasoactive substances (bacterial endotoxins, histamine, lactate) enter the bloodstream, the vascular endothelium is damaged due to the simultaneous expansion of arterioles and compression of venules, blood fluid is perfused from the vessels into the surrounding tissues, the blood flow in the microcirculatory bed is disrupted. An important role in the disturbance of blood circulation in small blood vessels is played by circulating immune complexes representing the «antigen-antibody» complex. Low molecular weight circulating immune complexes settle in various organs and tissues of the body, lead to inflammation and damage the normal tissue structure. Most frequently, immune complexes affect the endothelium of blood vessels, renal glomeruli and joints. Distal limb vessels are primarily affected in cattle, leading to disturbance of skin trophism of the limbs and hooves, development of laminitis, while the hoof horn is weakly keratinized and cannot resist aggressive mechanical and chemical environmental factors. Damaged hooves are the gateway of infection for the agents of necrobacteriosis (*Fusobacterium necrophorum*), staphylococcosis (*Staphylococcus* spp.), streptococcosis (*Streptococcus* spp.) and other pathogens. In addition, favorable conditions evolve for the development of mixed infection due to reduction in the overall organism resistance, which is observed for both respiratory and distal limb infections.

Keywords: acute respiratory diseases of cattle, distal limb infections of cattle, necrobacteriosis, staphylococcosis, streptococcosis, pasteurellosis, bovine infectious rhinotracheitis, bovine viral diarrhea, bovine parainfluenza-3, bovine respiratory syncytial infection

Acknowledgement: The research was funded through the commercial contracts in the framework of the Program "Improvement of the system of animal infectious disease prevention".

For citations: Alekseev A. D., Petrova O. G., Barashkin M. I., Milshtein I. M., Moskvin V. D. Role of acute respiratory diseases in pathogenesis of distal limb infections in cattle. *Veterinary Science Today.* 2021; 10 (3): 190–196. DOI: 10.29326/2304-196X-2021-3-38-190-196.

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

For correspondence: Olga G. Petrova, Doctor of Science (Veterinary Medicine), Professor, Professor of the Department of Infectious and Non-Infectious Pathology, FSBEI HE "Urals State Agrarian University", 620075, Russia, Ekaterinburg, K. Liebknecht, 42, *e-mail: super.kafedra2013@yandex.ru*.

УДК 619:616.988:636.22/.28

Роль острых респираторных заболеваний в патогенезе инфекций дистального отдела конечностей крупного рогатого скота

А. Д. Алексеев[1], О. Г. Петрова[2], М. И. Барашкин[3], И. М. Мильштейн[4], В. Д. Москвин[5]
ФГБОУ ВО «Уральский государственный аграрный университет» (ФГБОУ ВО Уральский ГАУ), г. Екатеринбург, Россия
[1] *ORCID 0000-0002-0418-4498, e-mail: alekseev.urgau@mail.ru*
[2] *ORCID 0000-0003-3105-1751, e-mail: super.kafedra2013@yandex.ru*
[3] *ORCID 0000-0002-8865-3027, e-mail: dekanatvet@yandex.com*
[4] *ORCID 0000-0001-6293-718X, e-mail: 4u4@bk.ru*
[5] *ORCID 0000-0002-0450-6987, e-mail: vsloth@mail.ru*

РЕЗЮМЕ

По современным представлениям ацидоз рубца и метаболический ацидоз являются следствием кормления крупного рогатого скота преимущественно консервированными кислыми кормами, такими как силос и сенаж. Вместе с тем погрешности в кормлении не единственный этиологический фактор, приводящий к ацидозу. В ряде случаев у крупного рогатого скота метаболический ацидоз может развиваться на фоне респираторной патологии, вызываемой вирусными и бактериальными агентами. Основными патологическими процессами, вызываемыми острыми респираторными заболеваниями крупного рогатого скота, являются бронхиты, трахеиты и пневмонии. При поражении респираторного тракта в организме животных возникает гипоксия, что ведет к развитию эндогенной интоксикации, приводящей к ацидозу рубца, в результате чего в кровь поступают сосудисто-активные вещества (эндотоксины бактерий, гистамин, лактат), за счет одновременного расширения артериол и сжатия венул повреждается эндотелий сосудов, наблюдается перфузия из сосудов в окружающие ткани жидкости крови, нарушается кровоток в микроциркуляторном русле. Немаловажную роль в нарушении циркуляции крови в мелких кровеносных сосудах играют циркулирующие иммунные комплексы, представляющие собой комплекс антиген – антитело. Низкомолекулярные циркулирующие иммунные комплексы, оседая в разнообразных органах и тканях организма, приводят к воспалению и повреждают нормальную структуру тканей. Наиболее часто иммунные комплексы поражают эндотелий кровеносных сосудов, почечные клубочки и суставы. У крупного рогатого скота в первую очередь поражаются сосуды дистального отдела конечностей, что ведет к нарушению трофики кожи конечностей и копытец, развивается ламинит, при этом копытный рог слабо кератинизирован и не может противостоять агрессивным механическим и химическим факторам внешней среды. Поврежденные копытца являются воротами инфекции для возбудителей некробактериоза (*Fusobacterium necrophorum*), стафилококкоза (*Staphylococcus* spp.), стрептококкоза (*Streptococcus* spp.) и других патогенов. Кроме того, благоприятные условия для развития микст-инфекции создаются за счет снижения общей резистентности организма, что отмечается как при респираторной патологии, так и при патологии дистального отдела конечностей.

Ключевые слова: острые респираторные заболевания крупного рогатого скота, инфекции дистального отдела конечностей крупного рогатого скота, некробактериоз, стафилококкоз, стрептококкоз, пастереллез, инфекционный ринотрахеит КРС, вирусная диарея КРС, парагрипп-3 КРС, респираторно-синцитиальная инфекция КРС

Благодарность: Финансирование работы проводилось за счет хозяйственных договоров в рамках программы «Усовершенствование системы профилактики инфекционных болезней животных».

Для цитирования: Алексеев А. Д., Петрова О. Г., Барашкин М. И., Мильштейн И. М., Москвин В. Д. Роль острых респираторных заболеваний в патогенезе инфекций дистального отдела конечностей крупного рогатого скота. *Ветеринария сегодня*. 2021; 10 (3): 190–196. DOI: 10.29326/2304-196X-2021-3-38-190-196.

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

Для корреспонденции: Петрова Ольга Григорьевна, доктор ветеринарных наук, профессор, профессор кафедры инфекционной и незаразной патологии ФГБОУ ВО «Уральский государственный аграрный университет», 620075, Россия, г. Екатеринбург, ул. Карла Либкнехта, 42, *e-mail: super.kafedra2013@yandex.ru.*

INTRODUCTION

Bovine extremity diseases under the conditions of intensive animal farming are quite common and sometimes become widespread. Their main causes are gross violations of animal feeding and keeping, as well as infectious respiratory diseases.

According to our observations, 25.4% of cases of respiratory pathology in cattle are associated with infections of the distal extremities, primarily with necrobacteriosis, streptococcosis and staphylococcosis, which cause economic losses due to reduced performance and culling up to 30% of highly productive animals [1, 2].

One of the factors influencing the development of distal extremity infections in cattle is acute respiratory infections, such as infectious bovine rhinotracheitis (IBR), bovine viral diarrhea (BVD), bovine parainfluenza-3 (BPI-3), bovine respiratory syncytial virus (BRSV) infection, pasteurellosis, chlamydiosis and salmonellosis. Farms, which reported necrobacteriosis outbreaks were infected with bovine respiratory infections.

The aim of the research was the theoretical and practical substantiation of the pathogenic role of acute respiratory disease in infections of bovine distal extremities.

MATERIALS AND METHODS

The research was conducted at the Department of Infectious and Non-Infectious Pathology of the Ural State Agrarian University and farms of the Ural Region in the period from 2014 to 2021. Epizootological, pathomorphological, immunological and bacteriological research methods were used in the work.

The epidemic situation related to acute respiratory diseases was assessed by the statistical method; the annual reports of the Information and Analytical Center of the Rosselkhoznadzor Veterinary Surveillance Department (FGBI "Federal Center for Animal Health"), the BI Udmurt Veterinary Diagnostic Center, the FSBI "Chelyabinsk Interoblast Veterinary Laboratory", the GBI "Sverdlovsk Oblast Veterinary Laboratory" were studied.

The objects of the study were commercially raised cattle (tethered), blood, serum, hoof scrapes as well as pathological material collected from a 14-day-old calf killed due to pneumonia signs (pieces of lung and bronchial lymph nodes).

The pathological material from the killed calf was fixed in a 10% neutral buffered formalin, embedded in paraffin and histological preparations were prepared according to the generally accepted method. The sections were stained with hematoxylin and eosin, as well as using Van Gieson method, then examined by light microscopy according to the generally accepted method.

The blood of cows and calves was used for immunological studies. The amount of circulating immune complexes (CICs) in the serum was calculated by immunoprecipitation in 4% polyethylene glycol (PEG-6000), followed by photometry using SF-2000 spectrophotometer (OKB Spectrum LLC, Russia).

Bacteriological studies were carried out according to generally accepted methods.

The digital data of epizootological and laboratory studies are processed using mathematical statistics methods adopted in biology and medicine. The reliability of the results was determined by statistical processing using the Student's paired *t*-test. The results were considered reliable at $p \leq 0.05$.

The obtained statistical and experimental data were processed using the Microsoft Excel software, which is included in the Microsoft Office package.

RESULTS AND DISCUSSION

Acute respiratory diseases (ARDs) in the bovine infectious pathology in the Ural region rank second after infectious gastrointestinal diseases [1]. The infectious respiratory diseases are mostly spread in animals on the farms of the Udmurt Republic, where the following diseases take a lead in the nosological picture of infectious respiratory pathologies by the number of infected sites: pasteurellosis – 41.5%, BPI-3 – 16.7%, chlamydiosis – 14.1%, BVD – 11.8%, IBR – 10.6%, BRSV infection – 5.3% [2, 3].

In the livestock farms of the Chelyabinsk Oblast, the following diseases were registered: pasteurellosis – 46.3%, IBR – 21.9%, BVD – 12.3%, chlamydiosis – 7.3%, BPI-3 – 7.3%, BRSV infection – 4.9%. In the Sverdlovsk Oblast, pasteurellosis – 63.7%, BPI-3 – 14.9%, BVD – 8.5%, IBR – 7.4%, chlamydiosis – 5.5% were detected on cattle farms (Table 1).

When assessing the BRSV infection prevalence on farms of the Ural Economic region, it was found that among all bovine infectious respiratory pathologies, the frequency of this disease detection in the Udmurt Republic is 5.3%, in the Chelyabinsk Oblast is 4.9%. In the Sverdlovsk Oblast, according to the data of the GBI "Sverdlovsk Regional Veterinary Laboratory", BRSV infection was not reported [4, 5].

Serological, molecular biological, microbiological and immunological tests suggest a great involvement of pathogens of different taxonomic groups in respiratory pathology. The most frequently detected associations of BRSV infection are with IBR, pasteurellosis and other infectious agents (Tables 2, 3).

In large dairy farms, the detection of BRSV antibodies in cattle depended on the level of the infection with IBRV and pasteurellosis agent, as well as the presence in herds of individuals latently infected with bovine respiratory syncytial virus. Herewith, the level of infection of animals with the IBRV ranged from 10.6 to 21.9%, with pasteurellosis – from 41.5 to 46.3% [5].

No differences in the geographical spread of the disease among cattle of different ages were established. The greatest number of positive serological reactions to IBR, BVD, BRSV viruses and wound infections of cattle distal extremities were detected in large dairy farms with a herd high density and milk yield. The infection rate of cattle in medium and small livestock farms was lower, but the higher the milk yield was, the higher was the risk of the above mentioned respiratory diseases occurring. The spread of diseases was facilitated by the crowding of animals and the presence of latent virus carriers in the herd [4].

The results of various studies have proved that the range of infectious agents that cause infectious respiratory diseases and wound infections of bovine distal extremities is quite wide. Infections of bacterial etiology are often secondary, but can be concomitant or independent. This depends on the animal density in facilities, the presence or absence of specific vaccination against viral and bacterial infections, as well as on-farm factors. In this regard, when planning anti-epidemic measures, it is essential to conduct a full range of laboratory diagnostic tests (virological, bacteriological) in order to determine the nosological structure of a concrete outbreak of respiratory infections and the etiological role of each infectious pathogen.

Table 1
Rate of acute respiratory infections in cattle at livestock establishments

Disease	Udmurt Republic, %	Chelyabinsk Oblast, %	Sverdlovsk Oblast, %
Pasteurellosis	41.5	46.3	63.7
Chlamydiosis	14.1	7.3	5.5
BPI-3	16.7	7.3	14.9
BVD	11.8	12.3	8.5
IBR	10.6	21.9	7.4
BRSV infection	5.3	4.9	–

The difference is reliable $p \leq 0.05$

Table 2
Viral and bacterial associations of BRSV in livestock establishments of the Republic of Udmurtia

Viral-bacterial associations BRSV in the Udmurt Republic	As % of the total ARD affected farms, %
BRSV infection + pasteurellosis + chlamydiosis	1.8
BRSV infection + IBR + pasteurellosis	1.6
BRSV infection + pasteurellosis	1.6
BRSV infection + IBR + BVD + pasteurellosis	1.5
BRSV infection + IBR + pasteurellosis + chlamydiosis	1.4
BRSV infection + IBR + BVD + pasteurellosis + chlamydiosis	0.8
BRSV infection + IBR + BVD + chlamydiosis	0.8
BRSV infection + IBR + BVD	1.5
BRSV infection + BVD + pasteurellosis	0.5
BRSV infection + IBR + BPI-3 + pasteurellosis	0.4

The difference is reliable $p \leq 0.05$.

Histological examination of the pathological material (lung and bronchial lymph nodes), collected in a livestock farm of the Chelyabinsk Oblast from a 14-day-old calf killed due to signs of respiratory disease, revealed signs of pneumonia characteristic of pasteurellosis (Fig. 1, 2), syncytia were found in the lung tissues, which is consistent with BRSV infection (Fig. 3, 4) [3–5].

Based on the results of the histological examination, it was concluded that the nature of the processes detected in the tested preparations corresponds to an associated viral-bacterial infection (BRSV infection + pasteurellosis) [3–5]. This diagnosis was confirmed by laboratory research methods (materials are not covered by this paper).

In addition to respiratory infections, an infectious pathology of the distal extremities was registered in the animals on the farm where the studies were conducted. The bacteriological test of hoof scrapings revealed the causative agent of necrobacteriosis – *Fusobacterium necrophorum*.

Thus, in this farm, a mixed infection was registered – acute infectious respiratory diseases in association with wound infections of the distal extremities (BRSV infection + pasteurellosis + necrobacteriosis).

At the next stage of the work, the concentration of CICs was calculated in the blood of pregnant cows two months before calving and of calves aged 1 month (Table 4). As it can be seen, the concentration of CICs in cows and calves was higher than the physiological norm, which is a sign of their insufficient elimination out of the body. This increases the risk of deposition of these complexes in normal tissues. An important pathogenetic factor in the development of wound infections of the bovine distal extremities in some cases are acute respiratory infections.

According to modern concepts, rumen acidosis and, as a consequence, metabolic acidosis are the result of feeding cattle mainly with preserved acidic feeds, such as silage and haylage [6–10]. Cellulolytic bacteria (*Ruminococcus*, etc.), as well as fungi of the *Neocallimastigaceae* family are sensitive to the pH of the environment: the acidic environment inhibits their growth, and therefore the cellulase activity of the rumen contents decreases. The bovine gastrointestinal tract stops digesting fiber, the digestibility of the diet decreases sharply. The acidic environment also suppresses the growth of *Streptococcus bovis* bacteria, which are representatives of the rumen microbiome, participate in the breakdown of pectin, protein and starch and thereby contribute to the acidification of the environment. *Streptococcus bovis* is replaced by lactic acid bacteria of the *Lactobacillus* genus, which are more resistant to acidic environments. Such conditions are favorable for the reproduction of *Fusobacterium necrophorum* bacteria in the rumen, which can enter the bloodstream, as well as of other microorganisms that can produce toxins.

Table 3
Viral and bacterial associations of BRSV in livestock establishments of the Chelyabinsk Oblast

Viral-bacterial associations BRSV in the Chelyabinsk Oblast	As % of the total ARD affected farms, %
BRSV infection + IBR + pasteurellosis	4.9
BRSV infection + IBR + BVD + BPI-3 + pasteurellosis + chlamydiosis	2.4

The difference is reliable $p \leq 0.05$.

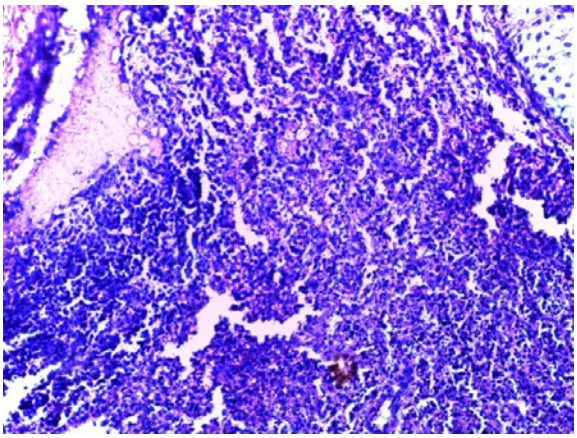


Fig. 1. Foci of purulent pneumonia (hematoxylin – eosin staining, magnification 200×)

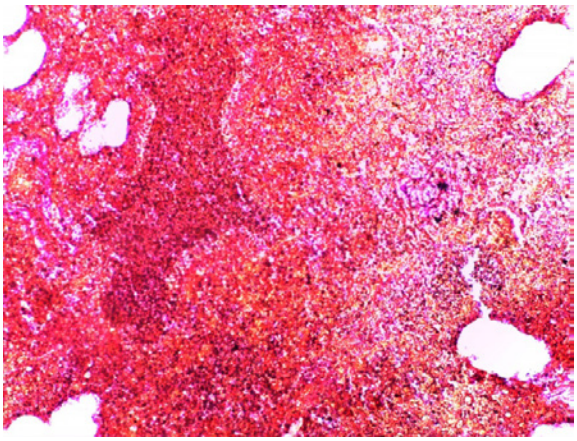


Fig. 2. Focal hemorrhagic pneumonia (staining according to Van Gieson, magnification 200×)

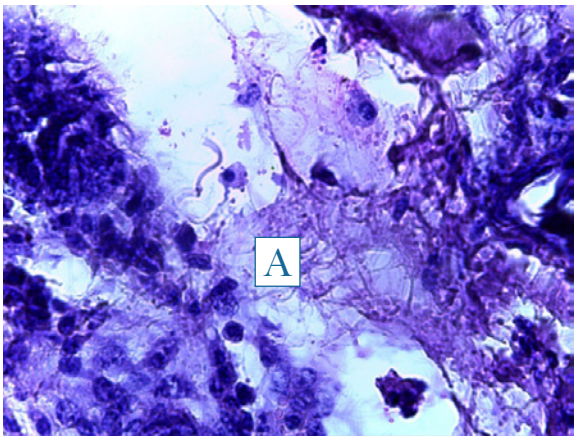


Fig. 3. Macrophages in the lung and syncytia (A), characteristic of respiratory syncytial infection (hematoxylin – eosin staining, magnification 630×)

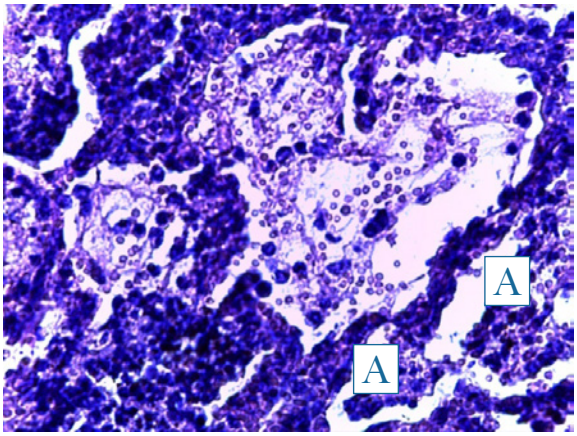


Fig. 4. Macrophages, erythrocytes and syncytia (A) in the lumen of the alveoli, typical of respiratory syncytial infection (hematoxylin – eosin staining, magnification 400×)

However, errors in feeding are not the only etiological factor leading to acidosis. For example, several authors have found that in parallel to respiratory pathology caused by viral and bacterial agents, acidosis develops in cattle [8, 9, 11, 12].

The main pathological processes in the respiratory tract caused by bovine acute respiratory infections are bronchites, tracheites and pneumonias. When the respiratory tract is affected, hypoxia occurs in the body of animals, which leads to the development of endogenous intoxication, resulting in rumen acidosis [11, 12], followed by introduction of vasoactive substances (bacterial endotoxins, histamine, lactate) into blood [7–10]. Due to the simultaneous expansion of arterioles and compression of venules, the vascular endothelium is damaged, perfusion of blood fluid from the vessels into the surrounding tissues occurs, blood flow in the microcirculatory bed is disrupted [7].

An important role in poor blood circulation in small blood vessels is played by CICs, which are antigen – antibody complexes. The CICs formation is one of the normal responses of the body's immune system to the introduction of a pathogen. At the same time, CICs increased concentration, which occurs during a high antigenic load or malfunction of their elimination mechanisms out of the body, leads to pathological changes in the tissues and organs of animals, which is caused by high biological activity of immune complexes. Most of the CICs are quickly removed from the bloodstream of the body due to the reticulohistiocytic system, which combines various heterogeneous groups of the body cells, in particular Kupfer

Table 4
Concentration of circulating immune complexes in the blood of cows and calves

Indicator	Cows ($n = 10$)	Calves ($n = 10$)
CICs, c.u.	212.400 ± 0.645	234.460 ± 5.905

cells capable of active phagocytosis. CICs have both immunostimulating and immunosuppressive properties. The greatest pathogenic effect is exerted by complexes capable of activating the complement system and reacting with blood cells that have receptors for binding immunoglobulins or complement. The main CICs damaging effect is complement and neutrophil-dependent mechanism. Complement associated CICs exhibit chemotactic properties, resulting in the accumulation of neutrophils in the affected areas and to the release of hydrolytic enzymes from them that destroy the body tissues. At the same time, CICs can cause pathology regardless of the presence of neutrophils and complement [13]. Low-molecular-weight CICs, settling in various organs and tissues of the body, lead to inflammation and damage the normal tissue structures. The blood vessel endothelium, renal glomeruli and joints are most often damaged by immune complexes [14].

In cattle, the vessels of the distal extremities are primarily affected, which leads to trophism disorder of hoof and limb skin. That's why laminitis develops, while the hoof horn tissue is weakly keratinized and cannot resist aggressive mechanical and chemical environmental factors [7, 10]. Damaged hooves are the gateway of infection for the agents of necrobacteriosis (*Fusobacterium necrophorum*), staphylococcosis (*Staphylococcus* spp.), streptococcosis (*Streptococcus* spp.) and other pathogens [7, 10, 12, 15]. In addition, favorable conditions for the development of mixed infection are created by reduced organism resistance, which is observed both during respiratory pathology and during pathologies of the distal extremities [15].

CONCLUSION

Studies have shown that one of the pathogenic factors contributing to the development of infections of bovine distal extremities is respiratory diseases, leading to reduced natural resistance of the body and subsequent metabolic acidosis.

Comprehensive prophylaxis of infections of bovine distal extremities should include mandatory laboratory diagnostics for acute respiratory diseases.

REFERENCES

1. Petrova O. G., Barachkin M. I., Drozdova L. I., Alecseev A. D. Respiratory syncytial infection. Dissemination, relevance, problems. *Advances in Agricultural and Biological Sciences*. 2017; 3 (4): 25–34. DOI: 10.22406/aabs-17-3.4-25-34.

2. Krasnoperov A. S., Maryin E. M., Zabrodin E. A., Barashkin M. I., Milshtein I. M., Pryanichnikov V. P., Teterev N. N. Diseases of the hoof in cattle [Zabolevaniya kopytec u krupnogo rogatogo skota]. *BIO*. 2020; 7 (238): 26–33. eLIBRARY ID: 44074061. (in Russian)

3. Alexeev A. D., Petrova O. G., Drozdova L. I. The particular manifestations of acute respiratory viral infections of cattle in modern conditions. *Agrarian Bulletin of the Urals*. 2015; 6 (136): 38–40. eLIBRARY ID: 24000590. (in Russian)

4. Alekseev A. D., Petrova O. G., Drozdova L. I. Respiratory syncitial infection and its role in pathogenesis of acute respiratory diseases of cattle stock. *Medicus*. 2016; 3 (9): 31–33. eLIBRARY ID: 25957400. (in Russian)

5. Alexeev A. D., Petrova O. G., Drozdova L. I. Bovine respiratory syncytial infection and its importance in the epidemiology of acute respiratory disease. *Bulletin of Omsk State Agrarian University*. 2015; 4 (20): 39–44. eLIBRARY ID: 25108641. (in Russian)

6. Balash A., Batiz G., Bridl E., Chaki T., Gombosh Sh., Kurtfalvi A. Maintenance, feeding and the most important veterinary issues in the breeding of the Holstein-Friesian cattle breed. Ed. by E. Bridle; translated by Ya. Zakhar. Budapest: Agrota; 1994. 238 p. (in Russian)

7. Samolovov A. A., Lopatin S. V. Lameness – reflection of system metabolic diseases of the dairy cattle. *Innovations and Food Safety*. 2013; 2 (2): 76–80. Available at: https://innfoodsecr.elpub.ru/jour/article/view/29. (in Russian)

8. Evglevsky A. A., Skhira V. N., Evglevskaya E. P., Vanina N. V., Mikhailova I. I., Suleimanova T. A., Pereverzeva J. A. Metabolic acidosis of high yielding cows: causes, consequences, prophylaxes. *Veterinariya*. 2017; 5: 45–48. eLIBRARY ID: 29155246. (in Russian)

9. Evglevsky A. A., Evglevskaya E. P., Mikhailova I. I., Vanina N. V., Erizhenskaya N. F., Suleymanova T. A. Disturbance of acid-base balance in the cow: causes, consequences and treatment. *Veterinarnaya patologiya*. 2017; 1 (59): 53–58. eLIBRARY ID: 29737143. (in Russian)

10. Melnik N. V., Samuylenko A. Ya., Melnik R. N., Grin S. A., Klyukina V. I., Nikitin A. I., et al. Necrobacteriosis in animals. Treatment and prevention. Ed. by RAS Academician A. Ya. Samuylenko. Krasnodar: Kuban SAU; 2018; 279 p. Available at: https://www.elibrary.ru/download/elibrary_36686875_13270852.pdf. (in Russian)

11. Alekhin Yu. N., Zhukov M. S., Lebedeva A. Yu. Functional state of proventriculi at different stages of bronchopneumonia development and during post-treatment period in calves. *Veterinariya, zootekhniya i biotekhnologiya*. 2016; 11: 13–19. eLIBRARY ID: 27257704. (in Russian)

12. Zolotarev A. I., Chernitskiy A. E., Retsky M. I. Acid-base status and blood gas composition in calves with bronchitis. *Veterinariya*. 2013; 7: 47–52. eLIBRARY ID: 19403634. (in Russian)

13. Mager S. N., Dementieva E. S. Physiology of immune system: the study book. Saint Petersburg: Lan'; 2014. 192 p. (in Russian)

14. Male D., Brostoff J., Roth D., Roitt I. Immunology. 7th ed. Elsivier Ltd; 2006. 564 p.

15. Barashkin M. I., Petrova O. G. Features of the epizootiology of infectious diseases of the distal extremities of cattle in industrial technology of content. *Agrarian Bulletin of the Urals*. 2016; 3 (145): 27–31. eLIBRARY ID: 25797139. (in Russian)

Received on 30.04.2021
Approved for publication on 26.07.2021

INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Anatoliy D. Alekseev, Candidate of Sciences (Veterinary Medicine), Associate Professor, Department of Infectious and Non-Infectious Pathology, FSBEI HE Ural SAU, Ekaterinburg, Russia.

Алексеев Анатолий Дмитриевич, кандидат ветеринарных наук, доцент кафедры инфекционной и незаразной патологии ФГБОУ ВО Уральский ГАУ, г. Екатеринбург, Россия.

Olga G. Petrova, Doctor of Science (Veterinary Medicine), Professor, Professor of the Department of Infectious and Non-Infectious Pathology, FSBEI HE Ural SAU, Ekaterinburg, Russia.

Mikhail I. Barashkin, Doctor of Science (Veterinary Medicine), Professor, Head of the Department of Surgery, Obstetrics and Microbiology, FSBEI HE Ural SAU, Ekaterinburg, Russia.

Igor M. Milshtein, Candidate of Sciences (Veterinary Medicine), Associate Professor, Department of Surgery, Obstetrics and Microbiology, FSBEI HE Ural SAU, Ekaterinburg, Russia.

Vladislav D. Moskvin, Post-Graduate Student, Department of Infectious and Non-Infectious Pathology, FSBEI HE Ural SAU, Ekaterinburg, Russia.

Петрова Ольга Григорьевна, доктор ветеринарных наук, профессор, профессор кафедры инфекционной и незаразной патологии ФГБОУ ВО Уральский ГАУ, г. Екатеринбург, Россия.

Барашкин Михаил Иванович, доктор ветеринарных наук, профессор, заведующий кафедрой хирургии, акушерства и микробиологии ФГБОУ ВО Уральский ГАУ, г. Екатеринбург, Россия.

Мильштейн Игорь Маркович, кандидат ветеринарных наук, доцент кафедры хирургии, акушерства и микробиологии ФГБОУ ВО Уральский ГАУ, г. Екатеринбург, Россия.

Москвин Владислав Дмитриевич, аспирант кафедры инфекционной и незаразной патологии ФГБОУ ВО Уральский ГАУ, г. Екатеринбург, Россия.

DOI: 10.29326/2304-196X-2021-3-38-197-202

Immune status improvement in piglets through the use of interferon-containing products during specific prevention of porcine pleuropneumonia

A. G. Shakhov[1], L. Yu. Sashnina[2], V. A. Prokulevich[3], Yu. Yu. Vladimirova[4], M. I. Adodina[5]

[1,2,4,5] Federal State Budgetary Scientific Institution "All-Russian Veterinary Research Institute of Pathology, Pharmacology and Therapy" (FSBSI "ARVRIPP&T"), Voronezh, Russia

[3] Belarusian State University (BSU), Minsk, Republic of Belarus

[1] *ORCID 0000-0002-6177-8858, e-mail: a.g.shakhov@mail.ru*

[2] *ORCID 0000-0001-6477-6156, e-mail: l.yu.sashnina@mail.ru*

[3] *ORCID 0000-0003-3462-3540, e-mail: prokulevichv@bsu.by*

[4] *ORCID 0000-0001-8888-7264, e-mail: uliavcoach379@gmail.com*

[5] *ORCID 0000-0002-1791-0866, e-mail: mari.adodina@mail.ru*

SUMMARY

Specific prevention is one of the most effective methods for the control of infectious diseases causing considerable economic damage to commercial pig farms, among which is porcine pleuropneumonia. In order to improve the effectiveness of preventive vaccination, various immunomodulators that differ in their origin and mechanism of action are used. The paper presents the results of the study of the effect of such products as biferon-S and prostimul containing species-specific recombinant interferons on the immune status of piglets during specific prevention of porcine pleuropneumonia. Tests were carried out in clinically healthy 30–35-day-old piglets immunized with Ingelvac® APPX vaccine (Boehringer Ingelheim Vetmedica GmbH, Germany). It was found that the use of biferon-S and prostimul together with the vaccine administration is accompanied by immune status improvement in the animals, which is manifested as an increase, in comparison with vaccinated animals that received no interferon-containing products (base case), in serum levels of γ-globulins – by 34.6 and 53.7% (in case of prostimul and β-globulins – by 10.1%), total immunoglobulins – by 32.8 and 37.8%, large circulating immune complexes – by 37.5 and 52.6%, a less significant increase in the levels of small complexes and, as a result, pathogenicity coefficient reduction by 5.4 and 12.4%, respectively. Tests for post-vaccination immunity levels in piglets showed a 3.8-fold increase in the levels of specific antibodies against the antigen of porcine pleuropneumonia agent, and in case of the vaccine administration in combination with biferon-S and prostimul – a 4.0-fold and 4.9-fold increase, respectively. The use of prostimul was accompanied by a more considerable improvement of immune status in the piglets, and this is attributable to the fact that vitamins A, E and C, which have antioxidant properties and improve the effectiveness of interferons, natural resistance and specific immunity, are included in its composition in addition to recombinant type 1 cytokine.

Keywords: porcine pleuropneumonia, piglets, specific prevention, vaccine, immune status, biferon-S, prostimul, vitamins A, E and C

Acknowledgements: The study was funded by FSBSI "ARVRIPP&T" within the framework of "Development of methodological approaches to prevention and treatment of immunodeficiency disorders in pigs during the critical periods in raising" research work.

For citation: Shakhov A. G., Sashnina L. Yu., Prokulevich V. A., Vladimirova Yu. Yu., Adodina M. I. Immune status improvement in piglets through the use of interferon-containing products during specific prevention of porcine pleuropneumonia. *Veterinary Science Today.* 2021; 10 (3): 197–202. DOI: 10.29326/2304-196X-2021-3-38-197-202.

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

For correspondence: Larisa Yu. Sashnina, Doctor of Science (Veterinary Medicine), Head of Immunology Laboratory, FSBSI "ARVRIPP&T", 394087, Russia, Voronezh, ul. Lomonosova, 114b, *e-mail: l.yu.sashnina@mail.ru*.

УДК 619:616.98:579.843.96:579.887.111:636.4:616-087.371:636:612.017.11/.12

Повышение иммунного статуса у поросят интерферонсодержащими препаратами при специфической профилактике актинобациллезной плевропневмонии

А. Г. Шахов[1], Л. Ю. Сашнина[2], В. А. Прокулевич[3], Ю. Ю. Владимирова[4], М. И. Адодина[5]

[1,2,4,5] ФГБНУ «Всероссийский научно-исследовательский ветеринарный институт патологии, фармакологии и терапии» (ФГБНУ «ВНИВИПФиТ»), г. Воронеж, Россия

[3] Белорусский государственный университет (БГУ), г. Минск, Республика Беларусь

[1] *ORCID 0000-0002-6177-8858, e-mail: a.g.shakhov@mail.ru*
[2] *ORCID 0000-0001-6477-6156, e-mail: l.yu.sashnina@mail.ru*
[3] *ORCID 0000-0003-3462-3540, e-mail: prokulevichv@bsu.by*
[4] *ORCID 0000-0001-8888-7264, e-mail: uliavcoach379@gmail.com*
[5] *ORCID 0000-0002-1791-0866, e-mail: mari.adodina@mail.ru*

РЕЗЮМЕ

Специфическая профилактика является одним из наиболее эффективных методов борьбы с инфекционными заболеваниями, наносящими значительный экономический ущерб промышленным свиноводческим хозяйствам, к числу которых относится актинобациллезная плевропневмония свиней. Для повышения эффективности вакцинопрофилактики применяются различные иммуномодулирующие средства, отличающиеся по происхождению и механизму действия. В статье представлены результаты изучения влияния препаратов биферона-С и простимула, содержащих видоспецифичные рекомбинантные интерфероны, на иммунный статус поросят при специфической профилактике актинобациллезной плевропневмонии. Исследования проведены на клинически здоровых поросятах 30–35-суточного возраста, иммунизированных вакциной Ingelvac® APPX (Boehringer Ingelheim Vetmedica GmbH, Германия). Установлено, что применение биферона-С и простимула одновременно с введением вакцины сопровождается повышением иммунного статуса животных, проявляющимся увеличением по сравнению с базовым вариантом содержания в сыворотке крови γ-глобулинов на 34,6 и 53,7% (при назначении простимула и β-глобулинов – на 10,1%), общих иммуноглобулинов – на 32,8 и 37,8%, крупных циркулирующих иммунных комплексов – на 37,5 и 52,6%, менее значимым увеличением комплексов мелких размеров и снижением в результате этого коэффициента патогенности на 5,4 и 12,4% соответственно. Исследование напряженности поствакцинального иммунитета у поросят показало, что уровень специфических антител к антигену возбудителя актинобациллезной плевропневмонии повысился в 3,8 раза, а при введении вакцины в сочетании с бифероном-С и простимулом – в 4,0 и 4,9 раза соответственно. Применение простимула сопровождалось более существенным повышением иммунного статуса у поросят, обусловленным наличием в его составе кроме рекомбинантного цитокина первого типа витаминов А, Е и С, обладающих антиоксидантными свойствами, повышающих эффективность интерферонов, естественную резистентность и специфический иммунитет.

Ключевые слова: актинобациллезная плевропневмония, поросята, специфическая профилактика, вакцина, иммунный статус, биферон-С, простимул, витамины А, Е и С

Благодарность: Работа выполнена за счет средств ФГБНУ «ВНИВИПФиТ» по теме «Разработка методических подходов к профилактике и терапии иммунодефицитных состояний у свиней в критические периоды выращивания».

Для цитирования: Шахов А. Г., Сашнина Л. Ю., Прокулевич В. А., Владимирова Ю. Ю., Адодина М. И. Повышение иммунного статуса у поросят интерферонсодержащими препаратами при специфической профилактике актинобациллезной плевропневмонии. *Ветеринария сегодня*. 2021; 10 (3): 197–202. DOI: 10.29326/2304-196X-2021-3-38-197-202.

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

Для корреспонденции: Сашнина Лариса Юрьевна, доктор ветеринарных наук, заведующий лабораторией иммунологии ФГБНУ «ВНИВИПФиТ», 394087, Россия, г. Воронеж, ул. Ломоносова, 114б, *e-mail: l.yu.sashnina@mail.ru.*

INTRODUCTION

Porcine pleuropneumonia caused by *Actinobacillus pleuropneumoniae* has high economic and epidemiological significance for commercial pig farms [1–4]. *A. pleuropneumoniae,* one of the most important respiratory bacterial pathogens of pigs, can cause monoinfection and, in association with influenza, porcine reproductive and respiratory syndrome viruses and mycoplasmas, plays the leading role in the development of the complex of porcine respiratory diseases [4, 5]. Specific prevention of porcine pleuropneumonia is the main method for the infection control [4].

Routine vaccination against porcine pleuropneumonia carried out in piglets aged 3 to 4 weeks coincides with the late suckling period when, against the background of physiological immunodeficiency or stress response to weaning, lipid peroxidation activation and a decline in antioxidant defence and the levels of humoral and cellular factors of non-specific resistance occur [6, 7].

Immunodeficiency recorded before vaccination negatively affects the development of specific immunity; ligfol, ligaverin, selekor having adaptogenic and immunomodulatory properties were tested and found to contribute to specific immunity improvement in sows and piglets immunized against escherichiosis and salmonellosis [8, 9].

The following immunomodulators used during vaccination are effective for increasing antibody levels and antibody persistence: salmozan, glicopin, immunofan, roncoleukin, polyribotan, etc. [10].

When studying the possibility of using recombinant cytokines as adjuvants during vaccination, A. S. Simbirtsev et al. found that interleukin-1 used during immunization against viral hepatitis B has the highest adjuvant activity: it neutralizes the negative effect of a biological on the functional activity of neutrophils and contributes to the increase in specific antibody titers [11].

Biferon-S containing recombinant porcine *α*- and *γ*-interferons was found to have a positive impact on specific immunity development when used during vaccination against porcine circovirus disease [12], colibacteriosis [13], circovirus infection and mycoplasmosis [14].

The study is aimed at investigating the effect of biferon-S and prostimul on the immune status of piglets during specific prevention of porcine pleuropneumonia.

MATERIALS AND METHODS

The tests were carried out in 2020 in an industrial pig production complex in the Tambov Oblast that had been porcine pleuropneumonia infected since 2016 due to the introduction of replacement gilts being *A. pleuropneumoniae* serotype 2 carriers. The animals for the tests were selected from the production group of piglets ($n = 1300$) moved to the nursery at the same time. Each animal's body weight was 9.0 ± 0.94 kg. The tests were carried out in 150 clinically healthy 30–35-day old piglets without distinction as to their sex; 3 groups, each comprising 50 animals, were formed. Control group animals (base case) were immunized with Ingelvac® APPX vaccine (Boehringer Ingelheim Vetmedica GmbH, Germany) administered twice at a 14-day interval at a dose of 2 ml; test group 1 piglets received biferon-S at a dose of 0.1 ml/kg as an adjuvant simultaneously with the vaccine administration, test group 2 piglets received the same dose of prostimul according to the similar scheme.

Ingelvac® APPX vaccine is manufactured using *A. pleuropneumoniae* serotype 1, 2, 3, 4, 5, 7 cultures, ApxI, ApxII, ApxIII toxoids inactivated with formalin (0.2% by volume) with addition of aluminum hydroxide (0.5% by volume) and isotonic sodium chloride solution.

Biferon-S contains porcine recombinant *α*- and *γ*-interferons with total antiviral activity of at least 1.0×10^4 $TCID_{50}/cm^3$ in the solvent supplemented with stabilizing agents.

Prostimul contains a recombinant cytokine as an active ingredient, ascorbic acid, vitamins A, E and C, sodium benzoate, sodium sulfite, ethylenediaminetetraacetic acid, non-ionogenic solvent, glycerin and water.

Biferon-S and prostimul are manufactured by OOO "Scientific and Production Centre "ProBioTekh" (the Republic of Belarus).

The animals were clinically observed for 2 months.

Blood samples were collected from piglets ($n = 6$) of each group before the administration of the vaccine and the above-mentioned products (baseline), as well as 14 days after their second administration and tested at the Research Centre of the FSBSI "ARVRIPP&T".

Sera from the piglets were tested for total protein, protein fractions, circulating immune complexes (CICs): large (3%), medium (3.5%) and small (4%) according to the Methodical recommendations for immune status evaluation and improvement in animals [15], total immunoglobulins (Ig) [16], CIC pathogenicity coefficient (C4/C3 ratio) [17]. Titers of antibodies against *A. pleuropneumoniae* were determined with enzyme-linked immunosorbent assay (ELISA) with subsequent reading of results with a Uniplan-TM spectrophotometer according to the instruction for ID Screen® APP Screening Indirect test kit (France).

The statistical analysis of the findings was performed using Statistica v 6.1 software; the significance of differences was estimated using Student's *t*-criterion.

RESULTS AND DISCUSSION

The piglets' clinical status during the experiment was within the norm.

Baseline biochemical tests showed no significant differences in the levels of total protein, *β*-globulin, *γ*-globulin and total immunoglobulins in the piglets of both test groups (Table 1).

Control group piglets demonstrated lower albumin levels than test group piglets, the lowest CIC pathogenicity coefficient and higher *α*-globulin and large CIC levels.

Test group 1 piglets demonstrated lower levels of large and medium CICs than test group 2 and control group animals and, consequently, the highest pathogenicity coefficient.

With advancing age, the biochemical status of the animals underwent changes influenced by vaccination and interferon-containing products (Table 1).

Total protein content increased by 16.2% in control group piglets, by 12.7% – in test group 1 piglets,

Table 1
Biochemical blood parameters in piglets

Parameters	Groups		
	Control group	Test group 1	Test group 2
Before vaccination			
Protein, g/l	49.50 ± 1.49	51.10 ± 1.45	49.90 ± 0.65
Albumins, %	47.90 ± 1.34	52.00 ± 2.93	54.40 ± 0.79
Globulins: %			
α	16.90 ± 0.94	13.10 ± 1.26	13.30 ± 0.45
β	21.90 ± 0.67	21.30 ± 0.95	19.90 ± 0.99
γ	13.30 ± 0.86	13.60 ± 1.09	12.30 ± 0.45
Total immunoglobulins, mg/ml	25.60 ± 1.23	23.80 ± 1.41	22.50 ± 0.68
CICs, 3.5% mg/ml	0.370 ± 0.029	0.26 ± 0.04	0.430 ± 0.037
CICs, 3.0% mg/ml	0.280 ± 0.001	0.16 ± 0.01	0.19 ± 0.02
CICs, 4.0% mg/ml	0.37 ± 0.03	0.37 ± 0.02	0.43 ± 0.06
C4/C3	1.32 ± 0.14	2.210 ± 0.009	2.09 ± 0.18
After vaccination			
Protein, g/l	57.50 ± 0.81**	57.60 ± 1.23**	59.10 ± 1.43***
Albumins, %	46.10 ± 0.88	48.80 ± 1.37	47.60 ± 0.97***
Globulins: %			
α	15.20 ± 0.53	11.50 ± 0.45	11.80 ± 0.73
β	21.90 ± 0.46	21.40 ± 0.57	21.90 ± 0.62
γ	16.90 ± 0.84*	18.30 ± 0.83**	18.90 ± 0.52***
Total immunoglobulins, mg/ml	30.90 ± 0.78**	31.60 ± 0.70***	31.00 ± 0.69***
CICs, 3.5% mg/ml	0.290 ± 0.023*	0.230 ± 0.027	0.240 ± 0.036**
CICs, 3.0% mg/ml	0.36 ± 0.02*	0.22 ± 0.02*	0.29 ± 0.01**
CICs, 4.0% mg/ml	0.59 ± 0.03***	0.50 ± 0.02**	0.53 ± 0.02
C4/C3	1.76 ± 0.11*	2.09 ± 0.15	1.83 ± 0.09

* $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$ – against baseline values.

by 18.4% – test group 2 piglets; this is indicative of hepatic protein synthesis activation, especially in the animals that received the vaccine in combination with prostimul containing vitamins A, E and C that have antioxidant properties.

Significant changes were observed in the proteinogram in the vaccinated animals, especially in those that had received the vaccine in combination with biferon-S and prostimul. Control group animals demonstrated a decrease in the levels of albumins by 3.8% and *α*-globulins – by 10.1%, and an increase in the levels of *γ*-globulins, which contain predominantly antibodies, by 27.1%. Test group 1 and 2 piglets demonstrated a decrease in the levels of albumins by 6.2 and 12.5%, of *α*-globulins – by 12.2 and 11.3%, and an increase in the levels of *γ*-globulins by 34.6 and 53.7%, respectively. The levels of *β*-globulins containing complement components and a part of immunoglobulins in control group and test group 1 animals practically did not differ from baseline values; the levels of *β*-globulins in piglets immunized in combination with prostimul administration increased by 10.1%.

The changes observed in the proteinogram in the vaccinated animals and in the animals immunized with simultaneous administration of interferon-based products are indicative of immune system transformation which is the most pronounced in case of the vaccine administration in combination with prostimul and manifested as a significant increase in the levels of *γ*-globulins and an increase in the level of *β*-globulins responsible for humoral protection.

The vaccinated animals of the control group demonstrated an increase in total immunoglobulin levels by 20.7%, in case of vaccine administration in combination with biferon-S and prostimul – by 32.8 and 37.8%, respectively, and this is indicative of a significant enhancement of humoral protection in the piglets, especially when specific prevention is carried out in combination with the use of interferon-containing products, mainly prostimul.

The positive effect of immunomodulators on the immune status of animals vaccinated against porcine pleuropneumonia is demonstrated by the results of tests of circulating immune complexes that are the product of antigen, antibody and complement reactions and play an important role in the maintenance of homeostasis in the body.

In particular, control group piglets demonstrated a significant increase in the levels of small CICs – by 59.5%, of large CICs – by 28.6% and a decrease in the levels of medium CICs by 21.6% in comparison with baseline values. At the same time, CIC pathogenicity coefficient (C4/C3 ratio) increased by 33.3%, and this is probably due to a high antigen load in piglets.

Table 2
Specific immunity parameters in piglets

Parameters	Titres of antibodies against *A. pleuropneumoniae*		
	control group	test group 1	test group 2
Before vaccination	11.90 ± 2.10	12.11 ± 2.96	9.40 ± 2.23
After vaccination	45.10 ± 3.82*	48.90 ± 2.49*	46.10 ± 2.21*

* $p < 0.0001$ – against baseline values.

The animals immunized in combination with the use of biferon-S demonstrated a more significant, as compared with control group animals, increase in the levels of large CICs (by 37.5%), a less pronounced increase in the levels of small CICs (by 35.1%) and a decrease in the levels of medium CICs – by 11.5%; at the same time, their pathogenicity coefficient decreased by 5.4% in comparison with baseline values.

In test group 2 piglets subjected to vaccination with simultaneous administration of prostimul, large CIC levels increased by 52.6% and were significantly higher than those in test group 1 and control group animals, their medium CIC levels decreased by 44.2%, an increase in small CIC levels (by 23.3%) was less significant, and that resulted in pathogenicity coefficient reduction by 12.4%.

Serological test results presented in Table 2 are indicative of humoral protection improvement in the piglets after vaccination, especially in combination with administration of interferon-based products.

It was found that titers of specific antibodies against *A. pleuropneumoniae* antigen in control group piglets increased by a factor of 3.8 in comparison with baseline values, and when the vaccine was administered in combination with biferon-S and prostimul – by a factor of 4.0 and 4.9, respectively.

Thus, the use of interferon-containing products during specific prevention of porcine pleuroneumonia is accompanied by immune status improvement in piglets due to recombinant porcine interferons and a number of excipients included in their composition.

Alpha interferon contained in biferon-S has immunomodulatory properties: it increases the activity of natural killer cells, T helpers, phagocytes, the intensity of B lymphocyte differentiation [18, 19]. Gamma interferon has a pronounced immunomodulatory effect, activating macrophages, cytotoxic T lymphocytes, natural killer cells [18, 20, 21].

Alpha and beta interferons contained in prostimul have immunoregulatory effect: they modulate antibody production, enhance cellular cytotoxity of T lymphocytes and natural killer cells, inhibit the proliferation of lymphocytes, T-cell suppression and facilitate preferential differentiation of T helpers into Th1 lymphocytes [22].

Vitamins A, E and C contained in prostimul improve the antioxidant status of the body by restraining accumulation of lipid peroxidation products [23, 24], have antioxydant effect on immune system cells by protecting them from oxygen-dependent types of apoptosis [25, 26], as well as potentiate the effectiveness of inteferons [23]; as a result, prostimul can do more, than biferon-S, to improve the immune status of piglets during specific prevention of porcine pleuropneumonia.

CONCLUSION

The study showed that the use of biferon-S and prostimul together with administration of the vaccine against porcine pleuropneumonia is accompanied by immune status improvement in piglets, which is manifested as an increase, in comparison with vaccinated animals that received no interferon-containing products (base case), in serum levels of *γ*-globulins, and when prostimul and *β*-globulins were used – of total immunoglobulins, specific antibodies against *A. pleuropneumoniae* antigen,

CIC pathogenicity reduction. The use of prostimul that contains vitamins A, E and C having antioxidant properties potentiates the effectiveness of interferons, improves natural resistance and specific immunity.

REFERENCES

1. Sidorov M. A., Skorodumov D. I. Haemophilus infections in animals [Gemofilezy zhivotnyh]. M.: Agropromizdat; 1986. 174 p. (in Russian)

2. Lebedev N. V., Potekhin A. V, Frolovtseva A. A. Porcine *Actinobacillus pleuropneumnia*: diagnosis prophylaxis and control measures. *Veterinary Science Today*. 2012; 3 (3): 24–30. eLIBRARY ID: 22480810. (in Russian)

3. Potekhin A. V., Kovalishin V. F. *Actinobacilosis pleuropneumonia* (APP): diagnosis, prevention and control measures. *Veterinary Science Today*. 2014; 3 (10): 18–29. eLIBRARY ID: 22445539.

4. Gottschalk M., Broes A. Actinobacillosis. *In: Diseases of Swine*. Eds. J. J. Zimmerman, L. A. Karriker, A. Ramirez, K. J. Schwartz, G. W. Stevenson, J. Zhang. 11th ed. New York: John Wiley&Sons; 2019; Chapter 48: 749–766. DOI: 10.1002/9781119350927.ch48.

5. Orlyankin B. G., Aliper T. I., Mishin A. M. Porcine infectious respiratory diseases: etiology, diagnosis and prevention [Infekcionnye respiratornye bolezni svinej: etiologiya, diagnostika i profilaktika]. *Pig-Breeding*. 2010; 3: 67–69. eLIBRARY ID: 17711236. (in Russian)

6. Retsky M. I., Buzlama V. S., Shakhov A. G. The role of antioxidant status in adaptive heterogenicity and immune resistance in animals [Znachenie antioksidantnogo statusa v adaptivnoj geterogennosti i immunologicheskoj rezistentnosti zhivotnyh]. *Veterinarnaya patologiya*. 2003; 2 (6): 63–65. eLIBRARY ID: 9164252. (in Russian)

7. Shakhov A. G., Sashnina L. Yu., Vladimirova Yu. Yu., Tarakanova K. V. Haemomorphological, biochemical and immune status in piglets under stress, caused by weaning from sows and crossover to nursery. *Issues of Legal Regulation in Veterinary Medicine*. 2019; 3: 182–186. DOI: 10.17238/issn2072-6023.2019.3.182. (in Russian)

8. Nikolaeva O. N., Andreeva A. V. The dynamics of indicators of humoral immunity at prevention of associative infections of young growth of farm animals. *Russian Electronic Scientific Journal*. 2014; 8 (14): 186–193. eLIBRARY ID: 26494610. (in Russian)

9. Shakhov A. G., Retsky M. I., Masyanov Yu. N., Brigadirov Yu. N., Zaiko A. M. The use of immunomodulators and antioxidants to improve the effectiveness of immunization in pigs [Ispol'zovanie immunomodulyatorov i antioksidantov dlya povysheniya effektivnosti immunizacii svinej]. *Topical Issues of Animal Infectious Pathology and Immunology*: *Proceedings of the International Research-to-Practice Conference (May 16–17, 2006)*. M.: Izograf; 2006; 522–525. eLIBRARY ID: 41447034. (in Russian)

10. Deyeva A. V., Mekhdikhanov G. G., Sokolov V. D., Belousova R. V. The use of immunomodulators in production animals [Primenenie immunomodulyatorov produktivnym zhivotnym]. *Veterinariya*. 2008; 6: 8–12. eLIBRARY ID: 11149494. (in Russian)

11. Simbirtsev A. S., Petrov A. V., Pigareva N. V., Nikolaev A. T. New opportunities for using recombinant cytokines as adjuvants for vaccination. *Biopreparats (Biopharmaceuticals)*. 2011; 1 (41): 16–20. eLIBRARY ID: 20369106. (in Russian)

12. Kudin K. V., Kudina I. V., Prokulevich V. A. The effect of recombinant cytokine-based product Biferon-S on the growth, development and immunization of piglets against PCV-2 [Vliyanie preparata «Biferon-S» na osnove rekombinantnyh citokinov na rost, razvitie i immunizaciyu porosyat k CVS-2]. *Biotechnology: Advances and Development Prospects: Proceedings of the III International Research-to-Practice Conference* (*November 22–23, 2018*). Pinsk: Polessky State University; 2018; 78–81. eLIBRARY ID: 36624367. (in Russian)

13. Zaitsev V. V., Biletsky M. O., Biletsky O. R., Zaitseva A. V. The effect of procine recombinant alfa- and gamma-interferons on the immunogenicity of *Escherichia coli* antigens. *Uchenye zapiski Educational Establishment "Vitebsk State Academy of Veterinary Medicine"*. 2018; 54 (1): 9–13. eLIBRARY ID: 34999020. (in Russian)

14. Shakhov A., Shabunin S., Zheynes M. Correction of cellular immunity with biferon-C in piglets in case of specific prevention of circovirosis and mycoplasmosis. *E3S Web of Conferences DAIC-2020.* 2020; 222:02056. DOI: 10.1051/e3sconf/202022202056.

15. Shakhov A. G., Masyanov Yu. N., Retsky M. I., Brigadirov Yu. N., Anufriyev A. I., Belyayev V. I., et al. Methodical recommendations for immune status evaluation and improvement in animals [Metodicheskie rekomendacii po ocenke i korrekcii immunnogo statusa zhivotnyh]. *In: New Methods of Research on Veterinary Medicine Issues. P. III. Methods of Research on the Issues of Non-Contagious Pathology in Production Animals*. M.: RAAS; 2007; 216–292. (in Russian)

16. Kondrakhin I. P., Arkhipov A. V., Levchenko V. I., Talanov G. A., Frolova L. A., Novikov V. E. Veterinary clinical laboratory diagnostic methods [Metody veterinarnoj klinicheskoj laboratornoj diagnostiki]: Reference book. Under general editorship of. I. P. Kondrakhin. M.: KolosS; 2004. 520 p. (in Russian)

17. Skibo Y. V., Kurmaeva N. S., Tsibulkina V. N., Mustafin I. G., Abramova Z. I. The characteristics of serum circulating immune complexes of patients with atopic asthma with different severity degree. *Kazan Medical Journal*. 2013; 94 (5): 744–748. DOI: 10.17816/KMJ1934. (in Russian)

18. Sologub T. V., Tsvetkov V. V., Deeva E. G. Interferon gamma-cytokine with antiviral, immunomodulatory and antitumor action. *I. P. Pavlov Russian Medical Biological Herald*. 2014; 22 (3): 56–60. DOI: 10.17816/PAVLOVJ2014356-60. (in Russian)

19. Harrison G. A., McNicol K. A., Deane E. M. Type I interferon genes from the egg-laying mammal, *Tachyglossus aculeatus* (short-beaked echidna). *Immunol. Cell Biol*. 2004; 82 (2): 112–118. DOI: 10.1046/j.0818-9641.2004.01230.x.

20. Ike K., Uchida Y., Nakamura T., Imai S. Induction of interferon-gamma (IFN-gamma) and T helper 1 (Th1) immune response by bitter gourd extract. *J. Vet. Med. Sci*. 2005; 67 (5): 521–524. DOI: 10.1292/jvms.67.521.

21. Laouar Y., Sutterwala F. S., Gorelik L., Flavell R. A. Transforming growth factor-beta controls T helper type 1 cell development through regulation of natural killer cell interferon-gamma. *Nat. Immunol*. 2005; 6 (6): 600–607. DOI: 10.1038/ni1197.

22. Ketlinsky S. A., Simbirtsev A. S. Cytokines [Citokiny]. St. Petersburg: Foliant; 2008. 552 p. (in Russian)

23. Malinovskaya V. V. Ontogeny of interferon system and issues of therapy in neonatal period [Ontogenez

sistemy interferona i problemy terapii v neonatal'nom periode]. *In: Interferon-2011*. Ed. by RAMS Member, prof. F. I. Yershov and prof. A. N. Narovlyansky. M.; 2012; 35–51. Available at: http://interferons.ru/downloads/glava1.pdf. (in Russian)

24. Gavrilova O. A. Features of lipid peroxidation process in normal conditions and in various pathological conditions in children (review of literature). *Acta Biomedica Scientifica*. 2017; 2 (4): 15–22. DOI: 10.12737/article_59fad50f919f18.64819381. (in Russian)

25. Martynova Ye. A., Morozov I. A. The role of nutrition in the maintenance of the functional activity of immune system and development of adequate immune response [Rol' pitaniya v podderzhanii funkcional'noj aktivnosti immunnoj sistemy i razvitii polnocennogo immunnogo otveta]. *Russian Journal of Gastroenterology, Hepatology, Coloproctology. Annex No. 14. Current Issues of Digestive Physiology and Pathology: Proceedings of the XVI Session of the Academic School-Workshop named after A. M. Ugolev (Puschino-on-Oka, May 14–17, 2001)*. 2001; 11 (4): 28–38. Available at: https://gastroportal.ru/nauchnye-uchrezhdeniya-shkoly/akademicheskaya-shkola-seminar-im-a-m-ugoleva/pitanie-i-immunitet-rol-pitaniya-v-podderzhanii-funktsionalnoy-aktivnosti-immunnoy-sistemy-i-razvitii-polnotsennogo-immunnogo-otveta.html. (in Russian)

26. Strelchenko E. A. Study of the effect of vitamin C on human physiological systems. *Scientific works of KubSTU*. 2019; 1: 319–330. eLIBRARY ID: 36944395. (in Russian)

Received on 23.03.2021
Approved for publication on 16.06.2021

INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Alexey G. Shakhov, Doctor of Science (Veterinary Medicine), Professor, Associate Member of the Russian Academy of Sciences, Chief Researcher, Immunology Laboratory, FSBSI "ARVRIPP&T", Voronezh, Russia.

Larisa Yu. Sashnina, Doctor of Science (Veterinary Medicine), Head of Immunology Laboratory, FSBSI "ARVRIPP&T", Voronezh, Russia.

Vladimir A. Prokulevich, Doctor of Science (Veterinary Medicine), Professor, Head of Department of Microbiology, Belarusian State University, Minsk, Republic of Belarus.

Yulia Yu. Vladimirova, Junior Researcher, Immunology Laboratory, FSBSI "ARVRIPP&T", Voronezh, Russia.

Maria I. Adodina, Senior Researcher, Immunology Laboratory, FSBSI "ARVRIPP&T", Voronezh, Russia.

Шахов Алексей Гаврилович, доктор ветеринарных наук, профессор, член-корреспондент РАН, главный научный сотрудник лаборатории иммунологии ФГБНУ «ВНИВИПФиТ», г. Воронеж, Россия.

Сашнина Лариса Юрьевна, доктор ветеринарных наук, заведующий лабораторией иммунологии ФГБНУ «ВНИВИПФиТ», г. Воронеж, Россия.

Прокулевич Владимир Антонович, доктор биологических наук, профессор, заведующий кафедрой микробиологии БГУ, г. Минск, Республика Беларусь.

Владимирова Юлия Юрьевна, младший научный сотрудник лаборатории иммунологии ФГБНУ «ВНИВИПФиТ», г. Воронеж, Россия.

Адодина Мария Ивановна, старший научный сотрудник лаборатории иммунологии ФГБНУ «ВНИВИПФиТ», г. Воронеж, Россия.

DOI: 10.29326/2304-196X-2021-3-38-203-208

Biological properties of swine vesicular disease virus strain 2348 Italy/2008

Ye. N. Kalinina[1], **S. N. Fomina**[2]
FGBI "Federal Centre for Animal Health" (FGBI "ARRIAH"), Vladimir, Russia
[1] *ORCID 0000-0001-8088-6400, e-mail: haritonova@arriah.ru*
[2] *ORCID 0000-0002-2122-9096, e-mail: fomina@arriah.ru*

SUMMARY
Swine vesicular disease (SVD) is a viral infectious disease, which, if acute, is manifested by the clinical pattern similar to a number of vesicular diseases including foot-and-mouth disease. In case of subclinical disease, there are no evident clinical signs, therefore the diagnosis is problematic, and there can be the risk of the disease introduction into the Russian Federation with the infected pigs. The key measure for the prevention of SVD introduction involves control diagnostic testing of all animals imported in the country that makes it necessary to keep updated the currently used methods and tools for the disease laboratory diagnosis. The paper demonstrates data on experimental infection of pigs with SVDV strain 2348 Italy/2008 that belongs to the most recent one of the four known phylogenetic groups. The virus was kindly provided by the World Reference Laboratory for Foot-and-Mouth Disease (Pirbright, Great Britain), and it was adapted to the monolayer continuous cell cultures of porcine origin (IB-RS-2 and PSGK-30). The pigs were intradermally infected with concentrated cultured virus at a dose of 10^9 $TCID_{50}$. The infected animals demonstrated clinical signs typical for the acute disease. There was evidence that the virus was not transmitted to the intact animal in case husbandry conditions were met that allowed to avoid the infection transmission by the fecal-oral and contact mechanisms. As a result of the experiment, reference sera were collected at different time intervals post infection and their activity was determined using virus microneutralization test in cell culture and ELISA. Aphthae collected from the infected animals were deposited into the Strain collection of the Reference Laboratory for Foot-and-Mouth Disease, FGBI "ARRIAH".

Keywords: swine vesicular disease, laboratory diagnosis, experimental infection, enzyme-linked immunosorbent assay, virus microneutralization test in cell culture

Acknowledgements: The work was funded by the federal government as a part of the research activities "Development of the method for the detection of swine vesicular disease virus antibodies using microneutralization assay" (SA 081-00010-19-00 of 28.12.2018).

For citation: Kalinina Ye. N., Fomina S. N. Biological properties of swine vesicular disease virus strain 2348 Italy/2008. *Veterinary Science Today*. 2021; 10 (3): 203–208. DOI: 10.29326/2304-196X-2021-3-38-203-208.

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

For correspondence: Yelena N. Kalinina, Candidate of Science (Veterinary Medicine), Junior Researcher, Reference Laboratory for FMD Diagnosis, FGBI "ARRIAH", 600901, Russia, Vladimir, Yur'evets, *e-mail: haritonova@arriah.ru*.

УДК 619:616.98:578.835.1:616-078

Изучение биологических свойств штамма «№ 2348 Италия/2008» вируса везикулярной болезни свиней

Е. Н. Калинина[1], **С. Н. Фомина**[2]
ФГБУ «Федеральный центр охраны здоровья животных» (ФГБУ «ВНИИЗЖ»), г. Владимир, Россия
[1] *ORCID 0000-0001-8088-6400, e-mail: haritonova@arriah.ru*
[2] *ORCID 0000-0002-2122-9096, e-mail: fomina@arriah.ru*

РЕЗЮМЕ
Везикулярная болезнь свиней – инфекционная вирусная болезнь, сопровождающаяся при остром течении клинической картиной, общей для ряда заболеваний с везикулярным синдромом, включая ящур. При субклиническом течении явные признаки болезни отсутствуют, что затрудняет постановку диагноза и может создавать угрозу заноса инфекционного заболевания на территорию Российской Федерации с инфицированными свиньями. Основной мерой предотвращения заноса везикулярной болезни свиней является проведение контрольных диагностических исследований всех ввозимых на территорию страны животных, что требует постоянного поддержания в актуальном состоянии используемых методов и средств лабораторной диагностики

заболевания. В статье представлены данные по экспериментальному заражению естественно восприимчивых животных штаммом «№ 2348 Италия/2008» вируса везикулярной болезни свиней, принадлежащего к наиболее поздней из четырех известных филогенетических групп. Вирус получен из Всемирной справочной лаборатории по ящуру (Пирбрайт, Великобритания) и адаптирован к перевиваемым монослойным культурам клеток свиного происхождения IB-RS-2 и ПСГК-30. Свиней заражали интрадермально концентрированным культуральным вирусом в дозе 10^9 $ТЦД_{50}$. У зараженных животных наблюдали клинические признаки, характерные для острого течения болезни. Показано отсутствие передачи вируса интактному животному при соблюдении условий содержания, предотвращающих фекально-оральный и контактный механизмы передачи инфекции. В результате опыта получены референтные образцы сыворотки крови свиней в разные сроки после инфицирования, определена их активность в реакции микронейтрализации вируса в культуре клеток и в иммуноферментном анализе. Отобранный от зараженных животных афтозный материал заложен на хранение в рабочую коллекцию штаммов микроорганизмов референтной лаборатории диагностики ящура ФГБУ «ВНИИЗЖ».

Ключевые слова: везикулярная болезнь свиней, лабораторная диагностика, экспериментальное заражение, иммуноферментный анализ, реакция микронейтрализации вируса в культуре клеток

Благодарность: Исследование выполнено за счет средств федерального бюджета в рамках проведения научно-исследовательских работ по теме «Разработка метода выявления антител к везикулярной болезни свиней в реакции микронейтрализации» (ГЗ № 081-00010-19-00 от 28.12.2018).

Для цитирования: Калинина Е. Н., Фомина С. Н. Изучение биологических свойств штамма «№ 2348 Италия/2008» вируса везикулярной болезни свиней. *Ветеринария сегодня*. 2021; 10 (3): 203–208. DOI: 10.29326/2304-196X-2021-3-38-203-208.

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

Для корреспонденции: Калинина Елена Николаевна, кандидат ветеринарных наук, младший научный сотрудник референтной лаборатории диагностики ящура ФГБУ «ВНИИЗЖ», 600901, Россия, г. Владимир, мкр. Юрьевец, *e-mail: haritonova@arriah.ru.*

INTRODUCTION

Swine vesicular disease (SVD) is a contagious viral disease associated with a complex of symptoms common for a number of diseases with vesicular syndrome, and, first of all, for foot-and-mouth disease (FMD) being one of the economically significant animal diseases. Due to the similar clinical manifestations, the differential diagnosis of the vesicular diseases is impossible without a number of virus-specific tools for laboratory tests [1–5].

In spite of high contagiousness and increased virus resistance to the environmental factors, the morbidity of the susceptible animals varies substantially. The direct losses due to SVD are not significant, because in case of the disease outbreak the animals demonstrate only temporal loss of productivity and low mortality. The indirect damage due to the disease eradication measures are however significant [3, 4, 6–8]. The factor aiding to the spread of the infection involves the SVD's capacity for subclinical development being long undetected. During the latest officially reported epidemics the asymptomatic disease resulted in the mass SVD spread in susceptible animals, and the disease was reported only based on the serological monitoring data [9, 10]. Therefore, the largest World Reference Laboratories involved in examination and diagnosis of the diseases with vesicular syndrome developed, implemented and maintain up-to-date tools for SVD laboratory diagnosis [5].

The SVD virus, as well as many members of *Picornaviridae* family, is characterized by relatively high antigenic variability. There are four separate phylogenetic groups in the singular serotype, which developed successively at different time points and significantly differ antigenically [5, 10]. In the Russian Federation, SVD is considered to be an exotic disease and it has never been reported, but it was observed in the USSR (Odessa Oblast) in 1972. Strain 463 Odessa/1972 isolated and identified during the epidemics was placed into SVD phylogenetic group II, the isolates from which are antigenically different from the subsequent isolates of genetic groups III and IV [5, 10–12]. Hence, of specific academic and practical interest for SVD diagnosis is the virus strain 2348 Italy/2008 that belongs to the latest of the known groups, i.e. to group IV; and according to the published reports it causes mainly asymptomatic and subclinical disease on the pig farms [7].

The goal of the work was representation of the SVD clinical pattern in naturally susceptible animals following experimental infection with SVDV strain 2348 Italy/2008 and examination of the dynamics of the antibody formation in the infected pigs. The experiment poses a unique opportunity for the collection of sera from the experimental pigs at different, precisely known time points following infection, and such sera are of some value as reference controls that can be used for diagnostic purposes.

MATERIAL AND METHODS

Virus. In the experiment we used the SVDV provided by the World Reference Laboratory for Foot-and-Mouth Disease (Pirbright, Great Britain). The virus was adapted to monolayer continuous cell cultures and deposited National Collection of Microorganism Strains of the FGBI "ARRIAH" as 2348 Itlay/2008 strain. The infectivity titer was determined in IB-RS-2 cell culture-containing 96-well culture plates using micromethod. The passage VII virus cultured in IB-RS-2 cell culture and having infectivity ti-

ter (7.73 ± 0.60) lg $TCID_{50}$/50 µl was used for experimental infection and hyperimmunization of the naturally susceptible animals.

Cell culture. In order to adapt SVDV strain 2348 Italy/2008 the following porcine cell cultures produced in the cell cultivation sector of the Innovation Department of the FGBI "ARRIAH" were used: PKC (primary porcine kidney cell culture), PSGK-30 (continuous porcine cell culture) and IB-RS-2 (continuous porcine kidney cell culture). The cell sensitivity to the virus was determined by a series of "blind" passages in 25 cm^3 plastic culture flasks containing maintenance medium. The blind passages were carried out until apparent cytopathic effect (CPE). The infected cell culture was incubated at (37.0 ± 0.5) °C for at least 72 hours. The duration of each consecutive passage was reduced as the time necessary for the development of 80–100% CPE reduced.

Animals. Three 35–40 kg Yorkshire and Landrace gilts at the age of 4 months old were used for the experimental infection, and they had been delivered from the infectious disease-free farms of the Vladimir Oblast.

All animal experiments were performed in strict compliance with interstate standard on laboratory animal keeping and handling GOST 33215-2014, adopted by Interstate Council on Standardization, Metrology and Certification and pursuant to the requirements of Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

Experimental infection of pigs. The animals were kept isolated at the animal keeping facilities of the FGBI "ARRIAH" for 29 days, and they had free access to water and feed. Pigs 1 and 2 were intradermally infected with the concentrated virus at a dose of 10^9 $TCID_{50}$ inoculated in the snout, coronary bands of both forelegs and one hind leg. For hyperimmune serum production the convalescent animals were additionally immunized on day 21 post infection (dpi), i.e. 100-fold concentrated virus antigen supplemented with incomplete Freund's adjuvant in 1:1 ratio was intramuscularly inoculated. Intact animal 3 was transferred to the isolator with the infected gilts on 4 dpi and they were further kept together. Before and during the experiment, blood samples were regularly collected from all animals at 2–6 day intervals. The blood samples were used for serological tests.

Virus microneutralization test in cell culture. Virus microneutralization test (MNT) in 2348 Italy/2008 SVD virus-based cell culture and sensitive continuous cell culture IB-RS-2 was used for pig serum testing for virus-neutralizing antibodies. The pig serum samples were tested in 96-cell culture plate by serial dilutions with the virus suspension inoculated at pre-calculated working dose. The plate was kept in CO_2-incubator with 5% CO_2 at (37.0 ± 0.5) °C for 1 hour in order the virus neutralizing antibodies contained in the tested sera could interact with the virus. Then the cell culture of (0.8–1.0) × 10^6 cells/cm^3 was inoculated. The plate was again transferred to the CO_2-incubator at (37.0 ± 0.5) °C for 60–72 hours. The reaction was examined using the inverted microscope and the number of cells with the preserved monolayer was calculated. The antibody titer was calculated using Karber's method.

Enzyme-linked immunosorbent assay. Enzyme-linked immunosorbent assay (ELISA) was used for the determination of SVDV antibodies in animal sera. The assay was performed according to the 'Methodical Guidance on Detection of SVDV Antibodies Using Competitive Sandwich ELISA with Monoclonal Antibodies 5B7' (CS-ELISA), and using ELISA kit for SVDV antibody detection (FGBI "ARRIAH", Russia). Commercial PrioCHEK SVDV Ab Kit (Prionics Lelystad B.V., Netherlands) was used as a reference diagnostic test-kit. The test-kits were used according to the manufacturer's instructions.

RESULTS AND DISCUSSION

SVDV adaptation to cell cultures. SVDV strain 2348 Italy/2008 was adapted by means of blind passages in PKC cell culture (3 passages), PSGK-30 (5 passages) and IB-RS-2 (8 passages). For further work the cell culture was selected, to which the virus adapted most rapidly and which required less time for 80–100% CPE development. Herewith, the infectivity titers remained constant during at least five successive passages. The data in Table 1 demonstrate that SVDV strain 2348 Italy/2008 better adapted to the continuous cell lines PSGK-30 and IB-RS-2.

There were no signs of the virus reproduction in PKC cell culture during three passages. During cultivation in PSGK-30 and IB-RS-2 cultures, the virus induced 100% CPE in 48 hours. By passage 3, the time period necessary

Table 1
Adaptive capacity of SVDV strain 2348 Italy/2008 in cell cultures of porcine origin

Cell culture	Passage	Time for 80–100% CPE development, hours	Infectivity titer determined using MNT cell culture, lg $TCID_{50}$/50 µl
PKC	I	none	–
PSGK-30	I	48	n/t
IB-RS-2	I	48	n/t
PKC	II	none	–
PSGK-30	II	48	n/t
IB-RS-2	II	24	n/t
PKC	III	none	–
PSGK-30	III	21	n/t
IB-RS-2	III	18	7.80 ± 0.60
PSGK-30	IV	18	n/t
IB-RS-2	IV	18	7.80 ± 0.60
PSGK-30	V	18	n/t
IB-RS-2	V	18	7.73 ± 0.60
IB-RS-2	VI	18	6.98 ± 0.60
IB-RS-2	VII	18	7.73 ± 0.60
IB-RS-2	VIII	18	6.75 ± 0.60

n/t – not tested.

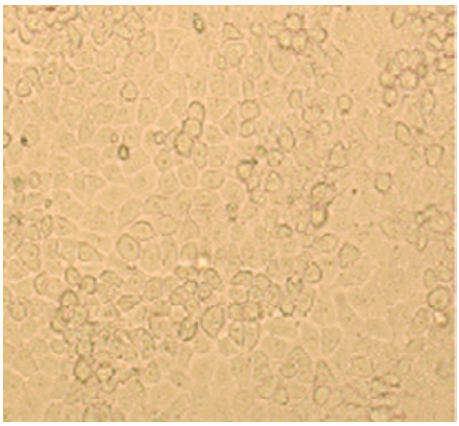


Fig. 1. IB-RS-2 cell culture after 96 hours of cultivation (magnification 200×)

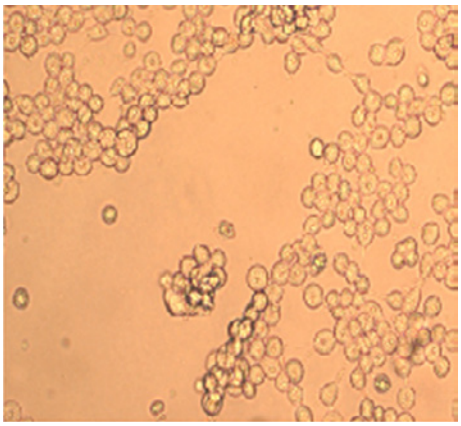


Fig. 2. CPE of SVDV in IB-RS-2 cell culture in 15 hours post inoculation (magnification 200×)

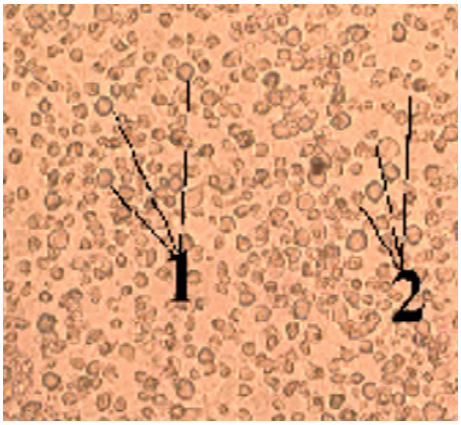


Fig. 3. CPE of SVDV in IB-RS-2 cell culture in 18 hours post inoculation (magnification 200×):
1 – rounded cells, 2 – in the process of degradation

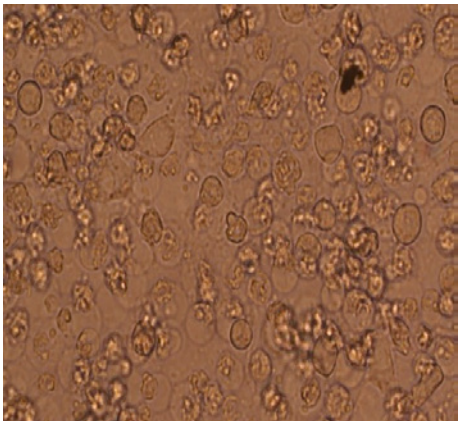


Fig. 4. CPE of SVDV in IB-RS-2 cell culture in 18 hours post inoculation (magnification 400×)

for the complete destruction of the cell monolayer reduced to 21 hours for PSGK-30 and to 18 hours for IB-RS-2. IB-RS-2 cell culture was selected for further work due to its sensitivity to SVDV. The pattern of the cellular degenerative changes caused by 2348 Italy/2008 was typical for SVD agent. In 12–15 hours post inoculation, initially irregular polygon-shaped monolayer cells of IB-RS-2 culture (Fig. 1) slightly stretched under the effect of the accumulated virus and took the shape of a spindle (Fig. 2). Hereafter, the cells rounded, detached from the plastic walls of the culture flask (Fig. 3) and finally shrunk and degraded (Fig. 4).

During six passages in monolayer continuous IB-RS-2 cell culture the SVDV strain 2348 Italy/2008 demonstrated relatively constant infectivity ranging from (6.98 ± 0.60) to (7.80 ± 0.60) lg $TCID_{50}$/50 µl. Seventh passage 100-fold concentrated virus cultured in IB-RS-2 cell culture and having the infectivity titer (7.73 ± 0.60) lg $TCID_{50}$/50 µl was used for the infection of pigs. Cell suspension containing sixth passage SVDV strain 2348 Italy/2008 and having the infectivity titer (6.98 ± 0.60) lg $TCID_{50}$/50 µl was used for microneutralization test.

Infection of naturally susceptible animals. The pigs were observed daily during the whole experiment. The observation involved clinical examination with thermometry, assessment of the general condition of the animals, pattern of movements and neurological signs.

In 2 dpi, animals 1 and 2 infected with the concentrated cultured SVDV strain 2348 Italy/2008 demonstrated clinical signs typical for the acute disease. Reduced motion activity and hyperthermia were reported in both pigs. Initial aphthae were formed at the sites of intradermal inoculation: in pig 1 – on the snout and coronary bands, in pig 2 – on coronary band of three limbs. Generalized infection and formation of secondary aphthae were reported on 5 dpi. Significant improvement of the pigs' health conditions was reported only after the formation of the secondary aphthae and before 7 dpi. Diarrhea was reported in animal 2 on 4 dpi and starting from 5 dpi the animal demonstrated lameness due to hoof inflammation. Herewith, the animal mostly remained laying and laboriously rose on carpal joints. On 11 dpi the animals demonstrated pododermatitis complicated with purulent process formed due to secondary infection. In pig 1 diarrhea was reported and lameness was demonstrated from 7 dpi; they were not so expressed but lasted longer, until 11 dpi, due to pododermatitis formation. During the whole experiment, none of the animals demonstrated any nervous signs, and they did not refuse from feed. Starting

from 5 dpi, both animals demonstrated papular eruption on the belly and in the pelvic area that was later accompanies with itching.

Animal 3 demonstrated no clinic signs of SVD, hyperthermia or behavioral changes.

During the experimental infection samples of biological materials were collected from the diseased animals: aphthous epithelium and lymph. Molecular and genetic tests of the biomaterials confirmed presence of SVDV genome that was similar to the genome of SVDV strain 2348 Italy/2008. The obtained epithelium sample were deposited to the collection of the strains of microorganisms at the Laboratory for FMD Diagnosis, FGBI "ARRIAH".

Serological tests. Blood samples used for serum production were collected from pigs before the experiment and during the whole experiment starting from 4 dpi. Total of 26 samples with different serological activity were collected. Studies showed correlation between the SVDV virus antibody test results obtained using various test methods. The results are demonstrated in Table 2.

The obtained results demonstrated that the diseased pigs had virus-specific antibodies that were detected by all test-kits: in animal 1 – starting from 7 dpi; in animal 2 – starting from 4 dpi. The antibody titers increased until 11 dpi, and they further gradually decreased [8]. Herewith, animal 2 demonstrated higher virus-specific antibody titer as compared to animal 1. For the production of the hyperimmune sera against SVDV the convalescent animals were additionally immunized on 21 dpi. Additional immunization intensified the immune response as expected, and antibody titers in the sera of both pigs reached significant values that was demonstrated by all test results.

Pig 3 did not demonstrate any antibodies by any of the tests that led to the conclusion that the animal was not diseased with SVD, although it was housed with the diseased pigs. It could be supposed that the animal was not infected because of the housing conditions that minimized the virus transmission: the pigs were housed in dry and daily cleaned spacious room. This excluded the contact route of the infection transmission. In spite of the fact that SVD infected animals typically shed the virus with the feces for a long time and the fecal-oral transmission route remains the major one, the concentration of the virus particles in the feces is relatively low as a number of researchers reported [3, 6]. Feeding and watering of the pigs was arranged in such a way as to allow the animals to consume the combined feed in short order and to supply the water in the drinking bowls on an ongoing basis thus continuously renewing it. That excluded contamination of both feed and water by the excrements and therefore prevented the infection transmission from the diseased animals to the contact one either with water or with feed.

CONCLUSION

The research results demonstrated that swine vesicular disease induced by SVDV strain 2348 Italy/2008 was characterized by typical clinical signs and accompanied with the expressed immune response. Early virus-specific antibody response was reported in the infected pigs on 4 dpi. Length of the log and stationary phases of the productive stage of antibody formation following the primary contact with SVDV amounted to about two weeks: the level of virus-specific antibodies reached its maximum on 11–15 dpi and steadily decreased. Following repeated virus inoculation the animals demonstrated evident dynamics in the increase of the virus-specific antibodies that was maintained until the experiment completion. Since SVD is an

Table 2
Activity of sera collected from pigs at different time intervals post disease and immunization with 2348 Italy/2008 SVDV strain and tested using different test-kits ($n > 3$)

Animal	dpi	ELISA results						MNT results	
		ARRIAH test-kit		CS-ELISA		PrioCHEK SVDV Ab Kit			
		lg	result	lg	result	lg	result	lg	result
1	0	< 1	neg.	< 1	neg.	< 1	neg.	< 1	neg.
	4	< 1	neg.	< 1	neg.	1.00	pos.	1.81	pos.
	7	2.20	pos.	2.08	pos.	1.90	pos.	2.58	pos.
	11	2.68	pos.	2.08	pos.	2.08	pos.	2.71	pos.
	15	2.51	pos.	2.08	pos.	1.78	pos.	2.58	pos.
	21*	2.34	pos.	1.78	pos.	1.48	pos.	2.41	pos.
	25	2.68	pos.	2.34	pos.	1.90	pos.	3.01	pos.
	29	3.71	pos.	3.28	pos.	2.98	pos.	3.79	pos.
	31	4.19	pos.	3.71	pos.	3.89	pos.	3.79	pos.
2	0	< 1	neg.	< 1	neg.	< 1	neg.	< 1	neg.
	4	1.78	pos.	1.30	pos.	1.30	pos.	2.11	pos.
	7	2.68	pos.	2.98	pos.	2.98	pos.	2.71	pos.
	11	2.98	pos.	2.81	pos.	2.98	pos.	2.89	pos.
	15	2.68	pos.	2.68	pos.	2.81	pos.	2.71	pos.
	21*	2.68	pos.	2.08	pos.	2.51	pos.	2.71	pos.
	25	2.98	pos.	2.68	pos.	2.51	pos.	3.49	pos.
	29	4.01	pos.	3.58	pos.	3.11	pos.	3.91	pos.
	31	4.19	pos.	3.58	pos.	3.71	pos.	3.91	pos.
3	0	< 1	neg.	< 1	neg.	< 1	neg.	< 1	neg.
	4	< 1	neg.	< 1	neg.	< 1	neg.	< 1	neg.
	7	< 1	neg.	< 1	neg.	< 1	neg.	< 1	neg.
	11	< 1	neg.	< 1	neg.	< 1	neg.	< 1	neg.
	17	< 1	neg.	< 1	neg.	< 1	neg.	< 1	neg.
	21	< 1	neg.	< 1	neg.	< 1	neg.	< 1	neg.
	24	< 1	neg.	< 1	neg.	< 1	neg.	< 1	neg.
	27	< 1	neg.	< 1	neg.	< 1	neg.	< 1	neg.

* booster immunization.

exotic disease for the Russian Federation, the sera of the convalescent experimental animals are of definite value as they can be used as reference sera during the disease laboratory diagnosis.

REFERENCES

1. Swine vesicular disease. *In: OIE. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals.* 2019; Chapter 3.8.8: 1608–1617. Available at: http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.08.08_SVD.pdf.

2. Semakina V. P., Akimova T. P., Solomatina I. Yu., Karaulov A. K. Risk of introducing highly dangerous animal vesicular diseases into the Russian Federation. *Veterinary Science Today*. 2019; 1 (28): 3–15. DOI: 10.29326/2304-196X-2019-1-28-3-15.

3. De Clercq K. Swine vesicular disease: incidence, pathogenicity, epidemiology, diagnosis, economic impact. *Conf. OIE*. 2000; 263–270. Available at: https://www.oie.int/doc/ged/D5674.PDF.

4. Lin F., Kitching R. P. Swine vesicular disease: an overview. *Vet. J*. 2000; 160 (3): 192–201. DOI: 10.1053/tvjl.2000.0505.

5. EFSA Panel on Animal Health and Wellfare (AHAW). Scientific Opinion on Swine Vesicular Disease and Vesicular Stomatitis. *EFSA Journal*. 2012; 10 (4):2631. DOI: 10.2903/j.efsa.2012.2631.

6. Dekker A. Pathogenesis, diagnosis and epizootiology of swine vesicular disease: PhD Thesis. Lelystad: University of Utrecht; 2000. 122 p. Available at: https://dspace.library.uu.nl/bitstream/handle/1874/330939/Dekker.pdf?sequence=1&isAllowed=y.

7. Bellini S., Alborali L., Zanardi G., Bonazza V., Brocchi E. Swine vesicular disease in northern Italy: diffusion through densely populated pig areas. *Rev. Sci. Tech. Off. Int. Epiz*. 2010; 29 (3): 639–648. DOI: 10.20506/rst.29.3.2006.

8. Niedbalski W. Diagnostic value of the isotype-specific ELISA for the detection of antibodies against swine vesicular disease virus (SVDV). *Bull. Vet. Inst. Pulawy*. 2002; 46 (1): 37–44.

9. Bellini S., Santucci U., Zanardi G., Brocchi E., Marabelli R. Swine vesicular disease surveillance and eradication activities in Italy. *Rev. Sci. Tech. Off. Int. Epiz*. 2007; 26 (3): 585–593. PMID: 18293607. DOI: 10.20506/RST.26.3.1766.

10. Brocchi E., Zhang G., Knowles N. J., Wilsden G., McCauley J. W., Marquardt O., et al. Molecular epidemiology of recent outbreaks of swine vesicular disease: two genetically and antigenically distinct variants in Europe, 1987–94. *Epidemiol. Infect*. 1997; 118 (1): 51–61. DOI: 10.1017/s0950268896007170.

11. Niedbalski W. Phylogenetic analysis of Polish isolates of swine vesicular disease virus (SVDV). *Bull. Vet. Inst. Pulawy*. 1999; 43 (2): 139–145.

12. Lugovskaya N. N. Antigenic relationship of strain O-72 to European and Asian strains of swine vesicular disease virus. *Proceedings of the Federal Centre for Animal Health*. 2012; 10: 113–121. eLIBRARY ID: 18881518. (in Russian)

Received on 20.04.2021
Approved for publication on 07.07.2021

INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Yelena N. Kalinina, Candidate of Science (Veterinary Medicine), Junior Researcher, Reference Laboratory for FMD Diagnosis, FGBI "ARRIAH", Vladimir, Russia.

Svetlana N. Fomina, Candidate of Science (Veterinary Medicine), Head of Reference Laboratory for FMD Diagnosis, FGBI "ARRIAH", Vladimir, Russia.

Калинина Елена Николаевна, кандидат ветеринарных наук, младший научный сотрудник референтной лаборатории диагностики ящура ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Фомина Светлана Николаевна, кандидат ветеринарных наук, заведующий референтной лабораторией диагностики ящура ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

DOI: 10.29326/2304-196X-2021-3-38-209-215

Comparative study of PCR test kits for ASFV DNA detection

M. S. Krasnikova[1], S. P. Yatsentyuk[2], M. B. Bryusova[3], A. D. Kozlova[4], M. A. Gergel[5]

FSBI "The Russian State Centre for Quality and Standardization of Veterinary Drugs and Feed" (FSBI "VGNKI"), Moscow, Russia

[1] *ORCID 0000-0002-6248-419X, e-mail: m.krasnikova@vgnki.ru*

[2] *ORCID 0000-0002-4819-2131, e-mail: pcr-lab@vgnki.ru*

[3] *e-mail: m.bryusova@vgnki.ru*

[4] *ORCID 0000-0002-4793-2345, e-mail: adkozlova@vgnki.ru*

[5] *ORCID 0000-0002-8033-1154, e-mail: m.gergel@vgnki.ru*

SUMMARY

The paper presents comparative test results of 12 domestically produced diagnostic kits/PCR test systems for DNA detection of the African swine fever virus with regard to the following parameters: completeness and correctness of instructions for use; labeling and package contents; convenience of using the kit; shelf life stability of reagents; stability of reagents after transportation and repeated freezing – thawing; batch-to-batch repeatability; sensitivity of various test materials and specificity of kits. The study of the instructions for use and kit contents revealed incompleteness of some instructions. It was noted that some manufacturers make serious errors in the instructions, which can significantly affect the interpretation of test results. It was also observed that there is insufficient control of the manufacturing process, which results in the production of faulty kits, as well as kits with poor-quality components and errors in the labeling. Thus, during the study, one kit showed its inactivity, demonstrating the absence of accumulation curves of the fluorescent signal during amplification of both positive controls and DNA of ASFV isolates. When the specificity was assessed, all the kits showed absence of non-specific reactions and acceptable sensitivity when testing various types of ASFV-containing material (blood, suspensions of pork spleen and pork casings used in sausage production). The stability test showed a sharp deterioration in the quality of operation of one kit within the shelf life period, and a significant decrease in the fluorescence signal was detected during repeated freeze – thaw cycles for another kit. Comparison of the repeatability results of different kit batches of the same manufacturer showed significant discrepancies for 41.5% of all kits. It was found that only 33% of the studied kits for ASFV DNA detection were compliant. The results of this study demonstrate the need for control of the manufactured diagnostic kits used in state programs for animal disease monitoring.

Keywords: PCR test system, African swine fever, sensitivity, specificity, stability

Acknowledgements: The study was carried out with the support of the Rosselkhoznadzor within the research work: "Study of quality parameters of domestic and foreign diagnostic PCR test kits used in laboratory veterinary practice in the Russian Federation in order to develop common requirements for quality parameters and methods of their control". The authors express their gratitude to the FGBI "ARRIAH" and the FSBSI FRCVM for providing the ASF virus samples.

For citation: Krasnikova M. S., Yatsentyuk S. P., Bryusova M. B., Kozlova A. D. Gergel M. A. Comparative study of PCR test kits for ASFV DNA detection. *Veterinary Science Today*. 2021; 10 (3): 209–215. DOI: 10.29326/2304-196X-2021-3-38-209-215.

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

For correspondence: Svetlana P. Yatsentyuk, Candidate of Science (Biology), Head of the Department of Genodiagnostics of Infectious Animal Diseases, Department of Biotechnology, FSBI "VGNKI", 123022, Russia, Moscow, Zvenigorodskoye shosse, 5, *e-mail: pcr-lab@vgnki.ru.*

УДК 619:578.842.1:616-076

Сравнительное исследование ПЦР-наборов для выявления ДНК вируса АЧС

М. С. Красникова[1], С. П. Яцентюк[2], М. Б. Брюсова[3], А. Д. Козлова[4], М. А. Гергель[5]

ФГБУ «Всероссийский государственный Центр качества и стандартизации лекарственных средств для животных и кормов» (ФГБУ «ВГНКИ»), г. Москва, Россия

[1] *ORCID 0000-0002-6248-419X, e-mail: m.krasnikova@vgnki.ru*

[2] *ORCID 0000-0002-4819-2131, e-mail: pcr-lab@vgnki.ru*

[3] *e-mail: m.bryusova@vgnki.ru*

[4] *ORCID 0000-0002-4793-2345, e-mail: adkozlova@vgnki.ru*

[5] *ORCID 0000-0002-8033-1154, e-mail: m.gergel@vgnki.ru*

РЕЗЮМЕ

В работе представлены результаты сравнения 12 отечественных диагностических наборов/ПЦР-тест-систем для выявления ДНК вируса африканской чумы свиней по таким показателям, как полнота и грамотность инструкций по применению; маркировка и комплектация; удобство использования наборов; стабильность работы реагентов в течение срока хранения; стабильность реагентов после транспортировки и многократного замораживания – оттаивания; межсерийная сходимость; чувствительность при тестировании различного материала и специфичность наборов. Изучение инструкций по применению и комплектации наборов выявило неполноту некоторых инструкций. Отмечено, что отдельные производители допускают в инструкциях серьезные ошибки, которые могут существенно повлиять на интерпретацию результатов исследования. Также отмечена недостаточность контроля производственного процесса, результатом которой является выпуск неработоспособных наборов, а также наборов с низким качеством компонентов и ошибками в их маркировке. Так, при проведении исследования один набор показал свою неработоспособность, демонстрируя отсутствие кривых накопления флуоресцентного сигнала как при амплификации положительных контролей, так и ДНК изолятов вируса АЧС. При оценке специфичности все наборы показали отсутствие неспецифических реакций и приемлемую чувствительность при тестировании различных типов материала (крови, суспензий свиной селезенки и черевы свиной, используемой при производстве колбасных изделий), содержащих вирус АЧС. Проверка стабильности показала резкое ухудшение качества работы одного набора в пределах срока годности, для другого набора выявлено существенное снижение уровня флуоресцентного сигнала при многократном замораживании – оттаивании. Сравнение сходимости результатов работы разных серий наборов одного производителя показало существенные расхождения для 41,5% наборов. Установлено, что лишь у 33% рассмотренных наборов для выявления ДНК вируса АЧС отсутствуют какие-либо недостатки. Результаты проведенной работы демонстрируют необходимость контроля выпускаемых диагностических наборов, используемых в государственных программах мониторинга заболеваний животных.

Ключевые слова: ПЦР-тест-система, африканская чума свиней, чувствительность, специфичность, стабильность

Благодарность: Работа выполнена при поддержке Россельхознадзора в рамках научно-исследовательской работы по теме: «Изучение параметров качества отечественных и зарубежных диагностических ПЦР-наборов, применяемых в лабораторной ветеринарной практике на территории РФ, с целью разработки единых требований к параметрам качества и методам их контроля». Авторы выражают благодарность ФГБУ «ВНИИЗЖ» и ФГБНУ ФИЦВиМ за предоставленные образцы вируса АЧС.

Для цитирования: Красникова М. С., Яцентюк С. П., Брюсова М. Б., Козлова А. Д., Гергель М. А. Сравнительное исследование ПЦР-наборов для выявления ДНК вируса АЧС. *Ветеринария сегодня*. 2021; 10 (3): 209–215. DOI: 10.29326/2304-196X-2021-3-38-209-215.

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

Для корреспонденции: Яцентюк Светлана Петровна, кандидат биологических наук, заведующий отделом генодиагностики инфекционных болезней животных отделения биотехнологии ФГБУ «ВГНКИ», 123022, Россия, г. Москва, Звенигородское шоссе, 5, *e-mail: pcr-lab@vgnki.ru.*

INTRODUCTION

African swine fever (ASF) is a highly contagious porcine disease caused by the virus of *Asfarviridae* family that affects both domestic and wild pigs of all ages. ASF causes serious economic and production losses and is listed in the Terrestrial Animal Health Code of the World Organization for Animal Health (OIE) as a notifiable disease.

Given that no effective treatment and vaccine currently exist for the disease, ASF prevention largely depends on timely disease outbreak containment and eradication. In the Russian Federation, both domestic pigs and wild boars are annually subjected to diagnostic testing. According to the data of the reports (Form 1-vet A) summarized by the FGBI "Veterinary Centre" more than 670 thousand tests were carried out in 2019 and 473 thousand tests were carried for 9 months of 2020. In 2020, according to the Information Analysis Centre of the Rosselkhoznadzor Department for Veterinary Surveillance, 161 ASF outbreaks in domestic pigs and 110 ASF outbreaks in wild boars in the Russian Federation were reported and notified to the OIE [1].

Currently, polymerase chain reaction (PCR) is widely used for ASF diagnosis. Veterinary laboratories commonly use domestically produced reagent kit (test-kits) for PCR tests. The laboratories carrying out diagnostic tests in the framework of the official programmes shall be accredited by the Federal Service for Accreditation (RusAccreditation). Documents laying down test procedures and test methods shall be indicated in the scope of accreditation of the testing laboratory. Often such documents present themselves instructions to the test-kits or reagent kits and the laboratories shall strictly follow such instructions. The problem is that current diagnostic test systems do not undergo mandatory official registration and certification, there is no list of requirements for them, the instruction text is not approved and the kits are not subjected to independent checks for their quality. This could result in release of substandard kits that in case of their use in tests could be responsible for ineffective diagnosis.

There are several types of PCR test systems/kits for ASF diagnosis on the Russian PCR diagnostica market that contain various components including those with electrophoretic detection and real time hybridization-fluorescent detection of amplification products; full-optional kits containing both PCR reagent kit and DNA extraction kit and, in case of electrophoretic detection, electrophoresis

kit, as well as kits designed for PCR only. For the latter kits, the manufacturer could provide recommendations in the instructions on the nucleic acid extraction kit to be used together with its PCR kit or extraction method to be used or indicate that any kit for nucleic acid extraction can be used. PCR control samples are essential kit components. However, there is also no uniformity here: some kits include an endogenous and/or exogenous internal control sample (ICS) allowing control of sample collection procedure and extraction quality, other kits do not contain ICS that reduces the reliability of diagnostic testing. No comparative assessment of the diagnostic kits for ASFV DNA detection available on the Russian diagnostica market has been carried out yet.

The study was aimed at comparative assessment of domestically produced diagnostic PCR kits for ASFV DNA detection for the following parameters: completeness and correctness of instructions for use; labeling and kit contents; convenience of the kit usage; stability of the reagents during the shelf life declared by the manufacturer; stability of reagents after transportation and repeated freezing – thawing; batch-to-batch repeatability when different batches of the kits from the same manufacturer were used; detection limit (sensitivity) when the kit was used for testing various materials and specificity of kits.

MATERIALS AND METHODS

The PCR kits for ASFV DNA detection produced by the following Russian manufacturers were assessed: the Federal Budget Institution of Science "Central Research Institute of Epidemiology" of the Federal Service for Customers' Rights Protection and Human Wellbeing (Rospotrebnadzor), "Lytech" Co. Ltd., Syntol JSC, "FBio" Co. Ltd., Innovative Diagnostic Systems Ltd., "VetFactor" Ltd., "VMT" Ltd., "Vetbiochem" Ltd., "Technology Centre" Ltd., "Organic-Test" Ltd. The assessed kits and their manufacturers were coded when the study results were discussed.

VetMAX ASFV Detection Kit (Thermo Fisher Scientific Inc., USA) validated and certified by the OIE (approval number: 20200114) was used as a reference diagnostic test-system.

A panel of the following 38 different samples, including bacterium and virus strains, was used for testing of the kits for their specificity: 'Skif' strain of Aujeszky's disease virus, 'Ilyinogorsky' strain of porcine transmissible gastroenteritis virus, 'IS' strain of porcine epidemic diarrhea virus, 'KS' and 'LK-VNIIVViM' strains of classical swine fever virus, 'VL90-94' strain of porcine parvovirus, 'G10 P11' strain of rotavirus, 94881 strain of porcine reproductive and respiratory syndrome virus, 1010 strain of porcine circovirus, *Bordetella bronchiseptica* ATCC 4617, *Brachyspira pilosicoli* ATCC 51139, *Brucella abortus* 82 ser. 022, *Brucella suis* 1330 strain, *Campylobacter jejuni* '70.2T strain', *Chlamydia psittaci* 'LS-87 strain', *Clostridium perfringens* 'Amo' strain, *Escherichia coli* 0157:H7, *Erysipelothrix rhusiopathiae* ATCC 8139, *Haemophilus parasuis* 'Uralsky' strain, *Histophilus somni* ATCC 700025, *Klebsiella pneumoniae* 'K2 5055' strain, *Lawsonia intracellularis* 'MS B3903' strain, *Leptospira interrogans* Pomona 'VGNKI-6 strain', *Listeria monocytogenes* 'USKHI-6' strain, *Mycobacterium avium* 'D4' strain, *Mycobacterium bovis* 1414 strain, *Mycobacterium paratuberculosis* 19698 strain, *Mycoplasma hyopneumoniae* 'J' strain, *Mycoplasma hyorhinis* field isolate, *Pasteurella multocida* ATCC 43137, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enterica* 'Dublin 6' strain, *Shigella sonnei* ATCC 25931, *Staphylococcus aureus* 'VKPMV 6646' strain, *Streptococcus pyogenes* ATCC 19615, *Yersinia enterocolitica* 'My O3 BNIPCHI Microbe' strain, *Yersinia pseudotuberculosis* 192 strain, as well as porcine genome DNA and a panel of 6 suspension samples containing ASF virus obtained from the FGBI "ARRIAH" collection (Kaliningrad 10/17, Oryol 07/18, Arm07, Krasnodar 07/17, Leningrad 02/19 isolates) and the Federal Research Centre for Virology and Microbiology ('Stavropol 01/08' strain).

The sensitivity (limit of detection) of the full-optional kits comprising both PCR reagent kit and DNA extraction kit in different matrices – biological materials (blood, spleen) and porcine small casing used for sausage production – was assessed for the full-optional kit as a whole. 'A DNA/RNA-S-Factor' DNA extraction kit ("VetFactor" Ltd., Russia) was used for the assessment of the test-kits intended for PCR assay only without any recommendations for DNA extraction in the instructions for their use. Ten-fold serial dilutions of ASFV Leningrad 02/19 isolate (initial titre 6.2 lg HAU_{50}/cm^3) were prepared in 10% porcine spleen and casing suspensions and in blood. DNA extraction and subsequent PCR were carried out in triplicate for each dilution of the materials of each type.

Serial dilutions of ASFV Kaliningrad 10/17 isolate (initial titre 5.8 lg HAU_{50}/cm^3) prepared with saline solution were used for comparative assessment of the amplification kits (without DNA extraction stage) for their sensitivity. 'Ribo-prep' kit (Federal Budget Institution of Science "Central Research Institute of Epidemiology" of the Rospotrebnadzor) was used for DNA extraction. Extracted DNA was used for PCR amplification using kits of different manufactures. Each virus dilution was tested with PCR in triplicate.

Nucleic acid was extracted from ten-fold dilutions of ASFV Leningrad 02/19 isolate prepared with saline solution for comparative assessment of the effectiveness of DNA extraction using extraction kits from different manufacturers. PCR was carried out with 'VetMAX ASFV Detection Kit' (Thermo Fisher Scientific Inc., USA), reference reagent kit.

DNA amplification was carried out with 'CFX96 C1000 Touch' (Bio-Rad Laboratories Inc., USA), 'Rotor-Gene Q' (QIAGEN GmbH, Germany) and 'Tertsik' (DNA-Technology LLC, Russia) depending on the PCR product detection method indicated by the kit manufacturer.

For testing reagent kits for their stability, results of positive and negative control amplification were assessed every three months within the kit shelf life period. To test reagents for their resistance to transportation temperature conditions recommended by their manufacturers as well as to repeated freezing – thawing every reagent was divided into three equal parts. The first part was kept at temperature recommended by the manufacture for the reagent storage period. The second part was subjected to multiple freeze-thaw cycles (up to 15 cycles). The third part was kept in a thermo insulating plastic foam box placed in ice for maximum transportation period indicated by the manufacturer. Comparative assessment of positive and negative control amplification results for each diagnostic kit was carried out in several repeats upon the testing completion.

Identical reagent kits of different batches were compared for their performance to assess batch-to-batch

reproducibility taking into account data of comparative assessment of positive controls and dilutions thereof as well as ASFV DNA-containing samples for their amplification.

Repeatability- and reproducibility-related precision was determined as closeness of the measurements obtained with multiple analyses of the same sample [2, 3]. The arithmetic mean of the threshold cycle Ct, the standard deviation and the coefficient of variation were calculated. The set of the obtained data was considered homogeneous when the coefficient of variation was less than or equal to 10%; sufficiently homogeneous – when the coefficient of variation was within 10–20%; sufficiently heterogeneous – when the coefficient of variation was within 20–33%; heterogeneous – when the coefficient of variation was higher than 33% [4].

RESULTS AND DISCUSSION

Twelve test-kits from 10 manufacturers were included in the study: 10 PCR test kits with real time hybridization-fluorescent detection and 2 PCR test kits with electrophoretic detection.

The study of the instructions for use to the reagent kits (test-systems) has revealed that some manufactures pay insufficient attention to their preparation as well as to their component labeling. There are serious errors in the instructions for use including those that contradict the regulations for the laboratories that use nucleic acid amplification methods [5]. Such errors could seriously affect the test results interpretation and lead to false positive or false negative results. Some instructions lack data on the sample preparation for DNA extraction. For test-kit No. 12, the manufacturer replaced the instruction for use by the leaflet that contained information on DNA amplification procedure and on interpretation of the results but lacked the description of the kit components as well as the reagent storage and transportation conditions.

Analysis of the kits' contents and ergonomics has showed that some manufacturers incorrectly estimate the control sample amounts without regard for the probable tests of single biological material samples in the laboratory.

Tests of all test-kits for their specificity showed that they, except for test-kit No. 12, correctly detected ASFV DNA in all tested samples containing ASFV isolates recovered on the territory of Russia at different times.

Amplification kit No. 12 demonstrated its malperformance when nine reagent kits of the said manufacturer were tested by different operators using different machines at different times. No fluorescent signal against ASFV was detected during the amplification of DNA extracted from the virus isolates as well as positive controls included in the test-kits. Therefore, PCR test-kits from the said manufacturer were excluded from the other tests.

Results of assessment of the amplification kits for their sensitivity without taking into account DNA extraction stage as well as assessment of the sets of primers for their sensitivity during testing of the DNA extracted from the materials of different types (blood, porcine spleen and casing suspensions) are summarized in Table 1.

Decrease in the virus detection limit was found for some kits when they were used for tests of the porcine

Table 1
Comparative results of sensitivity assessment of amplification kits when testing various ASFV-contaminated materials

Designation of coded test-system/kit	Sensitivity of ASFV DNA amplification kits			
	without DNA extraction stage	when DNA is extracted from the materials of different types		
	detected titre of ASFV Kaliningrad 10/17 isolate in saline solution (initial titre 5.8 lg HAU_{50}/cm^3)	detected titre of ASFV Leningrad 02/19 isolate (initial titre 6.2 lg HAU_{50}/cm^3)		
		in porcine blood	in porcine spleen suspension	in porcine casing suspension
No. 1	0.8	3.2	4.2	3.2
No. 2	0.8	3.2	3.2	3.2
No. 3	0.8	2.2	2.2	2.2
No. 4	0.8	2.2	2.2	1.2
No. 5	1.8	4.2	3.2	2.2
No. 6	1.8	3.2	4.2	3.2
No. 7	1.8	3.2	3.2	2.2
No. 8	1.8	3.2	3.2	4.2
No. 9	1.8	4.2	4.2	4.2
No. 10	1.8	3.2	3.2	2.2
No. 11	2.8	3.2	3.2	4.2

Table 2
Comparative test results of different batches of kits with real time hybridization-fluorescence detection

Designation of coded test-system/kit	Amplification of positive control sample detected as	Batch 1, Ct	Batch 2, Ct	Standard deviation	Coefficient of variation, %
No.1	ASF	17.36	18.03	0.34	1.89
	ICS	19.28	19.50	0.11	0.57
No. 2	ASF	26.89	28.87	0.99	3.55
	ICS	29.35	22.55	3.40	13.40
No. 3	ASF	19.22	17.35	0.93	5.11
	ICS	20.23	19.51	0.36	1.81
No. 4	ASF	32.68	34.14	0.73	2.18
	ICS	31.35	32.14	0.40	1.24
No. 5	ASF	23.15	23.24	0.04	0.19
	ICS	20.44	20.37	0.04	0.17
	exogenous ICS	21.76	21.83	0.03	0.16
No. 7	ASF	23.68	16.61	3.54	17.55
No. 9	ASF	16.87	20.32	1.73	9.28
No. 10	ASF	19.69	19.87	0.09	0.46
	ICS	24.17	26.34	1.09	4.30
No. 11	ASF	absence of detection	10.15	–	–

materials of different types. This fact becomes important in case of testing of porcine food products (sausages, minced meat) for African swine fever in which the virus concentration could be small. Therewith, some of amplification kits (No. 2, 3, 9) showed similar sensitivity regardless of the type of tested material. In general, it should be noted that all amplification kits demonstrated suitable sensitivity. Therefore, number and type of tested samples as well as contamination risk should be considered when choosing among them.

Since the stage of nucleic acid extraction plays an important role in the PCR testing, effectiveness of the DNA extraction with the kits of different manufacturers was assessed separately. Extraction effectiveness was assessed by comparing PCR results obtained using the reference kit, 'VetMAX ASFV Detection Kit'.

The effectiveness of DNA extraction with kits No. 7–9 was found to be lower than that one of the other tested kits. ASF virus extracted with the said kits was detected with 'VetMAX ASFV Detection Kit' at a titre of 4.2 lg HAU_{50}/cm^3. Whereas, ASF virus extracted with kits No. 1–6 and 10 was detected with 'VetMAX ASFV Detection Kit' at a titre of 2.2 lg HAU_{50}/cm^3. Despite of malperformance of PCR kit No. 12 the assessment showed its sufficiently high DNA extraction effectiveness when different extraction kits from this manufacturer were used: all reagent kits based on different DNA extraction methods demonstrated similar effectiveness (detected virus titre 3.2 lg HAU_{50}/cm^3).

It should be noted that all assessed DNA extraction kits complied with their intended use and allowed effective DNA extraction when they were used for tests of the materials of animal origin.

Tests of the kits for their stability during their shelf life showed drastic decrease in kit No. 8 performance at the last time point of storage (the 12th month of storage). Other kits demonstrated high and sufficient consistency of PCR results during the whole storage period with variation coefficient of 10% or within 10–20%.

Assessment of the kit components for their resistance to multiple freezing – thawing and storage under transportation conditions showed high stability of ten out of eleven kits. For kit No. 7, two-fold fluorescent signal decrease during amplification product detection was found after the kit reagents were subjected to multiple freezing – thawing as compared with the fluorescent signal obtained when aliquots of the original reagents were used.

Comparative testing of different batches of the kit from same manufacturer revealed batch-to-batch discrepancies in the kit performance for five out of eleven tested kits. Kit No. 6 with electrophoretic detection of amplification products showed high consistency of the results for two different batches, whereas kit No. 8 with analogous

detection method demonstrated discrepancies for positive extraction control included in the kit as a component: for one kit batch amplification product was detected when ten-fold dilution of this component was used and for other kit batch – amplification product was detected when the non-diluted component was used. This could be indicative of shortcomings during the control sample production and presence of large number of PCR inhibitors in the reagent of the first batch of the kit.

Table 2 shows results of the comparative testing of positive controls included in the real time hybridization-fluorescent detection PCR reagent kits for ASFV DNA detection.

For test-kit No. 7, the difference in Ct values when positive control was amplified with different batches of the kit was 7 (coefficient of variance higher than 10%); whereas, ASFV DNA amplification results were almost identical. Also, malperformance of internal control amplification system was revealed for one batch of the kit. Generally, this is indicative of unstable quality of control samples, set of primers and probes of the said manufacturer.

Comparison of different batches of kit No. 2 revealed the 4-fold difference in fluorescent signal during detection of positive sample amplification products (coefficient of variance higher than 10%).

Differences in PCR internal control amplification were revealed for different batches of kit No. 10: difference in Ct values when PCR internal control was detected was more than 14 (coefficient of variance was higher than 25%) that was indicative of unstable production quality of that component of the kit.

Low repeatability of the results was demonstrated for different batches of kit No. 11: the kits of one batch showed good performance whereas kits of other batch (three kits of the batch were tested by different operators) did not demonstrate exponential increase in fluorescent signal intensity curves both for positive controls included in the test-kits and for control samples containing ASF virus.

CONCLUSION

The study shows that only four out of twelve assessed reagent kits for ASFV DNA detection from three different manufactures of PCR test systems for veterinary use have no disadvantages impeding their maximum effective use.

It is noted that some PCR test-kit manufacturers pay insufficient attention to the drawing-up of the instruction for their kit use and to the kit contents. Insufficient control of the kit production process results in labelling incompliance, poor quality of the components and, finally, malperformance of control samples, reagents and the whole test-kit. It was shown that sensitivity of the same kits used for tests of different materials indicated in their instructions for use could differ by two orders of magnitude. Kits of all manufacturers demonstrated absence of non-specific reactions; tests of the kits for their stability and batch-to-batch repeatability of the results showed that only five out of twelve tested diagnostic kits complied with the said parameters.

Currently, comparative assessment of diagnostic test systems is carried out only in the laboratory or by the provider during analysis of proficiency testing runs. The study results demonstrate the importance of official registration and regular control of the manufactured diagnostic kits used for official programmes on animal disease monitoring. Similar regulatory procedures for diagnostic kit market exist in the EU, USA and Canada. Analysis of these procedures has showed that, first, the following is taking into account during veterinary diagnosticum assessment: its compliance with the intended use, specificity, sensitivity, reproducibility of the results obtained with the reagent kit. There is a comparable procedure in the Russian Federation: procedure for medical device registration aimed at marketing of quality and safe products in Russia. Development of similar procedure for official control of veterinary diagnostic kits is a currently important task.

REFERENCES

1. ASF epidemic situation in the Russian Federation in 2020 [Epizooticheskaya situaciya po ACHS v Rossijskoj Federacii v 2020 g.]. Available at: https://fsvps.gov.ru/fsvps-docs/ru/iac/asf/2020/12-31/01.pdf (date of access: 02.01.2021). (in Russian)

2. Polyakov I. V., Sokolova N. S. Practical guide to medical statistics [Prakticheskoe posobie po medicinskoj statistike]. L.: Meditsyna; 1975; 26–30. (in Russian)

3. Polyakova V. V., Shabrova N. V. Fundamentals of the theory of statistics [Osnovy teorii statistiki]: Study Guide. 2nd ed., revised and updated. Yekaterinburg: Ural University Publishing House; 2015. 145 p. (in Russian)

4. Sundar Rao P. S. S., Richard J. Introduction to biostatistics and research methods. 5th ed. New Delhi: PHI Learning Private Limited; 2012. 280 p.

5. Methodical Guidelines 1.3-2569-09 Organization of laboratory activities using methods for amplification of nucleic acids and handling materials containing microorganisms of I–IV pathogenicity groups [MU 1.3-2569-09 Organizaciya raboty laboratorij, ispol'zuyushchih metody amplifikacii nukleinovyh kislot pri rabote s materialom, soderzhashchim mikroorganizmy I–IV grupp patogennosti]: Methodical Guidelines. M.: Federal Center for Hygiene and Epidemiology of Rospotrebnadzor; 2010. 51 p. Available at: https://www.rospotrebnadzor.ru/documents/details.php?ELEMENT_ID=4801. (in Russian)

Received on 09.03.2021
Approved for publication on 26.05.2021

INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Maria S. Krasnikova, Candidate of Science (Biology), Senior Researcher, Department for Genodiagnostics of Infectious Animal Diseases of VGNKI, Department of Biotechnology, FSBI "VGNKI", Moscow, Russia.

Красникова Мария Сергеевна, кандидат биологических наук, старший научный сотрудник отдела генодиагностики инфекционных болезней животных отделения биотехнологии ФГБУ «ВГНКИ», г. Москва, Россия.

Svetlana P. Yatsentyuk, Candidate of Science (Biology), Head of the Department for Genodiagnostics of Infectious Animal Diseases of VGNKI, Department of Biotechnology, FSBI "VGNKI", Moscow, Russia.

Maria B. Bryusova, Candidate of Science (Biology), Leading Researcher, Head of the Department for Biological Object Studies FSBI "VGNKI", Moscow, Russia.

Aleksandra D. Kozlova, Candidate of Science (Biology), Senior Researcher, Department for Genodiagnostics of Infectious Animal Diseases of VGNKI Department of Biotechnology, FSBI "VGNKI", Moscow, Russia.

Mariya A. Gergel, Deputy Director and Head of Testing Center, FSBI "VGNKI", Moscow, Russia.

Яцентюк Светлана Петровна, кандидат биологических наук, заведующий отделом генодиагностики инфекционных болезней животных отделения биотехнологии ФГБУ «ВГНКИ», г. Москва, Россия.

Брюсова Мария Борисовна, кандидат биологических наук, ведущий научный сотрудник, заведующий отделом исследований биологических объектов ФГБУ «ВГНКИ», г. Москва, Россия.

Козлова Александра Дмитриевна, кандидат биологических наук, старший научный сотрудник отдела генодиагностики инфекционных болезней животных отделения биотехнологии ФГБУ «ВГНКИ», г. Москва, Россия.

Гергель Мария Александровна, заместитель директора, руководитель испытательного центра ФГБУ «ВГНКИ», г. Москва, Россия.

DOI: 10.29326/2304-196X-2021-3-38-216-223

Effect of pig serum storage conditions on detection of anti-ASFV antibodies by ELISA

A. R. Shotin[1], I. Yu. Zhukov[2], A. S. Pershin[3], Ali Mazloum[4], I. V. Shevchenko[5], A. S. Igolkin[6], O. A. Manuylova[7], K. N. Gruzdev[8]

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

[1] *ORCID 0000-0001-9884-1841, e-mail: shotin@arriah.ru*

[2] *ORCID 0000-0002-3817-2129, e-mail: zhukov@arriah.ru*

[3] *ORCID 0000-0002-5099-3050, e-mail: daredron@gmail.com*

[4] *ORCID 0000-0002-5982-8393, e-mail: mazlum@arriah.ru*

[5] *ORCID 0000-0001-6482-7814, e-mail: shevchenko@arriah.ru*

[6] *ORCID 0000-0002-5438-8026, e-mail: igolkin_as@arriah.ru*

[7] *ORCID 0000-0002-4616-5316, e-mail: o.manuylova@list.ru*

[8] *ORCID 0000-0003-3159-1969, e-mail: gruzdev@arriah.ru*

SUMMARY

One of the measures used to control and prevent African swine fever spread in the Russian Federation involves testing pig and boar sera using *inter alia* serological tools based on enzyme-linked immunosorbent assay (ELISA) for anti-viral antibody detection. However, there is no unified regulatory document specifying storage conditions for sera used in the ELISA for anti-ASFV antibody detection. There are also lack of published data on the maximum admissible shelf life of the pig sera, and the effect of storage conditions on the serological status of the pig sera as for ASF is understudied. The paper demonstrates results of the experiment aimed at the determination of the effect of storage temperatures and shelf life on the serological status of ASFV seropositive and seronegative pig sera when tested by INgezim PPA Compac (Ingenasa, Spain) ELISA as well as on the possibility of false results. During the experiment and analysis of its results, the new data were obtained, and they indicated from none to non-significant effect of the simulated storage conditions on the serological status of sera used for ASFV detection, while hemolyzed sera demonstrated more significant changes proportional to hemolysis degree and storage duration. Although the results of detection of antibodies against the agents of some diseases cannot be used in case of other pathogens, this study has a substantial applied significance as it allows to specify the dependence of the valid results of ASF serodiagnosis on the storage conditions of the samples.

Keywords: African swine fever, enzyme-linked immunosorbent assay, serum, storage conditions

Acknowledgements: The experiment was funded by the federal government as a part of the research activities "Applied Research" (SA No. 081-00010-19-00 от 28.12.2018).

For citation: Shotin A. R., Zhukov I. Yu., Pershin A. S., Mazloum Ali, Shevchenko I. V., Igolkin A. S., Manuylova O. A., Gruzdev K. N. Effect of pig serum storage conditions on detection of anti-ASFV antibodies by ELISA. *Veterinary Science Today*. 2021; 10 (3): 216–223. DOI: 10.29326/2304-196X-2021-3-38-216-223.

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

For correspondence: Andrey R. Shotin, Post-Graduate Student, Leading Biologist, Reference Laboratory for African Swine Fever, FGBI "ARRIAH", 600901, Russia, Vladimir, Yur'evets, *e-mail: shotin@arriah.ru.*

УДК 619:616.98:578.842.1:611.018.54:616-078

Влияние условий хранения сывороток крови свиней на выявление антител к вирусу АЧС методом ИФА

А. Р. Шотин[1], И. Ю. Жуков[2], А. С. Першин[3], Али Мазлум[4], И. В. Шевченко[5], А. С. Иголкин[6], О. А. Мануйлова[7], К. Н. Груздев[8]

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

[1] *ORCID 0000-0001-9884-1841, e-mail: shotin@arriah.ru*

[2] *ORCID 0000-0002-3817-2129, e-mail: zhukov@arriah.ru*

[3] *ORCID 0000-0002-5099-3050, e-mail: daredron@gmail.com*

[4] *ORCID 0000-0002-5982-8393, e-mail: mazlum@arriah.ru*

[5] *ORCID 0000-0001-6482-7814, e-mail: shevchenko@arriah.ru*

[6] *ORCID 0000-0002-5438-8026, e-mail: igolkin_as@arriah.ru*

[7] *ORCID 0000-0002-4616-5316, e-mail: o.manuylova@list.ru*

[8] *ORCID 0000-0003-3159-1969, e-mail: gruzdev@arriah.ru*

РЕЗЮМЕ

Одной из мер борьбы с распространением и профилактики африканской чумы свиней в Российской Федерации является проведение исследований проб, отбираемых от свиней и кабанов, в том числе серологическими методами с использованием иммуноферментного анализа (ИФА) для качественного определения содержания антител к вирусу. При этом в настоящее время на территории страны не существует нормативного документа, регламентирующего условия хранения проб сыворотки крови для постановки ИФА при определении содержания антител к вирусу АЧС. Также отсутствуют литературные данные о максимально допустимом сроке хранения проб сыворотки крови свиней, а влияние условий хранения на серологический статус сывороток крови домашних свиней в отношении АЧС изучено недостаточно. В статье представлены результаты эксперимента по определению влияния температурных режимов и длительности хранения серопозитивных и серонегативных в отношении вируса АЧС сывороток крови домашних свиней на их серологический статус при постановке тест-системой INgezim PPA Compac (Ingenasa, Испания) для твердофазного ИФА и вероятности получения ложных результатов. В ходе выполнения работы и анализа результатов получены новые данные, свидетельствующие об отсутствии или незначительном влиянии моделируемых режимов хранения на определение серологического статуса качественных проб сывороток крови в отношении вируса АЧС, в то время как гемолизированные пробы показали более заметное изменение, пропорциональное степени гемолиза и длительности хранения. Несмотря на то что полученные результаты по обнаружению антител к возбудителям одних болезней не применимы для других патогенов, данное исследование имеет существенное прикладное значение, позволяя установить зависимость получения достоверных результатов при серодиагностике АЧС от условий хранения проб.

Ключевые слова: африканская чума свиней, иммуноферментный анализ, сыворотка крови, условия хранения

Благодарность: Исследование выполнено за счет средств федерального бюджета в рамках проведения научно-исследовательских работ по теме: «Проведение прикладных научных исследований» (ГЗ № 081-00010-19-00 от 28.12.2018).

Для цитирования: Шотин А. Р., Жуков И. Ю., Першин А. С., Мазлум Али, Шевченко И. В., Иголкин А. С., Мануйлова О. А., Груздев К. Н. Влияние условий хранения сывороток крови свиней на выявление антител к вирусу АЧС методом ИФА. *Ветеринария сегодня*. 2021; 10 (3): 216–223. DOI: 10.29326/2304-196X-2021-3-38-216-223.

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

Для корреспонденции: Шотин Андрей Романович, аспирант, ведущий биолог референтной лаборатории по африканской чуме свиней ФГБУ «ВНИИЗЖ», 600901, Россия, г. Владимир, мкр. Юрьевец, *e-mail: shotin@arriah.ru.*

INTRODUCTION

African swine fever (ASF) is a contagious viral disease of domestic pigs and wild boar, manifested in susceptible animals as a hemorrhagic fever with a mortality rate of up to 100% [1]. The virus reservoirs are warthogs, bush and wild pigs, ticks of the *Ornithodoros* genus. The disease has a disastrous effect on pig farming as a whole, including establishments of all forms of ownership (starting from backyards to commercial industrial pig farms), leading to severe socio-economic consequences and compromising food security of the infected territory [2, 3].

African swine fever was first described by R. Montgomery in Kenya in 1921. Subsequently, ASF outbreaks were reported in most countries in Southern and Eastern Africa. In 1957, the porcine disease caused by the ASF virus was first registered in Europe. By mid-2020, the ASF virus was reported on the African, European and Asian continents, the Malay Archipelago and in the countries of Oceania [4].

To date, commercially available means of ASF specific prevention have not been developed. In this regard, the only way to control the disease is to implement a set of preventive measures, early diagnosis using modern and accurate methods, killing of infected and risk animals and introduction of strict restrictive measures (quarantine) [5, 6].

One of the measures to control ASF spread and prevention in the Russian Federation is to conduct monitoring tests [7] and quarantine tests (before shipment and/or when introducing new animals from/to farms), including serological methods using enzyme-linked immunosorbent assay (ELISA) for qualitative measurement of the viral antibodies in porcine sera [8, 9]. To date, there are many commercial ELISA test kits. One of the kits used in the FGBI "ARRIAH" Reference Laboratory for ASF is INgezim PPA Compac (Ingenasa, Spain) kit, which, according to various authors, has a 98 to 100% specificity.

According to H. C. Bergeron et al., false-positive results may be associated with poor sample quality [10]. According to the instructions for the INgezim PPA Compac kit and the recommendations of the World Organization for Animal Health (OIE), the use of hemolyzed or contaminated samples can lead to false-positive results in ELISA. As indicated in the Guidelines of the Food and Agriculture Organization of the United Nations (FAO), sera should be tested immediately after collection or put to storage at a temperature below minus 20 °C before testing, since an accurate diagnosis can be made only when the samples are in satisfactory condition [11].

However, according to the results of L. Mur et al. [12], when testing 158 sera samples using the INgezim PPA Compac kit, among which 49 were moderately and 28 were extensively hemolyzed, 11 gave a false-positive result and 6 were inconclusive. At the same time, there

was no correlation between hemolysis and false-positive results.

Based on general recommendations, it is known, that not frozen serum should be delivered to the diagnostic laboratory during the first 24 hours and in exceptional cases – no later than the third day after blood collection [11, 13, 14].

Current veterinary and sanitary rules of the Republic of Belarus regulating the procedure of serum and blood plasma collection allow freezing of samples and transportation of frozen samples. If it is not possible to freeze the serum and plasma samples, it is allowed to store and transport them at a temperature of 2 to 6 °C within no more than 48 hours after sample collection [15].

For serological testing, it is allowed to send whole blood to the laboratory without separating the serum, provided (difficult to implement) that it will not be shaken and will not undergo hemolysis on the way [16].

In the laboratory, the serum (without a clot) is stored in refrigerators at a temperature of (5 ± 3) °C for no more than 7 days before testing. For longer storage, the serum should be frozen at minus 20 °C or lower. Re-freezing of serum is not allowed [14].

It should be noted that currently there is no regulation in the Russian Federation regulating the conditions of serum sample storage for ELISA testing when determining the level of ASFV antibodies. Such standards are available, in particular, for brucellosis, where phenol or boric acid preserved serum samples are suitable for testing for 30 days, frozen samples – for 3 days after single thawing. Cloudy, bacteria and fungi contaminated, hemolysed blood sera are not suitable for testing for brucellosis [17].

To date, only the storage conditions for organ and blood samples are available, which are stored and transported in an insulated container at a temperature of 4 to 8 °C within no more than 24 hours after collection. For longer storage, the samples should be frozen [13].

There are no published data on the maximum permissible period for porcine serum sample storage, during which 95% of samples will demonstrate unchanged serological status when tested by ELISA. This parameter was established for samples containing antibodies against some other viruses, for example, against the hepatitis D virus; the analyte stability in serum at a temperature of 4 to 8 °C is 4 weeks, from 20 to 25 °C is 5–7 days [18].

The effect of storage conditions on the serological status of domestic pig sera for ASF testing has not been sufficiently studied. There are some published works on studies of the temperature effect (50 °C, 4 °C, minus 10 °C, freezing – thawing cycle), the storage time and the hemolysis degree effect on determination of antibodies against *Erysipelothrix rhusiopathiae* in pigs and *Suid herpesvirus* 1 in wild boar in hemolyzed and not-hemolyzed sera [19, 20]. The authors concluded that the storage conditions had a negligible effect on testing of high-quality serum samples, while the hemolyzed samples showed a more noticeable change proportional to the degree of hemolysis and the duration of storage. It should be noted that the results obtained for the detection of antibodies against some viruses are not applicable for other pathogens.

The aim of the work was to determine the effect of temperature conditions and duration of storage of ASF seropositive and seronegative samples of domestic pigs on their serological status when tested using INgezim PPA Compac solid-phase ELISA test kit (Ingenasa, Spain) and the probability of obtaining false results.

This study has a significant practical value, and allows to establish the relationship between the reliable results in ASF serological diagnostics and the sample storage conditions.

MATERIALS AND METHODS

Equipment. The storage conditions (4 °C, minus 20 °C and a multiple freeze – thaw cycles) were reproduced using a laboratory refrigerator HL-340 (POZIS, Russia). The sera during ELISA test were incubated in a thermoshaker PST-60HL-4 (BioSan, Latvia). The results were read using a Multiskan FC spectrophotometer (Thermo Fisher Scientific, Finland).

Samples. Ten seropositive samples collected on Days 25–26 after experimental infection of pigs with the strain "ARRIAH/ASF-VERO (40)" and ten seronegative samples prepared from the blood of clinically healthy domestic pigs (pig breeding complex, Moscow Oblast) were used. The samples had no signs of hemolysis and were obtained in accordance with the "Rules for Collection of Pathological Material, Blood, Feedstuffs and Their Submission for Laboratory Testing" [16].

Test kit. Serum samples were tested using the INgezim PPA Compac solid-phase ELISA test kit (Ingenasa, Spain) in duplicate. According to the kit instructions, the status of the tested sera was expressed using coefficient of inhibition calculated by the formula:

$$x\% = \frac{\text{NC} - \text{SAMPLE OD}}{\text{NC} - \text{PC}} \times 100,$$

where NC is the value of the absorbance units (AU) of the negative control;

PC is the value of the absorbance units (AU) of the positive control;

SAMPLE OD – the value of the absorbance units (AU) of the test serum.

Interpretation of the result:

– at $x \leq 40\%$, the result is considered negative (i.e. no specific antibodies were detected in the sample);

– at $x \geq 50\%$, the result is considered positive (i.e. specific antibodies were detected in the sample);

– at $40\% > x < 50\%$ the result is considered inconclusive.

Experimental sera were stored at the following temperature conditions:

1. Storage at minus 20 °C;
2. Storage at 4 °C;
3. Storage at room temperature (from 20 to 25 °C);
4. Multiple freeze – thaw cycles (daily freezing at minus 20 °C for 23 hours, followed by thawing at room temperature for an hour).

The analysis was carried out on the start day (Day zero), on the 5th, 15th, 29th and 53rd days after the start of the experiment.

TEST RESULTS

The first test to confirm the serological status of experimental sera was performed on Day 0.

The experimental samples were divided into 4 parts and stored at different temperature conditions for 53 days. Each group of experimental sera was analyzed according to the experiment design. The results are given in the Table.

Table
Serum testing using solid-phase ELISA

Storage condition	Sample status	Serum number	Average value of coefficient of inhibition (%), ($n = 2$)					Result
			Day 0	Day 5	Day 15	Day 29	Day 53	
Freeze – thaw cycle	Seronegative	1	17.0	26.4	23.7	22.7	28.9	–
		2	16.3	25.1	25.2	20.3	35.4	–
		3	23.2	20.8	18.2	21.2	22.3	–
		4	21.6	23.5	21.4	22.8	26.1	–
		5	22.7	26.1	23.3	22.9	27.3	–
		6	25.5	24.0	28.0	24.1	31.6	–
		7	20.3	22.3	21.1	20.1	19.1	–
		8	20.5	22.8	23.8	19.7	22.6	–
		9	17.3	20.6	18.1	16.1	20.5	–
		10	23.3	23.5	26.5	21.2	32.0	–
	Seropositive	11	94.1	88.1	90.5	92.0	90.6	+
		12	100.6	96.4	98.0	97.3	96.0	+
		13	97.1	91.1	92.8	92.7	91.4	+
		14	98.3	90.6	91.0	91.8	90.2	+
		15	88.1	93.7	95.0	94.9	92.3	+
		16	98.3	93.8	93.9	95.0	91.8	+
		17	86.7	85.4	83.8	87.2	85.5	+
		18	97.0	94.5	94.7	96.8	93.5	+
		19	94.8	91.6	94.7	93.5	89.9	+
		20	99.8	98.6	99.1	99.3	95.5	+
at minus 20 °C	Seronegative	1	17.0	20.8	19.9	18.8	25.4	–
		2	16.3	20.3	21.3	22.7	26.0	–
		3	23.2	24.0	22.2	24.2	29.4	–
		4	21.6	20.9	23.2	20.3	33.8	–
		5	22.7	16.5	20.0	19.8	7.1	–
		6	25.5	19.5	20.0	21.8	24.4	–
		7	20.3	21.1	22.2	23.4	28.7	–
		8	20.5	20.0	24.4	22.9	35.4	–
		9	17.3	10.2	11.4	15.8	15.9	–
		10	23.3	20.0	25.4	20.1	28.7	–
	Seropositive	11	94.1	89.2	90.9	91.8	89.2	+
		12	100.6	96.2	96.2	97.6	94.9	+
		13	97.1	89.9	91.2	91.7	89.7	+
		14	98.3	89.8	90.5	92.6	90.9	+
		15	88.1	93.3	94.2	95.3	92.6	+
		16	98.3	94.3	95.3	95.8	93.2	+
		17	86.7	82.9	80.5	83.0	81.9	+
		18	97.0	91.2	90.5	92.8	91.3	+
		19	94.8	91.6	93.8	94.9	93.1	+
		20	99.8	98.2	97.4	98.4	95.3	+

Storage condition	Sample status	Serum number	Average value of coefficient of inhibition (%), ($n = 2$)					Result
			Day 0	Day 5	Day 15	Day 29	Day 53	
at 4 °C	Seronegative	1	17.0	19.8	20.1	10.5	26.6	–
		2	16.3	19.9	19.1	11.2	29.8	–
		3	23.2	12.3	21.2	15.8	16.5	–
		4	21.6	19.3	22.3	13.0	23.5	–
		5	22.7	20.2	20.8	11.2	27.4	–
		6	25.5	16.5	19.7	13.4	26.9	–
		7	20.3	19.6	20.4	15.7	16.2	–
		8	20.5	20.5	20.1	14.1	26.1	–
		9	17.3	17.0	11.1	4.3	23.6	–
		10	23.3	17.0	21.5	13.5	25.9	–
	Seropositive	11	94.1	87.3	89.0	89.3	87.2	+
		12	100.6	93.3	95.9	96.8	94.3	+
		13	97.1	93.5	93.4	90.3	92.0	+
		14	98.3	90.2	90.2	88.6	88.1	+
		15	88.1	93.7	95.0	95.6	92.4	+
		16	98.3	92.5	95.3	93.8	91.3	+
		17	86.7	84.2	81.7	80.3	83.7	+
		18	97.0	93.1	93.4	94.4	92.8	+
		19	94.8	93.1	93.0	94.9	90.8	+
		20	99.8	96.2	96.8	97.9	93.5	+
at 20 °C	Seronegative	1	17.0	13.1	17.0	19.8	11.2	–
		2	16.3	18.8	20.9	20.6	13.0	–
		3	23.2	19.3	14.0	17.0	19.1	–
		4	21.6	18.6	19.9	22.1	20.8	–
		5	22.7	16.6	22.0	21.9	10.2	–
		6	25.5	19.4	18.1	16.6	15.4	–
		7	20.3	18.7	20.9	17.7	18.9	–
		8	20.5	18.9	20.3	21.1	19.2	–
		9	17.3	10.2	17.2	17.3	6.3	–
		10	23.3	17.3	22.0	18.0	18.8	–
	Seropositive	11	94.1	89.1	89.1	90.5	87.5	+
		12	100.6	97.9	97.7	98.7	92.6	+
		13	97.1	91.3	91.2	94.6	88.1	+
		14	98.3	88.3	88.4	90.9	85.9	+
		15	88.1	95.4	95.2	95.5	92.4	+
		16	98.3	95.2	95.5	95.8	92.3	+
		17	86.7	81.0	83.4	85.5	79.2	+
		18	97.0	92.3	95.3	95.7	90.8	+
		19	94.8	92.6	93.5	93.7	91.7	+
		20	99.8	96.9	97.8	98.7	94.0	+

"+" – positive result; "–" – negative result.

The obtained data demonstrate that when the coefficient of inhibition changed during the experiment, the serum status in solid-phase ELISA remained unchanged.

Following the general recommendations on storage of serum samples for laboratory diagnostics [11, 14, 15], the reference values were considered to be the results obtained by the testing of sera stored frozen. The variation of the values depended on the technical conditions of the method performance (temperature, humidity, kit storage time, etc.).

The dynamics of changes in the coefficients of inhibition of seronegative and seropositive samples is shown in Figure 1. From the presented data, related to negative sera, it can be seen that:

– the average coefficient of inhibition at the beginning of the experiment was 20.8%;

– for sera stored at minus 20 °C, the average coefficient of inhibition was 21.5%, the minimum – 7.1% (Day 53), the maximum – 35.4% (Day 53);

– for sera subjected to the freeze – thaw cycle, the average coefficient of inhibition was 22.8%, the minimum value was 16.1% (Day 29), the maximum value was 35.4% (Day 53);

– for sera stored at 4 °C, the average coefficient of inhibition was 19%, the minimum – 4.3% (Day 29), the maximum – 29.8% (Day 53);

– for sera stored at 20 °C, the average value was 18.3%, the minimum value was 6.3% (Day 53), the maximum value was 25.5% (Day zero).

The change in the difference in the inhibition coefficients from the reference samples for negative sera is shown in Figure 2.

It was established that during the whole study, the coefficient of inhibition for negative sera subjected to the freeze – thaw cycle was higher than the reference values, but did not exceed 4.2%, and on average differed by 1.7%; this indicator of sera stored at 4 °C was lower than the reference values, but did not exceed 8.7%, and on average differed by 3.1%. The inhibition coefficient of sera stored at 20 °C was lower than the reference values, but did not exceed 10.2%, and on average differed by 4%.

Based on the data obtained during the testing of porcine seropositive samples (Fig. 1), it can be seen that:

– the initial coefficient of inhibition was on average 95.5%;

– for sera stored at minus 20 °C, the average value was 92.8%, the minimum – 80.5% (Day 15), the maximum – 100.6% (Day zero);

– for sera subjected to the freeze – thaw cycle, the average coefficient of inhibition was 93.4%, the minimum value was 83.8% (Day 15), the maximum value was 100.6% (Day zero);

– for sera stored at 4 °C, the average coefficient of inhibition was 92.5%, the minimum – 80.3% (Day 29), the maximum – 100.6% (Day zero);

– for sera stored at 20 °C, the average coefficient of inhibition was 92.7%, the minimum was 79.2% (Day 53), the maximum was 100.6% (Day zero).

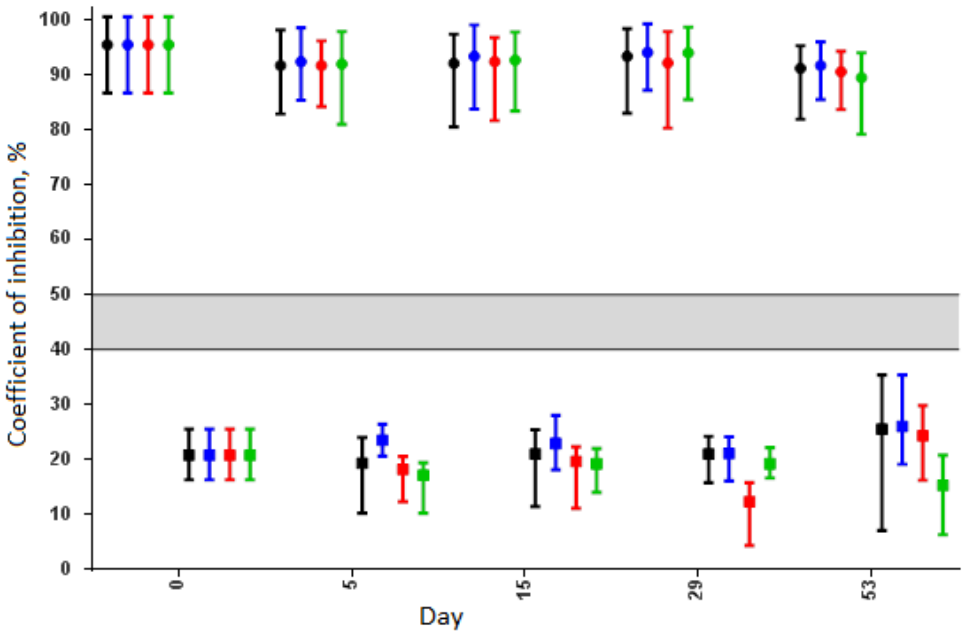


Fig. 1. Dynamics of inhibition coefficients for seronegative and seropositive samples. Lines in case of 40% and 50% inhibition are the negative/positive cut-off.

The difference in the coefficients of inhibition of seropositive samples stored at minus 20 °C is shown in Figure 3.

As it can be seen, the coefficient of inhibition of seropositive samples subjected to the freeze –thaw cycle was higher than the reference values throughout the entire study period, but did not exceed 1.3%, and on average differed by 0.8%.

The coefficient of inhibition of sera stored at 4 °C was higher than the reference value (maximum by 0.3%) for 15 days; later it became lower than it (maximum by 1.2%), and on average differed by 0.4%.

The coefficient of inhibition of sera stored at 20 °C was higher than the values of the reference samples until the Day 29 (maximum by 0.7%), and then lower by 1.8%, and on average differed by 0.1%.

In the group of seropositive samples stored at 4 °C, the coefficient of inhibition for some samples during the study varied from 4.3 to 23.6%. However, the status of the samples did not change throughout the experiment.

DISCUSSION

The simulation of various storage conditions of experimental pig sera, positive and negative for ASFV antibodies, provided the data on the absence of changes in their serological status over time.

The tested serum samples were stored at various temperature conditions (minus 20 °C, 4 °C, 20 °C, freeze – thaw cycle) for 53 days. Despite the differences in the coefficients of inhibition of negative and positive samples during the study from the reference values, a qualitative result was obtained by solid-phase ELISA, which suggests no or insignificant effect of the applied storage conditions on the results of the study.

The obtained data are consistent with the results of other authors who conducted similar studies in detection of antibodies to other infectious agents. In the work of E. J. Neumann and K. N. Bonistalli, serum antibodies were significantly more stable than previously thought, and the optical density values were stable even in case of gross violation of the storage temperature conditions [19, 20].

However, the methodology used in this study has some limitations. The test kit of a single manufacturer was used in the work, the serum antibody titers were not determined, and weakly positive samples were not tested.

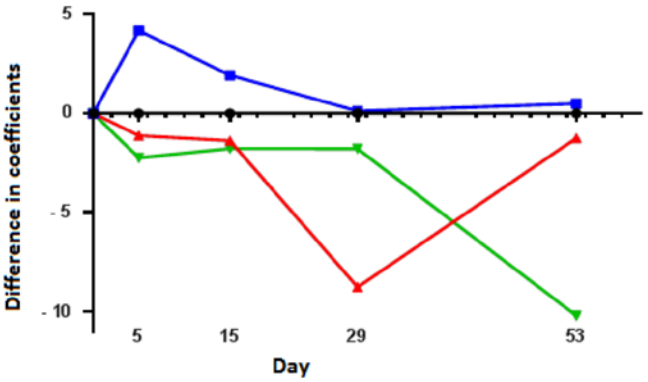
Therefore, to determine the reason of false results, it is necessary to conduct further studies and to analyze the preservation of serum samples for ASF testing, given that biological samples obtained from animals are not homogeneous in their composition, especially in the presence of pathogenic microflora.

CONCLUSION

The studies conducted showed that storage of porcine serum samples in simulated temperature conditions did not affect the status of positive sera with an initially high coefficient of inhibition and negative sera tested by solid-phase ELISA using the INgezim PPA Compac test kit (Ingenasa, Spain) during 53 days.

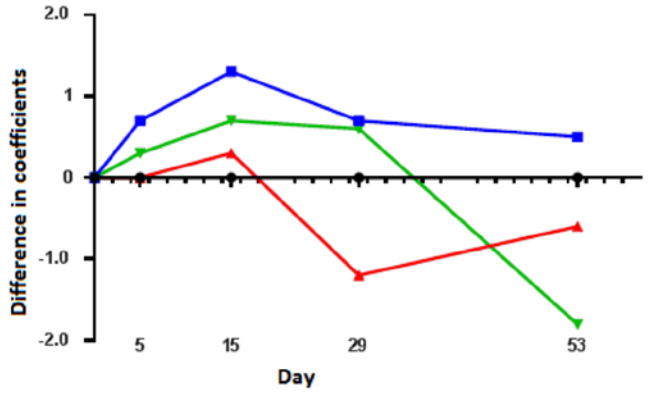
Sera of domestic pigs collected in accordance with the current rules can be stored under the above conditions for up to 53 days (observation period), provided that they are subsequently tested using an appropriate kit. This will simplify their transportation for a longer time if freezing is impossible. Also, if additional tests are needed, the repeated testing of a single sample becomes possible during long-term storage.

However, until the effect of storage conditions on the status of sera during ASF testing using other kits and methods (Western blotting, immunoperoxidase, immunochromatographic assays, ELISA using a complex antigen,



Conditions/day	0	5	15	29	53
minus 20 °C	0	0	0	0	0
freeze/thaw	0	4.2	1.9	0.1	0.5
4 °C	0	-1.1	-1.4	-8.7	-1.2
20 °C	0	-2.2	-1.8	-1.8	-10.2

Fig. 2. Changes of inhibition coefficients of seronegative sera as compared to reference sera (storage at minus 20 °C)



Conditions/day	0	5	15	29	53
minus 20 °C	0	0	0	0	0
freeze/thaw	0	0.7	1.3	0.7	0.5
4 °C	0	0	0.3	-1.2	-0.6
20 °C	0	0.3	0.7	0.6	-1.8

Fig. 3. Changes of inhibition coefficients of seropositive sera as compared to reference sera (storage at minus 20 °C)

etc.) has not been studied, the general recommendation remains the same – to deliver samples to the laboratory for research as soon as possible.

REFERENCES

1. Remyga S. G., Pershin A. S., Shevchenko I. V., Igolkin A. S., Shevtsov A. A. Clinical and post-mortem signs in European wild boars and domestic pigs infected with African swine fever virus. *Veterinary Science Today*. 2016; 3 (18): 46–51. Available at: https://veterinary.arriah.ru/jour/article/view/260. (in Russian)

2. Alonso C., Borca M., Dixon L., Revilla Y., Rodriguez F., Escribano J. M., ICTV Report Consortium. ICTV Virus Taxonomy Profile: *Asfarviridae*. *J. Gen. Virol*. 2018; 99 (5): 613–614. DOI: 10.1099/jgv.0.001049.

3. Preparation of African swine fever contingency plans. Ed. by M.-L. Penrith, V. Guberti, K. Depner, J. Lubroth. *FAO Animal Production and Health Manual*. *No. 8.* Rome: FAO; 2009. 70 p. Available at: http://www.fao.org/3/i1196e/i1196e.pdf.

4. World Animal Health Information and Analysis Department. African swine fever (ASF). Report No. 12: March 01 – March 14, 2019. Available at: http://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/Disease_cards/ASF/Report_12_Current_situation_ASF.pdf.

5. Galindo I., Alonso C. African swine fever virus: A review. *Viruses*. 2017; 9 (5):103. DOI: 10.3390/v9050103.

6. Gruzdev K. N., Karaulov A. K., Igolkin A. S. Experience in African swine fever control in the Russian Federation and its value for the other countries. *Veterinary Science Today*. 2020; 1 (32): 38–43. DOI: 10.29326/2304-196X-2020-1-32-38-43.

7. Shevtsov A. A., Petrova O. N., Remyga S. G., Pershin A. S., Gruzdev K. N., Igolkin A. S. Analysis of laboratory tests for several viral swine diseases in Russia in 2011–2017. *Veterinary Science Today*. 2018; 1 (24): 42–48. DOI: 10.29326/2304-196X-2018-1-24-42-48.

8. Pershin A., Shevchenko I., Igolkin A., Zhukov I., Mazloum A., Aronova E., et al. A long-term study of the biological properties of ASF virus isolates originating from various regions of the Russian Federation in 2013–2018. *Vet. Sci*. 2019; 6 (4):99. DOI: 10.3390/vetsci6040099.

9. Pershin A. S., Shevchenko I. V., Igolkin A. S., Zhukov I. Yu., Vlasova N. N., Manuylova O. A. Dynamics of antibodies production after experimental challenge with ASF virus. *Veterinaria Kubani*. 2019; 4: 3–7. DOI: 10.33861/2071-8020-2019-4-3-7. (in Russian)

10. Bergeron H. C., Glas P. S., Schumann K. R. Diagnostic specificity of the African swine fever virus antibody detection enzyme-linked immunosorbent assay in feral and domestic pigs in the United States. *Transbound. Emerg. Dis*. 2017; 64 (6): 1665–1668. DOI: 10.1111/tbed.12717.

11. Beltrán-Alcrudo D., Arias M., Gallardo C., Kramer S., Penrith M. L. African swine fever: detection and diagnosis – a manual for veterinarians. *FAO Animal Production and Health Manual. No. 19*. Rome: FAO; 2017. 88 p. Available at: http://www.fao.org/3/i7228e/i7228e.pdf.

12. Mur L., Boadella M., Martínez-López B., Gallardo C., Gortazar C., Sánchez-Vizcaíno J. M. Monitoring of African swine fever in the wild boar population of the most recent endemic area of Spain. *Transbound. Emerg. Dis*. 2012; 59 (6): 526–531. DOI: 10.1111/j.1865-1682.2012.01308.x.

13. GOST 28573-90 Pigs. Methods of laboratory diagnostics of african plague. M.: Standartinform; 2005. 9 p. Available at: https://files.stroyinf.ru/Data2/1/4294826/4294826183.pdf. (in Russian)

14. Examination of the population immunity against influenza in people in the Russian Federation [Izuchenie populyacionnogo immuniteta k grippu u naseleniya Rossijskoj Federacii]: methodical guidelines (MG 3.1.3490-17). M.: Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing; 2018. 34 p. Available at: https://www.meganorm.ru/Data2/1/4293738/4293738228.pdf. (in Russian)

15. Rules of veterinary and sanitary sampling for the control of harmful substances and their residues in live animals and products of animal origin [Veterinarno-sanitarnye pravila otbora prob dlya kontrolya soderzhaniya vrednyh veshchestv i ih ostatkov v zhivyh zhivotnyh i produktah zhivotnogo proiskhozhdeniya]: Regulation of the Ministry of Agriculture of the Republic of Belarus as of 23.09.2010 No. 57. Available at: https://mshp.gov.by/print/documents/technical-acts/e13f0df74187a8fa.html. (in Russian)

16. Rules of sampling pathological materials, blood, feed and their delivery for laboratory testing [Pravila vzyatiya patologicheskogo materiala, krovi, kormov i peresylki ih dlya laboratornogo issledovaniya]: approved by the Main Veterinary Department of the USSR Ministry of Agriculture on 24.06.1971. Available at: https://docs.cntd.ru/document/1200109238. (in Russian)

17. GOST 34105-2017 Animals. Laboratory diagnostics of brucellosis. Serological methods. M.: Standartinform; 2017. 23 p. Available at: http://docs.cntd.ru/document/1200145963. (in Russian)

18. GOST R 53079.4-2008 Clinical laboratory technologies. Quality assurance of clinical laboratory tests. Part 4. Rules for conducting of preanalytical stage. M.: Standartinform; 2009. 65 p. Available at: http://docs.cntd.ru/document/1200072566. (in Russian)

19. Boadella M., Gortázar C. Effect of haemolysis and repeated freeze – thawing cycles on wild boar serum antibody testing by ELISA. *BMC Res. Notes*. 2011; 4:498. DOI: 10.1186/1756-0500-4-498.

20. Neumann E. J., Bonistalli K. N. Effect of blood sample handling post-collection on *Erysipelothrix rhusiopathiae* antibody titres. *Vet. J.* 2009; 180 (3): 325–329. DOI: 10.1016/j.tvjl.2008.07.020.

Received on 22.04.2021
Approved for publication on 20.07.2021

INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Andrey R. Shotin, Post-Graduate Student, Leading Biologist, Reference Laboratory for African Swine Fever, FGBI "ARRIAH", Vladimir, Russia.

Шотин Андрей Романович, аспирант, ведущий биолог референтной лаборатории по африканской чуме свиней ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Ivan Yu. Zhukov, Candidate of Science (Biology), Junior Researcher, Reference Laboratory for African Swine Fever, FGBI "ARRIAH", Vladimir, Russia.

Andrey S. Pershin, Candidate of Science (Veterinary Medicine), Senior Researcher, Reference Laboratory for African Swine Fever, FGBI "ARRIAH", Vladimir, Russia.

Ali Mazloum, Candidate of Science (Biology), Researcher, Reference Laboratory for African Swine Fever, FGBI "ARRIAH", Vladimir, Russia.

Ivan V. Shevchenko, Candidate of Science (Biology), Researcher, Reference Laboratory for African Swine Fever, FGBI "ARRIAH", Vladimir, Russia.

Alexey S. Igolkin, Candidate of Science (Veterinary Medicine), Head of Reference Laboratory for African swine fever, FGBI "ARRIAH", Vladimir, Russia.

Olga A. Manuylova, Biologist, Reference Laboratory for African Swine Fever, FGBI "ARRIAH", Vladimir, Russia.

Konstantin N. Gruzdev, Doctor of Science (Biology), Professor, Chief Researcher, Information and Analysis Centre, FGBI "ARRIAH", Vladimir, Russia.

Жуков Иван Юрьевич, кандидат биологических наук, младший научный сотрудник референтной лаборатории по африканской чуме свиней ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Першин Андрей Сергеевич, кандидат ветеринарных наук, старший научный сотрудник референтной лаборатории по африканской чуме свиней ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Мазлум Али, кандидат биологических наук, научный сотрудник референтной лаборатории по африканской чуме свиней ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Шевченко Иван Вячеславович, кандидат биологических наук, научный сотрудник референтной лаборатории по африканской чуме свиней ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Иголкин Алексей Сергеевич, кандидат ветеринарных наук, заведующий референтной лабораторией по африканской чуме свиней ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Мануйлова Ольга Анатольевна, биолог референтной лаборатории по африканской чуме свиней ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Груздев Константин Николаевич, доктор биологических наук, профессор, главный научный сотрудник информационно-аналитического центра ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

DOI: 10.29326/2304-196X-2021-3-38-224-229

Comparative testing of vaccines based on viruses of genetic lineages G1 and Y280 for their potency against low pathogenic avian influenza H9N2

S. V. Frolov[1], L. O. Scherbakova[2], N. V. Moroz[3], V. N. Irza[4], V. Yu. Kulakov[5]
FGBI "Federal Centre for Animal Health" (FGBI "ARRIAH"), Vladimir, Russia
[1] *ORCID 0000-0001-6802-9940, e-mail: frolov@arriah.ru*
[2] *ORCID 0000-0001-5434-6179, e-mail scherbakova@arriah.ru*
[3] *ORCID 0000-0002-9672-8594, e-mail: moroz@arriah.ru*
[4] *ORCID 0000-0001-7489-1772, e-mail: irza@arriah.ru*
[5] *ORCID 0000-0002-1898-4576, e-mail: kulakov@arriah.ru*

SUMMARY
Due to the genetic diversity of low pathogenic avian influenza (LPAI) viruses of subtype H9N2, it deemed appropriate to study the potency of the vaccines based on the antigens of strains A/chicken/Amursky/03/12 and A/chicken/Chelyabinsk/314-1/20 that represent currently circulating in the Russian Federation genetic lineages Y280 and G1, respectively. While low pathogenicity of the agent does not allow demonstrating the vaccine protective properties by the direct methods generally used for potency assessment (e.g. morbidity and mortality), the indirect methods were used: determination of antigenic relatedness of the strains, level of the postvaccinal homologous and heterologous humoral immunity, analysis of the virus genome synthesis inhibition (reduction) in vaccinated birds following their challenge. The strains used in the vaccines were determined to have some antigenic differences, which were demonstrated in the hemagglutination inhibition (HI) assay during control of the postvaccinal immunity in birds. Both vaccines generally induced strong humoral immunity in vaccinated birds (9–10 $\log_2$ determined using HI assay) with some difference in the levels of the immune response following the use of homologous or heterologous antigens. It was also reliably determined that homologous immunity facilitated more expressed inhibition of the virus reproduction after the challenge. The level of inhibition (reduction) of the virulent LPAI virus genome synthesis in vaccinated birds following their challenge with H9N2 virus of genetic lineage G1 was higher in birds following homologous vaccination, while the time periods of the genome detection in the biomaterial samples were the same. It was demonstrated that due to antigenic and immunogenic differences between LPAI H9N2 strains, use of both antigenic components in the inactivated vaccines is appropriate.

Keywords: vaccines, low pathogenic avian influenza (LPAI), vaccine potency, humoral immunity

Acknowledgements: The study was funded by the FGBI "ARRIAH" within the framework of "Veterinary Welfare" research work.

For citation: Frolov S. V., Scherbakova L. O., Moroz N. V., Irza V. N., Kulakov V. Yu. Comparative testing of vaccines based on viruses of genetic lineages G1 and Y280 for their potency against low pathogenic avian influenza H9N2. *Veterinary Science Today*. 2021; 10 (3): 224–229. DOI: 10.29326/2304-196X-2021-3-38-224-229.

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

For correspondence: Sergey V. Frolov, Candidate of Science (Veterinary Medicine), Leading Researcher, Laboratory for Avian Diseases Prevention, FGBI "ARRIAH", 600901, Russia, Vladimir, Yur'evets, *e-mail: frolov@arriah.ru.*

УДК 619:616.98:578.832.1:636.52/.58:615.371:616-097.3

Сравнение иммуногенной активности вакцин против низкопатогенного гриппа птиц подтипа H9N2, изготовленных на основе вирусов генетических линий G1 и Y280

С. В. Фролов[1], Л. О. Щербакова[2], Н. В. Мороз[3], В. Н. Ирза[4], В. Ю. Кулаков[5]
ФГБУ «Федеральный центр охраны здоровья животных» (ФГБУ «ВНИИЗЖ»), г. Владимир, Россия
[1] *ORCID 0000-0001-6802-9940, e-mail: frolov@arriah.ru*
[2] *ORCID 0000-0001-5434-6179, e-mail scherbakova@arriah.ru*
[3] *ORCID 0000-0002-9672-8594, e-mail: moroz@arriah.ru*
[4] *ORCID 0000-0001-7489-1772, e-mail: irza@arriah.ru*
[5] *ORCID 0000-0002-1898-4576, e-mail: kulakov@arriah.ru*

РЕЗЮМЕ

В связи с генетическим разнообразием вирусов низкопатогенного гриппа птиц H9N2 представлялось целесообразным изучение иммуногенной активности вакцин, изготовленных на основе антигенов штаммов A/chicken/Amursky/03/12 и A/chicken/Chelyabinsk/314-1/20 – представителей генетических линий Y280 и G1 соответственно, циркулирующих на территории Российской Федерации. Поскольку низкая патогенность возбудителя не позволяет продемонстрировать протективные свойства вакцин прямыми методами оценки иммуногенности препаратов (например, заболеваемость и смертность), применяли косвенные методы: определение антигенной родственности штаммов, напряженности поствакцинального гуморального гомо- и гетерологичного иммунитета птиц, оценка подавления (редукции) синтеза генома вируса после контрольного заражения в организме вакцинированных птиц. Было установлено, что использованные в составе вакцин штаммы имели некоторые антигенные различия, которые были обнаружены в реакции торможения гемагглютинации при контроле поствакцинального иммунного ответа птиц. В целом обе вакцины индуцировали напряженный гуморальный иммунитет у привитых птиц (9–10 $\log_2$ в реакции торможения гемагглютинации) с некоторой разницей в величине иммунного ответа при использовании гомо- и гетерологичного антигенов. Также было достоверно установлено, что гомологичный иммунитет обеспечивал более выраженное подавление репродукции вируса при экспериментальном заражении. Степень подавления (редукции) синтеза генома вирулентного вируса низкопатогенного гриппа птиц в организме вакцинированных особей после их заражения вирусом H9N2 генетической линии G1 была выше у птиц, привитых гомологичной вакциной при одинаковых сроках детекции генома в пробах биоматериала. Показано, что с учетом антигенных и иммуногенных различий между штаммами вируса низкопатогенного гриппа птиц H9N2 целесообразно использование обоих антигенных компонентов в составе инактивированных вакцин.

Ключевые слова: вакцина, низкопатогенный вирус гриппа птиц подтипа H9N2, иммуногенность вакцины, гуморальный иммунитет

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

Для цитирования: Фролов С. В., Щербакова Л. О., Мороз Н. В., Ирза В. Н., Кулаков В. Ю. Сравнение иммуногенной активности вакцин против низкопатогенного гриппа птиц подтипа H9N2, изготовленных на основе вирусов генетических линий G1 и Y280. *Ветеринария сегодня*. 2021; 10 (3): 224–229. DOI: 10.29326/2304-196X-2021-3-38-224-229.

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

Для корреспонденции: Фролов Сергей Владимирович, кандидат ветеринарных наук, ведущий научный сотрудник лаборатории профилактики болезней птиц ФГБУ «ВНИИЗЖ», 600901, Россия, г. Владимир, мкр. Юрьевец, *e-mail: frolov@arriah.ru.*

INTRODUCTION

Low pathogenic avian influenza (LPAI) viruses H9N2 are widely spread in poultry in Africa, Asia and the Middle East. Based on the genetic features, there are two main genetic groups of H9N2 viruses – 'North American' and 'Eurasian', circulating in poultry and wild birds. The Eurasian group is divided into three main genotypes: G1, Y280 and Y439. Viruses of the G1 and Y280 genetic lineages demonstrate the widest geographical distribution, from East Asia to the Middle East [1–4].

In 2012, 2017 and 2018, LPAI cases in the poultry industry of the Russian Federation were caused by H9N2 virus of Y280 lineage [5–7]. In 2018, low pathogenic avian influenza virus H9N2 of G1 genetic lineage was isolated for the first time from wild birds in the Amur Oblast [8].

In 2019–2020, low pathogenic H9N2 influenza virus of G1 genetic lineage was detected in poultry raised on commercial poultry farms of the Ural region (the Chelyabinsk Oblast and the Perm Krai), as well as on farms of the European part of the country [5–7, 9].

Due to significant economic losses caused by this infection, some countries have been actively vaccinating against LPAI H9N2: Israel (since 2003), South Korea (since 2007). China has been a leader in prevention of LPAI H9N2 subtype since 1998 [1].

In 2012, the FGBI "ARRIAH" developed inactivated combined emulsion vaccine against LPAI H9N2 and Newcastle disease and launched its the serial production. H9N2 virus of Y280 lineage, isolated in 2012 from chickens on a poultry farm in the Amur region is used as a production virus strain. Currently, this vaccine is successfully used in commercial poultry farming of the Russian Federation.

Due to the genetic diversity of LPAI H9N2 viruses, it is appropriate to study the immunogenic activity of vaccines against H9N2 avian influenza, based on the antigens of different virus sublineages circulating in the Russian Federation. Low pathogenicity of the pathogen makes it impossible to demonstrate protective properties of the vaccines using direct methods of immunogenicity assessment (for example, morbidity and mortality). Therefore, indirect methods were used, i.e. determination of strain antigenic relatedness, assessment of humoral homo- and heterologous immunity levels in birds after vaccination, assessment of the virus genome synthesis inhibition in the vaccinated birds after challenge.

MATERIALS AND METHODS

Antigens. Antigens of the following strains of LPAI H9N2 subtype were used in the experiment:

– A/chicken/Chelyabinsk/314-1/20 (G1 genotype), infectivity before inactivation 8.75 lg EID_{50}/cm^3, hemagglutinating activity 9 $\log_2$ GAE, named as "Chelyabinsk-20";

– A/chicken/Amursky/03/12 (Y280 genotype), infectivity before inactivation 8.25 lg EID_{50}/cm^3, hemagglutinating activity 9 $\log_2$ GAE, named as "Amur-12".

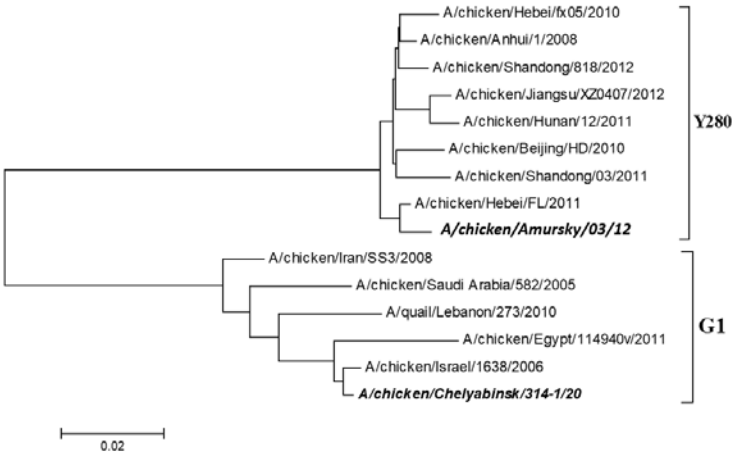


Fig. Phylogenetic tree constructed on the basis of LPAI H9 HA gene alignment using NJ method and MEGA 6.0 software

In order to standardize the antigen content in the inoculation dose (determined before inactivation on the basis of the infectious titer), the inactivated suspension of "Chelyabinsk-20" virus strain was diluted (by 3 times) before emulsification, so that the vaccine inoculation dose contained equal number of LPAI virus antigens.

Different genetic lineages of the viruses are shown in the figure.

Vaccines. The two vaccines used in the experiment contained a mixture of LPAI and Newcastle disease virus antigens (taken at equal volumes), which is the active component of the vaccine:

– an experimental vaccine with an antigen of "Chelyabinsk-20" strain, hereinafter referred to as "Chelyabinsk-20 (G1) Vaccine";

– an experimental vaccine with an antigen of "Amursky-12" strain, hereinafter referred to as "Amursky-12 (Y280) Vaccine".

During vaccine production, the active component (antigen) was combined with an oil adjuvant Montanide ISA 70 (Seppic, France) in the proportion 30:70 (% by weight) and emulsified in a high-speed laboratory mixer Silverson (England) at a speed of 6,000 rpm for 5 minutes.

LPAI virus for challenge. A virus of G1 genetic lineage, i.e. A/chicken/Chelyabinsk/314-1/20 H9N2 ("Chelyabinsk-20"), was used in the experiment in the form of a suspension prepared from a freeze-dried product with an original infectivity of 8.95 lg EID_{50}/cm^3.

Poultry. The experiment was carried out in egg laying chickens at the age of 80 days, taken from a farm free from acute forms of avian infectious diseases.

All animal experiments were performed in strict compliance with interstate standard on laboratory animal keeping and handling GOST 33215-2014, adopted by Interstate Council on Standardization, Metrology and Certification and pursuant to the requirements of Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

The experiment scheme. According to the tested vaccines, the poultry were divided into two groups (20 chickens in each). The vaccines were administered intramuscularly into the chest area in a volume of 0.5 cm^3.

On Day 28 post vaccination, the chickens of both groups were challenged with "Chelyabinsk-20" virus. The virus-containing suspension was administered orally in a volume of 1 cm^3, the infectious dose was 7.3 lg EID_{50}.

During the experiment, oropharyngeal and cloacal swabs were taken in both groups after the challenge, either daily or sometimes with a one day interval. The samples were tested in real-time reverse transcription polymerase chain reaction (real-time RT-PCR) to detect the challenge virus genome. Totally, 12 samples of each type of material were taken.

Before the challenge and on Day 15 after it, blood samples were taken from all the chickens to compare antibody titers to the LPAI virus in the hemagglutination inhibition test (HI test) with virus antigens of two different genetic lineages.

Test methods. The virus genome in the samples was detected and the amplification cycle threshold values were estimated in accordance with Methodical Instructions 45-16 "Instructions for RNA detection of avian influenza virus type A using real time RT-PCR" [10].

Serological tests. The hemagglutination inhibition test (HI) was performed according to a generally accepted procedure using a diagnostic kit manufactured by the FGBI "ARRIAH" to detect antibodies to avian influenza virus subtype H9 (H9N2 virus antigen of Y280 genetic lineage) and H9N2 virus antigen of G1 genetic lineage obtained during the vaccine production. Antibody titers of $\geq 4 \log_2$ ($\geq$ 1:16) were considered positive.

Statistical analysis. The significance of differences between quantitative indicators was analyzed. We used Miller's nonparametric multiple-comparison procedure [11] for the *k*-number of samples (groups), based on checking feasibility of the following inequality $|H_1 - H_2| / (\sqrt{(k\,(kn + 1) / 12)}) \geq q$, where H_1 and H_2 stand for the mean ranks of the compared samplings 1 and 2 in the general ordered series; *n* stands for samplings volumes ($n_1 = n_2 = n$); *q* is the table coefficient for *k* number and the given probability of forecast error (*p*) [11]. The differences were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Studying antigenic relationship between LPAI H9N2 strains and assessing strength of the post-vaccination immunity in poultry. On Day 28 post vaccination blood sera from poultry were tested with homologous and heterologous antigens, sera after challenge were tested with "Chelyabinsk-20" strain antigen. The results obtained are shown in Table 1. To facilitate processing of the data given in the table, vaccines and antigens are indicated by their clusters in the genetic lineages of H9N2 virus.

Based on the data in Table 1, it can be concluded that:

a) the antibody titers determined in homologous systems when testing blood sera taken on Day 28 after vaccination of poultry with both vaccines were almost the same, as demonstrated by the equality of logarithmic medians. The mean titer (*T*) was 9 $\log_2$ (1:512). It means that the poultry have demonstrated quite strong and roughly equal humoral immune response to each of the tested antigens;

b) when testing sera specified in point "*a*", the mean rank equivalents of (*H*) titers established in homologous systems for both antigens were reliably ($p < 0.05$) different

from the corresponding values observed when using heterologous antigens. For the "Chelyabinsk-20 (G1) Vaccine", *H* value was: $H_{homol.} = 24.2 > H_{heterol.} = 16.8$ and for the "Amursky-12 (Y280) Vaccine": $H_{homol.} = 24.4 > H_{heterol.} = 16.6$. It meant that, on average, heterologous reactions demonstrated significantly less activity, which, in turn, indicated certain antigenic differences between the studied strains.

For both antigens, the difference between medians of log titers in hetero- and homologous reactions was negative ($8\ \log_{2(heterol.)} - 9\ \log_{2(homol.)} = -1\ \log_2$), i.e. the heterologous reaction in both cases was twice less active (anti-$\log_2(-1) = 1/2$). This value can be interpreted by Archetti and Horsfall as an assessment of antigenic relationship (*r*), which for each strain was $r = 1/2 \times 100 = 50\%$. The obtained value suggests a slight antigenic difference between the studied viruses;

c) a test with "Chelyabinsk-20" strain antigen and sera from the poultry challenged with this LPAI strain, demonstrated that the mean rank equivalents of titers determined for the sera from poultry vaccinated with experimental and mass produced vaccines differed significantly ($p < 0.01$): $H_{homol.} = 15.4 < H_{heterol.} = 25.7$. This effect can be explained by the fact that after administration of the vaccine based on "Chelyabinsk-20" strain antigen, the poultry immunity was quite strong in relation to the homologous virus, and the pathogen penetration into the body did not cause any impact on the poultry immune system. The poultry that were administered mass-produced vaccine

Table 1
HI titres of LPAI virus antibodies in sera of vaccinated birds

Estimate of antibody titers (*T*, $\log_2$) on Day 28 post vaccination and on Day 15 after challenge, established in HI tests using homologous and heterologous antigens											
Day 28 post vaccination								Day 15 after challenge			
"Chelyabinsk-20" strain (G1) Vaccine				"Amursky-12" strain (Y280) Vaccine				"Chelyabinsk-20" strain (G1) Vaccine		"Amursky-12" strain (Y280) Vaccine	
G1 antigen		Y280 antigen		Y280 antigen		G1 antigen					
9	*25*[*]	9	*25*	8	*15.5*	7	*6*	9	*11.5*	10	*24*
10	*34*	7	*4.5*	11	*39.5*	8	*15.5*	10	*24*	9	*11.5*
8	*14*	8	*14*	9	*25.5*	7	*6*	8	*3*	12	*38*
8	*14*	9	*25*	9	*25.5*	7	*6*	9	*11.5*	11	*32.5*
7	*4.5*	8	*14*	8	*15.5*	7	*6*	9	*11.5*	8	*3*
9	*25*	7	*4.5*	8	*15.5*	10	*35*	9	*11.5*	10	*24*
9	*25*	8	*14*	9	*25.5*	9	*25.5*	10	*24*	12	*38*
11	*39*	10	*34*	10	*35*	9	*25.5*	9	*11.5*	9	*11.5*
11	*39*	9	*25*	8	*15.5*	8	*15.5*	11	*32.5*	9	*11.5*
9	*25*	7	*4.5*	9	*25.5*	7	*6*	9	*11.5*	12	*38*
9	*25*	7	*4.5*	7	*6*	7	*6*	10	*24*	10	*24*
9	*25*	10	*34*	9	*25.5*	10	*35*	9	*11.5*	10	*24*
10	*34*	8	*14*	9	*25.5*	9	*25.5*	10	*24*	10	*24*
11	*39*	8	*14*	10	*35*	7	*6*	8	*4*	11	*32.5*
8	*14*	10	*34*	9	*25.5*	10	*35*	9	*11.5*	12	*38*
8	*14*	7	*4.5*	11	*39.5*	8	*15.5*	11	*32.5*	10	*24*
10	*34*	8	*14*	10	*35*	7	*6*	10	*24*	11	*32.5*
7	*4.5*	8	*14*	9	*25.5*	7	*6*	7	*1*	11	*32.5*
9	*25*	7	*4.5*	7	*6*	8	*15.5*	9	*11.5*	9	*11.5*
9	*25*	10	*34*	9	*25.5*	10	*35*	9	*11.5*	12	*38*
9[**]	***(24.2)***	**8**	***(16.8)***	**9**	***(24.4)***	**8**	***(16.6)***	**9**	***(15.4)***	**10**	***(25.7)***
$p < 0.05$[***]				$p < 0.05$				$p < 0.01$			

* in italic is the rank (serial number) of the value in the combined and ordered sample of titers determined using homologous and heterologous systems;

** in bold is median sample ("*T*), in brackets is mean rank of random values (H);

*** statement of significance of the difference between average trends of titres in the relative systems (prediction error).

based on "Amursky-12" strain antigen demonstrated a slight increase in antibody titers, i.e. when infected, the heterologous virus had an additional impact on the poultry immune system. The corresponding medians of log titers ($\log_2$) were "$T_{homol.} = 9$ and "$T_{heterol.} = 10$.

Studying replication of LPAI virus in the vaccinated poultry. Within 14 days after challenge, 12 oropharyngeal and cloacal swabs were randomly taken to be tested in real time RT-PCR in order to detect influenza virus genome and assess its concentration. Amplification cycles threshold values (Ct) were estimated. A lower Ct value corresponded to a higher original concentration of the virus genetic material in the sample. The value of Ct ≥ 37 was considered negative, i.e. the virus genome was missing. To facilitate the analysis, the test results obtained within the time interval (j) were expressed as deviations from the negative assessment in the form of values $d_j = 37 - Ct_j$. Thus, the deviation values could range between $0 \le d \le 36$.

Table 2
Real-time RT-PCR-detection of avian influenza virus genome in the samples collected from vaccinated birds after challenge

Estimate of amplification cycles threshold values (Ct_j) in the form of deviations from the negative reaction ($d_j = 37 - Ct_j$), corresponding to the day post challenge (j, day), the type of swabs (oropharyngeal and cloacal) and the type of vaccines used for poultry vaccination. According to the types of samples, the ranks of d values and the results of corresponding comparisons are given								
j	Oropharyngeal swabs				Cloacal sawbs			
	"Chelyabinsk-20" strain (G1) Vaccine		"Amursky-12" strain (Y280) Vaccine		"Chelyabinsk-20" strain (G1) Vaccine		"Amursky-12" strain (Y280) Vaccine	
1	0	*4.5**	0	*4.5*	0	*4.5*	0	*4.5*
2	0.91	*14*	11.97	*24*	0	*4.5*	0	*4.5*
3	0.94	*15*	10.17	*22*	1.01	*14*	4.54	*23*
4	4.26	*21*	10.25	*23*	3.43	*21*	4.65	*24*
5	1.62	*19*	1.23	*18*	1.54	*18*	3.49	*22*
6	0	*4.5*	0.73	*12*	0	*4.5*	0.68	*13*
7	0.43	*11*	1.17	*17*	0.31	*11*	1.42	*16*
8	0	*4.5*	0	*4.5*	0	*4.5*	0.39	*12*
9	0	*4.5*	0.83	*13*	0	*4.5*	1.18	*15*
10	0.16	*9*	3.83	*20*	0.06	*9*	1.53	*17*
11	0	*4.5*	0.95	*16*	0	*4.5*	1.55	*19*
12	0	*4.5*	0.39	*10*	0.15	*10*	2.58	20
		(9.67)*		***(15.33)***		***(9.17)***		***(15.83)***
	*$p < 0.05$***				*$p < 0.025$*			

* in italic is the rank (serial number) of the value in the combined and ordered sample of d values determined for specific type of samples collected from birds immunized with G1 and Y280 vaccines;

** in brackets is mean rank of random values (H);

*** statement of significance of the difference (prediction error) between average trends of sample values.

Based on the data in Table 2, it can be concluded that:

a) after the challenge of vaccinated birds, the viral genome concentration in all types of the studied samples increased, reached a peak and decreased. The presence of the pathogen genome in both oropharyngeal and cloacal swabs suggested the virus presence in the body;

b) the oropharyngeal d-indicators determined during the first 4 days after the challenge formally exceeded similar values in cloacal swabs. This could be evidence of a slightly more active virus replication in the larynx, pharynx or upper respiratory tract of the bird. However, the statistical reliability of such an assumption could not be determined, due to a high variability of the primary values and the small sampling size;

c) effectiveness of the challenge virus reproduction depended on the homology with the vaccine antigen. Homologous immune background significantly reduced the pathogen development. Mean rank equivalents of d values established in oropharyngeal swabs obtained from the birds vaccinated with different vaccines significantly ($p < 0.05$) differed: $H_{homol.} = 9.67 < H_{heterol.} = 15.33$. A similar result ($p < 0.025$) was obtained when comparing the corresponding equivalents of d-values established during the analysis of cloacal swabs: $H_{homol.} = 9.17 < H_{heterol.} = 15.83$. Within 12 days of observation, the cumulative indicators of the challenge virus replication expressed as sums of d values at the homologous immunity in oropharyngeal and cloacal swabs were 8.32 and 6.5, respectively, similar values at heterologous immunity were 41.52 and 22.01.

These data are consistent with the results earlier obtained by the FGBI "ARRIAH" in experiments with vaccines against highly pathogenic avian influenza based on H5N1 virus (clades 2.2 and 2.3.2), when their protective properties against H5N8 virus were studied (clade 2.3.4.4b). The results of serological tests of post-vaccination immunity in HI test using different diagnostic antigens also confirmed antigenic variability of the avian influenza virus subtype H5. The results of acute experiments have shown that the match between the hemagglutinin of the vaccine antigen and the hemagglutinin of the field virus and its concentration in the vaccine is a decisive factor in ensuring protective properties of vaccines [12].

In our experiments, both vaccines significantly exceeded the minimum value of 5 $\log_2$ recommended by the OIE Guidelines for the immunity strength [13].

CONCLUSION

Antigens of low pathogenic avian influenza virus of H9N2 subtype (strains A/chicken/Chelyabinsk/314-1/20 G1 genotype and A/chicken/Amursky/03/12 Y280 genotype), that are used as active components in the inactivated combined emulsion vaccine against avian influenza H9N2 and Newcastle disease, induce strong humoral immunity.

These strains have some antigenic differences that can be detected by hemagglutination inhibition test when monitoring the post-vaccination immune response in birds. Homologous immunity more evidently suppresses virus reproduction during experimental infection.

Taking into account the identified antigenic differences of the studied strains, as well as further evolution of the pathogen, it is advisable to include both antigens in the active component of the inactivated avian influenza (H9) vaccines.

REFERENCES

1. Peacock T. H. P., James J., Sealy J. E., Iqbal M. A. A global perspective on H9N2 avian influenza virus. *Viruses*. 2019; 11 (7):620. DOI: 10.3390/v11070620.

2. Guo Y. J., Krauss S., Senne D. A., Mo I. P., Lo K. S., Xiong X. P. Characterization of the pathogenicity of members of the newly established H9N2 influenza virus lineages in Asia. *Virology*. 2000; 267 (2): 279–288. DOI: 10.1006/viro.1999.0115.

3. Nagy A., Mettenleiter T. C., Abdelwhab E. M. A brief summary of the epidemiology and genetic relatedness of avian influenza H9N2 virus in birds and mammals in the Middle East and North Africa. *Epidemiol. Infect*. 2017; 145 (16): 3320–3333. DOI: 10.1017/S0950268817002576.

4. Dong G., Luo J., Zhang H., Wang C., Duan M., Deliberto T. J. Phylogenetic diversity and genotypical complexity of H9N2 influenza A viruses revealed by genomic sequence analysis. *PLoS One*. 2011; 6 (2):17212. DOI: 10.1371/journal.pone.0017212.

5. Varkentin A. V., Irza V. N., Volkov M. S., Demchenko L. A. Case study of low pathogenic avian influenza H9N2 on commercial egg layers farm. *Poultry and Poultry Products*. 2020; 3: 10–13. DOI: 10.30975/2073-4999-2020-22-3-10-13. (in Russian)

6. Volkov M. S., Varkentin A. V., Irza V. N. Spread of low pathogenic avian influenza A/H9N2 in the world and Russian Federation. Challenges of disease eradication. *Veterinary Science Today*. 2019; 3 (30): 51–56. DOI: 10.29326/2304-196X-2019-3-30-51-56.

7. Zinyakov N. G., Osipova O. S., Akshalova P. B., Sosipatorova V. Yu., Andriyasov A. V., Andreychuk D. B., Chvala I. A. Analysis of genetic characteristics of influenza virus a/chicken/Chelyabinsk/30/2019 H9N2 isolated in Chelyabinsk Oblast. *Veterinary Science Today*. 2019; 4 (31): 49–53. DOI: 10.29326/2304-196X-2019-4-31-49-53.

8. Sharshov K., Kurskaya O., Sobolev I., Leonov S., Kabilov M., Alikina T., et al. First detection of a G1-like H9N2 virus in Russia, 2018. *Korean J. Vet. Res*. 2019; 59 (1): 37–42. DOI: 10.14405/kjvr.2019.59.1.37.

9. Marchenko V. Yu., Goncharova N. I., Evseenko V. A., Susloparov I. M., Gavrilova E. V., Maksyutov R. A., Ryzhikov A. B. Overview of the epidemiological situation on highly pathogenic avian influenza virus in Russia in 2018. *Problems of Particularly Dangerous Infections*. 2019; 1: 42–49. DOI: 10.21055/0370-1069-2019-1-42-49. (in Russian)

10. MI 45-16 Methodical instructions for the detection of avian influenza A virus RNA using real-time RT-PCR: approved by the Rosselkhoznadzor on 06.06.2016. Vladimir: FGBI "ARRIAH"; 2016. 13 p. (in Russian)

11. Hollander M., Wolfe D. A. Nonparametric Statistical Methods. 1st ed. New York: Wiley Sons Limited, Inc.; 1973. 503 p.

12. Irza V. N., Volkov M. S., Varkentin A. V., Frolov S. V., Altunin D. A. Protective properties of the domestic commercial and experimental vaccines against highly pathogenic subtype H5N1 and H5N8 avian influenza viruses isolated in 2015–2016 [Protektivnye svojstva otechestvennyh serijnyh i eksperimental'nyh vakcin v otnoshenii virusov vysokopatogennogo grippa ptic podtipov H5N1 i H5N8, izolirovannyh v 2015–2016 gg.]. *Poultry and Poultry Products*. 2017; 6: 12–15. Available at: http://vniipp.ru/images/statya/20176/st4.pdf. (in Russian)

13. Avian influenza (including infection with high pathogenicity avian influenza viruses). Chapter 3.3.4. *In: OIE. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals.* Available at: https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.03.04_AI.pdf.

Received on 17.05.2021
Approved for publication on 28.07.2021

INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Sergey V. Frolov, Candidate of Science (Veterinary Medicine), Leading Researcher, Laboratory for Avian Diseases Prevention, FGBI "ARRIAH", Vladimir, Russia.

Lidia O. Scherbakova, Candidate of Science (Biology), Leading Researcher, Reference Laboratory for Avian Viral Diseases, FGBI "ARRIAH", Vladimir, Russia.

Natalya V. Moroz, Candidate of Science (Veterinary Medicine), Head of Laboratory for Avian Diseases Prevention, FGBI "ARRIAH", Vladimir, Russia.

Viktor N. Irza, Doctor of Sciences (Veterinary Medicine), Chief Researcher, Information and Analysis Centre, FGBI "ARRIAH", Vladimir, Russia.

Vladimir Yu. Kulakov, Candidate of Science (Veterinary Medicine), Leading Researcher, Laboratory for Avian Diseases Prevention, FGBI "ARRIAH", Vladimir, Russia.

Фролов Сергей Владимирович, кандидат ветеринарных наук, ведущий научный сотрудник лаборатории профилактики болезней птиц ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Щербакова Лидия Олеговна, кандидат биологических наук, ведущий научный сотрудник референтной лаборатории вирусных болезней птиц ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Мороз Наталья Владимировна, кандидат ветеринарных наук, заведующий лабораторией профилактики болезней птиц ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Ирза Виктор Николаевич, доктор ветеринарных наук, главный научный сотрудник информационно-аналитического центра ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Кулаков Владимир Юрьевич, кандидат ветеринарных наук, ведущий научный сотрудник лаборатории профилактики болезней птиц ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

DOI: 10.29326/2304-196X-2021-3-38-230-238

Studies of biological properties of continuous suspension BHK-21/SUSP/ARRIAH cell line

M. I. Doronin[1], M. N. Guseva[2], D. V. Mikhalishin[3], A. S. Sharypov[4], N. S. Mudrak[5], N. Ye. Kamalova[6], B. L. Manin[7]

[1–3, 5–7] FGBI "Federal Centre for Animal Health" (FGBI "ARRIAH"), Vladimir, Russia

[4] FSBI "Central Scientific and Methodological Veterinary Laboratory" (FSBI CNMVL), Moscow, Russia

[1] *ORCID 0000-0002-4682-6559, e-mail: doronin@arriah.ru*

[2] *ORCID 0000-0002-3997-3390, e-mail: guseva_mn@arriah.ru*

[3] *ORCID 0000-0003-1718-1955, e-mail: mihalishindv@arriah.ru*

[4] *ORCID 0000-0002-1479-5479, e-mail: science@cnmvl.ru*

[5] *ORCID 0000-0002-9788-9197, e-mail: mudrak@arriah.ru*

[6] *ORCID 0000-0001-5671-2347, e-mail: kamalova@arriah.ru*

[7] *ORCID 0000-0002-5263-1491, e-mail: manin_bl@arriah.ru*

SUMMARY

The results of the studies of cytomorphological, karyological, cultural properties of continuous suspension BHK-21/SUSP/ARRIAH subline of newborn Syrian hamster kidney cells intended for foot-and-mouth disease, rabies, bovine parainfluenza-3, Aujeszky's disease virus reproduction, as well as for production of diagnostic veterinary biologicals are presented. When cultured in suspension, BHK-21/SUSP/ARRIAH cell subline undergoes selection towards hypoploidy: modal class is represented by cells with 41 chromosomes (32–40% of cells); the share of cells containing 40–42 chromosomes is 78–80%; the share of polyploids averages around 1%; the range of variation in the number of chromosomes is from 36 to 54. BHK-21/SUSP/ARRIAH cell subline cultured in suspension with cell seeding concentration of 0.6–0.8 million cells/cm^3 demonstrates growth rate of 6.67–11.00 and 96–99% cell viability. After 48 hours, G1-phase (diploid-2*n*) cells prevail in the cell population of the new subline (30.0–75.0% of cells); cells that undergo preparation for mitosis (S-phase) and mitosis (G2+M-phase) account for 3.0 to 20.0% of the entire population; the number of meganucleated and multinucleated cells (> 4*n*) at the beginning and at the end of the logarithmic phase increases to 2%. BHK-21/SUSP/ARRIAH cells recover rapidly after cryopreservation and demonstrate 95–99% viability and growth rate of 3.36–5.88 at passages 1 to 3 and 6.85–10.95 at passages 4 to 12. Continuous suspension BHK-21/SUSP/ARRIAH cell line ensures virus accumulation at the following titres: FMD virus – 7.30–8.00 lg $TCID_{50}$/cm^3, rabies virus – 7.25–8.00 lg $CCID_{50}$/cm^3, bovine parainflunza-3 virus – at least 6.00 lg $TCID_{50}$/cm^3, Aujeszky's disease virus – 7.50–7.80 lg $TCID_{50}$/cm^3.

Keywords: BHK-21/SUSP/ARRIAH cell line, biological properties of cell culture, foot-and-mouth disease, rabies, bovine parainflunza-3, Aujeszky's disease

Acknowledgements: The study was funded by the FGBI "ARRIAH" within the framework of "Veterinary Welfare" research work.

For citation: Doronin M. I., Guseva M. N., Mikhalishin D. V., Sharypov A. S., Mudrak N. S., Kamalova N. Ye., Manin B. L. Studies of biological properties of continuous suspension BHK-21/SUSP/ARRIAH cell line. *Veterinary Science Today.* 2021; 10 (3): 230–238. DOI: 10.29326/2304-196X-2021-3-38-230-238.

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

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

УДК 619:578.835.2:578.824.11:578.831.11:616.98:578.825.1:57.082.26

Исследование биологических свойств перевиваемой суспензионной линии клеток BHK-21/SUSP/ARRIAH

М. И. Доронин[1], М. Н. Гусева[2], Д. В. Михалишин[3], А. С. Шарыпов[4], Н. С. Мудрак[5], Н. Е. Камалова[6], Б. Л. Манин[7]

[1–3, 5–7] ФГБУ «Федеральный центр охраны здоровья животных» (ФГБУ «ВНИИЗЖ»), г. Владимир, Россия

[4] ФГБУ «Центральная научно-методическая ветеринарная лаборатория» (ФГБУ ЦНМВЛ), г. Москва, Россия

[1] *ORCID 0000-0002-4682-6559, e-mail: doronin@arriah.ru*

[2] *ORCID 0000-0002-3997-3390, e-mail: guseva_mn@arriah.ru*

[3] *ORCID 0000-0003-1718-1955, e-mail: mihalishindv@arriah.ru*

[4] *ORCID 0000-0002-1479-5479, e-mail: science@cnmvl.ru*

[5] *ORCID 0000-0002-9788-9197, e-mail: mudrak@arriah.ru*

[6] *ORCID 0000-0001-5671-2347, e-mail: kamalova@arriah.ru*

[7] *ORCID 0000-0002-5263-1491, e-mail: manin_bl@arriah.ru*

РЕЗЮМЕ

Представлены результаты изучения цитоморфологических, кариологических, культуральных характеристик перевиваемой суспензионной клеточной сублинии из почки новорожденного сирийского хомячка ВНК-21/SUSP/ARRIAH, предназначенной для репродукции вирусов ящура, бешенства, парагриппа-3 крупного рогатого скота, болезни Ауески, а также для изготовления диагностических ветеринарных биопрепаратов. Сублиния клеток ВНК-21/SUSP/ARRIAH при суспензионном культивировании проходит селекцию в направлении гипоплоидии: модальный класс соответствует 41 хромосоме (32–40% клеток); доля клеток с количеством хромосом 40–42 составляет 78–80%; доля полиплоидов – в среднем около 1%; пределы изменчивости хромосомного набора соответствуют диапазону от 36 до 54 хромосом. Клеточная сублиния ВНК-21/SUSP/ARRIAH при суспензионном культивировании с посевной концентрацией 0,6–0,8 млн кл./см^3 имеет кратность прироста 6,67–11,00 при жизнеспособности клеток 96–99%. В клеточном цикле популяции новой сублинии через 48 ч преобладает G1-фаза (диплоидная-2*n*), на которую приходится 30,0–75,0% клеток; в фазах подготовки к митозу (S-фаза) и митотического деления (G2+M-фаза) находится от 3,0 до 20,0% всей популяции; количество крупноядерных и многоядерных клеток (> 4*n*) в начале и конце стадии логарифмического роста увеличивается до 2%. Клетки сублинии ВНК-21/SUSP/ARRIAH быстро восстанавливаются после криоконсервирования с жизнеспособностью 95–99% и кратностью прироста 3,36–5,88 на первом – третьем пассажах и 6,85–10,95 – с четвертого по двенадцатый пассаж. Перевиваемая суспензионная линия клеток ВНК-21/SUSP/ARRIAH обеспечивает накопление вируса ящура в титрах 7,30–8,00 lg ТЦД$_{50}$/см^3, вируса бешенства – 7,25–8,00 lg ККИД$_{50}$/см^3, вируса парагриппа-3 крупного рогатого скота в титрах не менее 6,00 lg ТЦД$_{50}$/см^3, вируса болезни Ауески – 7,50–7,80 lg ТЦД$_{50}$/см^3.

Ключевые слова: клеточная линия ВНК-21/SUSP/ARRIAH, биологические свойства культуры клеток, ящур, бешенство, парагрипп-3 крупного рогатого скота, болезнь Ауески

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

Для цитирования: Доронин М. И., Гусева М. Н., Михалишин Д. В., Шарыпов А. С., Мудрак Н. С., Камалова Н. Е., Манин Б. Л. Исследование биологических свойств перевиваемой суспензионной линии клеток ВНК-21/SUSP/ARRIAH. *Ветеринария сегодня*. 2021; 10 (3): 230–238. DOI: 10.29326/2304-196X-2021-3-38-230-238.

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

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

INTRODUCTION

Continuous suspension BHK-21 clone 13 line derived from newborn Syrian hamster kidney cells by M. Stoker and J. Macpherson in England in 1961 is one of the cell cultures most commonly used worldwide [1]. The results of the study were published by the authors in scientific journals, such as *Virology* (1962) and *Journal of the National Cancer Institute* (1963). Later, the following analogues of this cell culture were developed:

– BHK-21/13 – a subline of continuous newborn Syrian hamster kidney cells developed at the Institute of Poliomyelitis and Viral Encephalitis of the Academy of Medical Sciences of the USSR (Moscow) [2];

– BHK-21/2-17b – a continuous monolayer-suspension clone of newborn Syrian hamster kidney cells developed at the All-Union Foot-and-Mouth Disease Research Institute (Vladimir) [2, 3];

– BHK-21/13-02 – a continuous monolayer-suspension subline of newborn Syrian hamster kidney cells developed at the Federal State Enterprise "Shchelkovo Biocombinat" for FMD and rabies virus reproduction [4];

– BHK-21/13-13 – a continuous monolayer-suspension subline of newborn Syrian hamster kidney cells developed at the Federal State Enterprise "Shchelkovo Biocombinat" for reproduction of type A, O, C, Asia-1, SAT-1, SAT-2, SAT-3 FMDV and "Shchelkovo-51" strain of rabies virus used for production of virus vaccines against foot-and-mouth disease and rabies [5, 6].

Continuous cell lines used in biotechnology differ in respect of morphology, karyology, growth properties, cultivation requirements, maintenance methods and susceptibility to viruses [7, 8]. The available BHK-21 cell sublines are characterized by high growth rates and allow for FMD and rabies virus reproduction. Full-scale production of culture vaccines against FMD, rabies, bovine parainfluenza-3 and Aujeszky's disease requires the use of a suspension BHK-21 cell subline to ensure the accumulation of the viruses with high infectivity titres. BHK-21/13, BHK-21/2-17b, BHK-21/13-02 and BHK-21/13-13 cell lines allow for preparation of viruses with the following infectivity titres: FMD virus – not more than 7.00 lg TCID$_{50}$/cm^3, rabies virus – 7.00 lg CCID$_{50}$/cm^3 [4, 5, 9, 10]. Data on the results of bovine parainfluenza-3 and Aujeszky's disease virus cultivation are not provided in the patents for the said BHK-21 cell sublines.

To respond to the production needs related to the reproduction of FMD, rabies, bovine parainfluenza-3 and Aujeszky's disease viruses with high infectivity titres, BHK-21/2-17b cell subline was developed at the All-Union Foot-and-Mouth Disease Research Institute by means of BHK-21 clone 13 cell culture selection, from which a new BHK-21/SUSP/ARRIAH subline was derived 30 years later by means of permanent cultivation in suspension. BHK-21/SUSP/ARRIAH cell subline was deposited in the Specialized Cell Culture Collection of the Center for Collective Use "Collection of Vertebrate Cell Cultures" of the Institute of Cytology of the Russian Academy of Sciences with No. RKKK(P) 797 D [11].

The study is aimed at examining the biological properties of continuous suspension BHK-21/SUSP/ARRIAH subline of newborn Syrian hamster kidney cells and evaluating the possibility of using it for animal virus reproduction.

MATERIALS AND METHODS

Cell cultivation in suspension was carried out using semi-continuous culture technique in glass and metal fermenters with the capacity of 40 to 2,000 l in separate cycles, 10–12 passages each. A culture growth medium containing 5% of bovine serum, blood protein hydrolysate at a concentration of 15–20 cm^3/dm^3, Hottinger's digest (2–10 cm^3/dm^3), 8 proteinogenic aminoacids, vitamins and mineral salts were used for cultivation. Cell seeding concentration was 0.6–0.8 million cells/cm^3. Every 12 hours, pH levels, live and dead cell concentrations, suspension sterility were measured. During cell reproduction, lactate was formed, and this contributed to pH decrease. Therefore, a 7.5% baking soda solution was added to the cell suspension and/or pH was adjusted by bubbling.

Cell morphology analysis. Cells were examined using phase contrast microscopy at different magnifications. In order to assess the nucleus-to-cytoplasm ratio, the suspension cells were treated with acridine orange. The acridine orange exposure resulted in bright greenish-yellow staining of nuclei visualized on the yellow background of cytoplasm. Trypan blue staining was used for cell viability determination [12].

Karyological analysis of cells was carried out using the technique described by P. S. Moorhead et al. [13] that allows for detection of metaphase chromosome. Log phase cells collected from the fermenters were transferred onto the solid substrate in the growth nutrient medium containing 0.001% of colchicine and incubated for 3–4 hours. Rounded metaphase cells were agglomerated by shaking and then concentrated by centrifugation of the suspension. The further process was carried out in centrifuge tubes as follows: 10 minutes – hypotonic treatment at 36 °C; 3 treatments with fixing solution, 10 minutes each, with centrifuging at 22–25 °C. The resulting suspension was applied to the chilled slides with a Pasteur pipette and stained with Giemsa's solution for 10–15 minutes. After the preparation had been prepared, 100 metaphase plates were photographed, the number of chromosomes in them was calculated using immersion microscopy at 90× magnification, and karyogram was plotted [14].

Cytometric analysis of the cell subline. The comparative analysis of cell cycle phases was carried out by means of cytometry [15, 16] (n = 22) using an Accuri C6 flow cytometer and the cell DNA detection kit "C6 Flow Cytometer Fluid Kit" (BD Accuri™, USA) according to the manufacturer's recommendations. DNA histograms were obtained for the subline cells 48 hours after subcultivation.

DNA eluates were analyzed in the cytometer using the programme "Analysis of cell cycle parameters and DNA content in live cells". The process lasted 2 hours; fluorescent signal was recorded. Cell distribution by G1/G0-, S- and G2/M-phases of the cell cycle was determined by measuring relative DNA content in the cells using DNA binding fluorescent dyes.

Cryopreservation of the cell suspension was carried out in 100 cm^3 vials using a cryomedium (a growth medium supplemented with 7–10% of dimethyl sulfoxide and 20% of fetal calf serum). The cell suspension was cooled to minus 70 °C at a cooling rate of 2 °C/min, to minus 150 °C – at 10 °C/min; then the vials were placed in liquid nitrogen at minus 196 °C. The cell suspension was thawed at 39–42 °C during 2 minutes.

For cell thawing, direct seeding method comprising the following steps was used: rapid thawing of the suspension at 37 °C using a water bath; mixing of 1.0 cm^3 of cells with 20 cm^3 of the culture growth medium containing 10% of calf serum (cell seeding concentration was 0.5–0.7 million cells/cm^3); incubation of cells for 12 hours with subsequent change of the medium in order to remove the cryopreservation agent.

Viruses. The following viruses were used: FMDV A/Turkey/2006, O/Saudi Arabia/2008, Asia-1/Tajikistan/2011 strains, rabies virus "ARRIAH" and "RV-97" strains, Aujeszky's disease virus "VK" and "K" strains, bovine parainfluenza-3 virus "VGNKI-4" strain.

RESULTS AND DISCUSSION

BHK-21/SUSP/ARRIAH cell morphology analysis in comparison with BHK-21/2-17b. Cell morphology of BHK-21/2-17b subline was studied at the time of patenting in 1986 (Fig. 1).

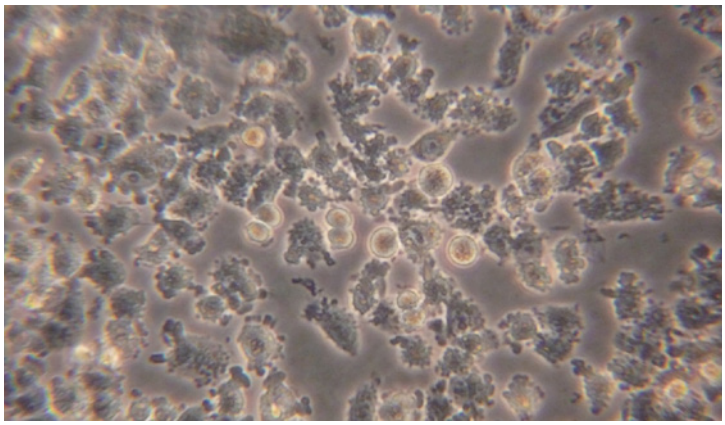


Fig. 1. Cell morphology of BHK-21/2-17b subline (passage 1, at the time of patenting in 1986; magnification 80×)

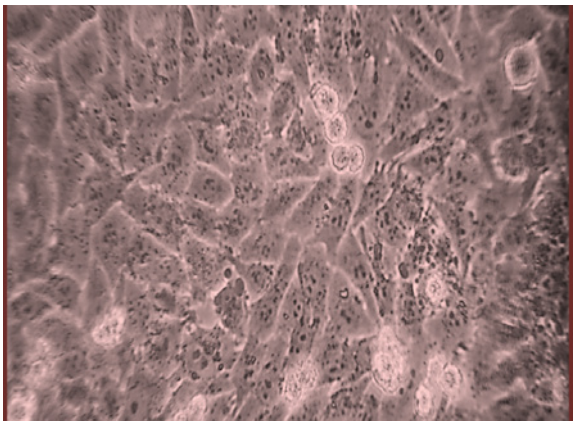


Fig. 2. Cell morphology of BHK-21/2-17b subline (passage 2; magnification 80×)

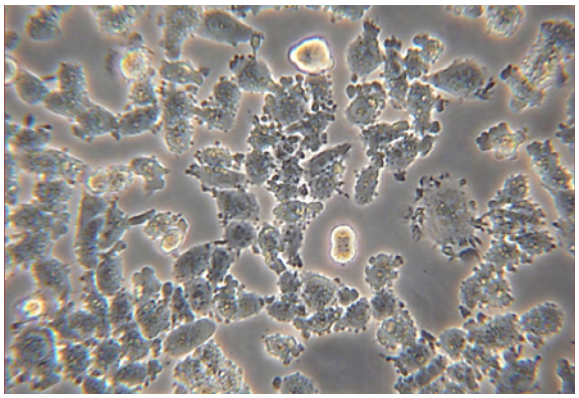


Fig. 3. Cell morphology of BHK-21/SUSP/ARRIAH subline (magnification 80×)

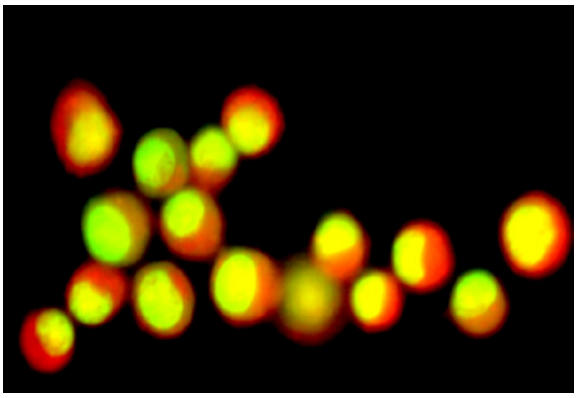


Fig. 4. Visualization of nuclei of suspension BHK-21/SUSP/ARRIAH subline cells stained with acridine orange

Morphologically, the cells were found to be amorphous; in other words, passage 1 cells seeded onto the substrate did not have a definite form, moved actively and attained a spherical form only during division. Passage 2 cells of this subline were fully adherent and attained an epithelioid form (Fig. 2). When all available substrate was covered with cells, some of the cells formed a suspension and demonstrated high proliferation rates (their growth rate was not less than 4–6).

The morphology of suspension BHK-21/SUSP/ARRIAH cells is shown in Figure 3. The morphology studies showed that the cells remained practically unchanged externally as compared with BHK-21/2-17b subline. Small cells of 8–10 μm in size prevailed in the population (up to 90%). It should be noted that, when passaged on the horizontal surface, the cells demonstrated low adhesion (the exceptions were polyploids) and moved intensively. Multiple mitotic divisions resulted in a significant increase in suspension cell concentration in the culture flask. Thus, the developed BHK-21/SUSP/ARRIAH cell subline is exclusively a suspension cell subline.

For nucleus-to-cytoplasm ratio assessment, 50 samples of the suspension of BHK-21/SUSP/ARRIAH cells grown in the glass and metal fermenters were collected and treated with acridine orange. The acridine orange exposure resulted in bright greenish-yellow staining of nuclei visualized on the yellow background of cytoplasm (Fig. 4).

Based on nucleus and cell diameter measurements, it was concluded that the new subline demonstrates a larger nucleus size in relation to cytoplasm and cell size. In most cases, the nucleus made up 60–80% of the cell volume.

Karyological analysis of BHK-21/SUSP/ARRIAH cells in comparison with BHK-21/2-17b. Chromosome preparations for the karyological analysis were prepared according to the technique described by P. S. Moorhead et al. [13]; then 100 metaphase plates were examined microscopically and microphotographed, the number of chromosomes was calculated, and karyogram was plotted.

Figure 5 shows BHK-21/2-17b cell population composition at the time of patenting in 1986. Multiple tests showed the stable predominance of 42-chromosome cell populations (28–40% of the entire population). The following chromosome numbers were detected: 36 chromosomes – in 1% of the populations, 37 chromosomes – in 2%, 38 chromosomes – in 9%, 39 chromosomes – in 3%, 40 chromosomes – in 8%, 41 chromosomes – in 27%, 42 chromosomes – in 40%, 43 chromosomes – in 7%, 44 chromosomes – in 2%, polyploids made up 1%.

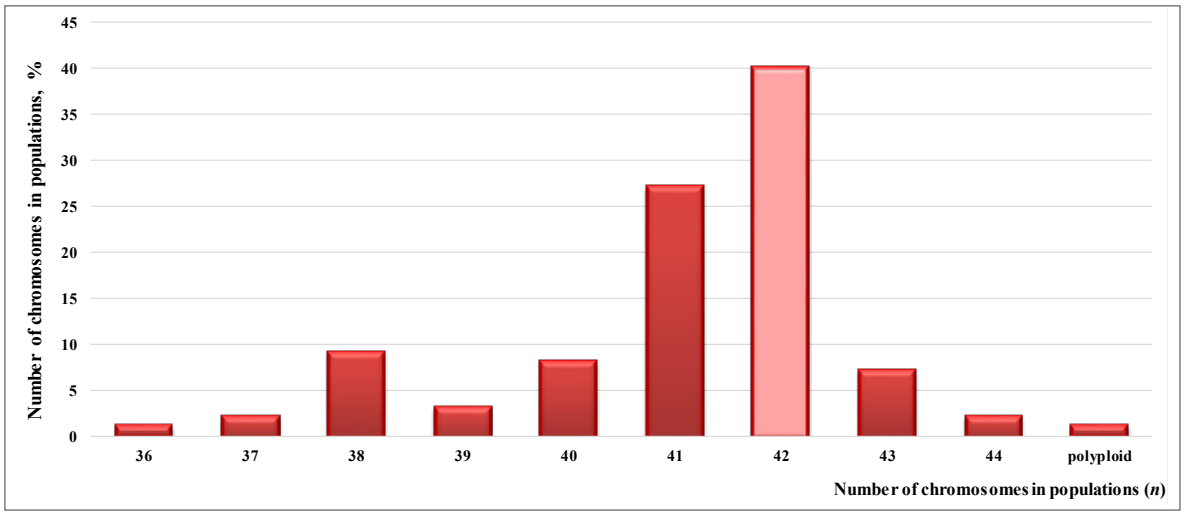


Fig. 5. Karyogram of BHK-21/2-17b subline cell population at the time of patenting (1986)

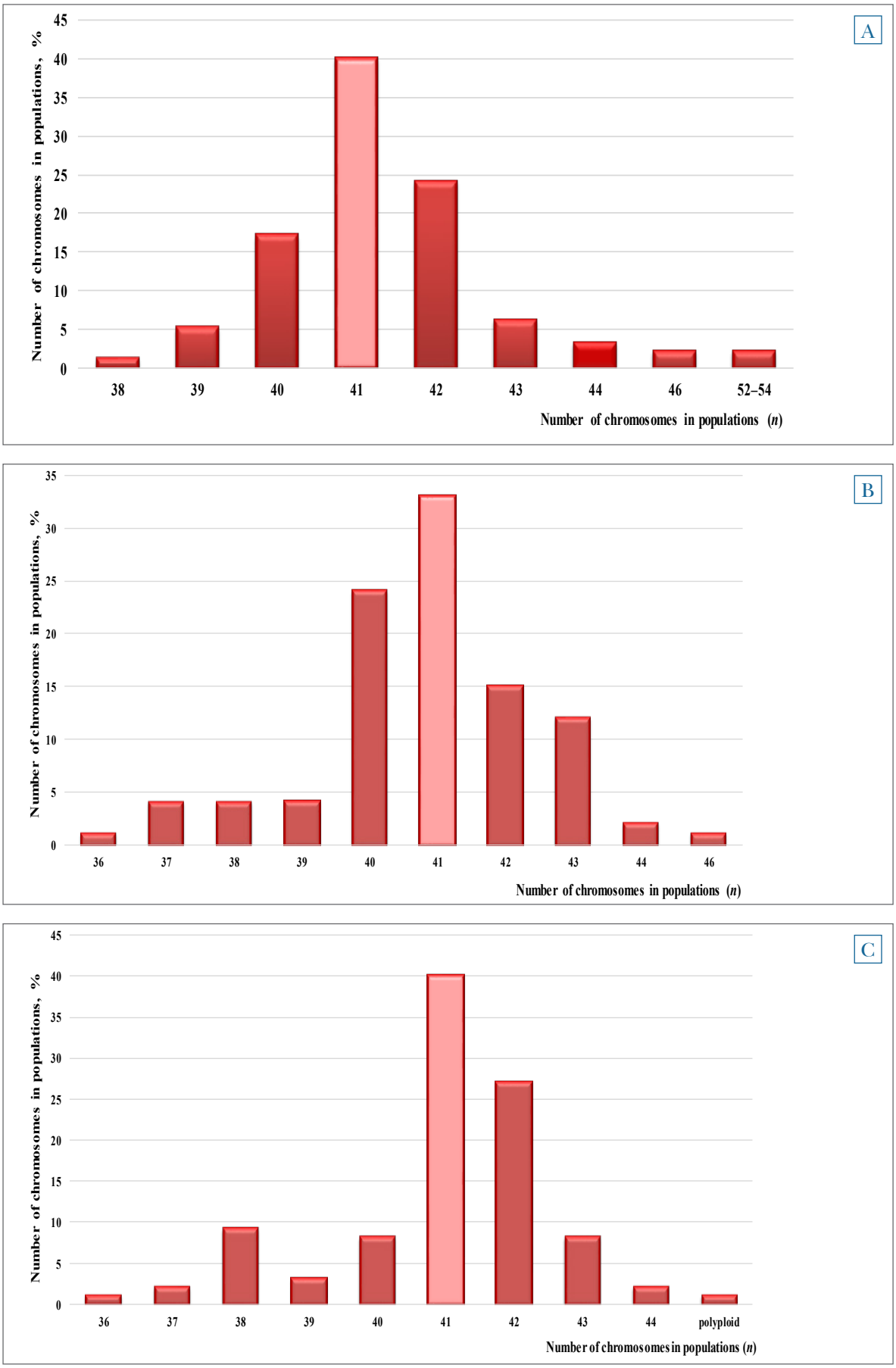


Fig. 6. Karyogram of BHK-21/SUSP/ARRIAH subline cells grown in 50 (A), 250 (B), 2,000 dm³ (C) fermenters

During three decades, monolayer culture of these cells was not used in production. As a result of suspension cultivation in the growth medium during about 100 consecutive passages, BHK-21/2-17b cells transformed into a new cell subline, BHK-21/SUSP/ARRIAH. The populations of cells of this line grown in 40, 250 and 2,000 dm^3 fermenters were subjected to karyological analysis. Fifty samples from each of the cell suspensions prepared 48 hours after seeding were used for the tests. It was found that the cells had undergone some karyological changes under homogeneous culture conditions. The modal class of the new subline is represented by cells with 41 chromosomes (32–40% of the population). The share of cells containing 40–42 chromosomes is 78–80%, the share of polyploids averages around 1%. The range of variation in the number of chromosomes in BHK-21/SUSP/ARRIAH cells is from 36 to 54. The following chromosome numbers were detected: 36 chromosomes – in 0–1% of the populations, 37 chromosomes – in 0–4%, 38 chromosomes – in 1–9%, 39 chromosomes – in 3–5%, 40 chromosomes – in 8–24%, 41 chromosomes – in 33–40%, 42 chromosomes – in 15–27%, 43 chromosomes – in 6–12%, 44 chromosomes – in 2–3%, 46 chromosomes – in 0–2%, and 52–54 chromosomes – in 0–2% (Fig. 6).

Cytometric analysis of BHK-21/SUSP/ARRIAH cells in comparison with BHK-21/2-17b. DNA histograms presented in Figure 7 and data in Table 1 were obtained in the course of comparative analysis of cell cycle phases of the two sublines 48 hours after cell suspension subcultivation.

Based on the data obtained, it was found that apoptotic cells and cell debris made up 51.0–60.0% of BHK-21/2-17b cells, that is 5.0–31.0% more than the percentage for BHK-21/SUSP/ARRIAH subline (20.0–55.0%). Cells undergoing G1-phase (pre-synthesis phase) made up 30.0–75.0% of BHK-21/SUSP/ARRIAH subline cells and 28.0–70.3% of BHK-21/2-17b subline cells. This phase is characterized by cell preparation for chromosome duplication, intensive synthesis of polypeptides, an increase in the number of mitochondria and ribosomes. The share of G1-phase cells in the new subline was 2.0–4.7% more as compared with BHK-21/2-17b subline. Apparently, some of BHK-21/2-17b cells, in between mitotic M-phase and the beginning of G1-phase, enter apoptosis, and this subsequently has impact on the growth rate of the entire population.

Cells undergoing S-phase (synthesis phase) that involves cell DNA replication in many replicons and the beginning of centriole duplication in the cell centre make up 2.0–33.0% of BHK-21/SUSP/ARRIAH subline cells and

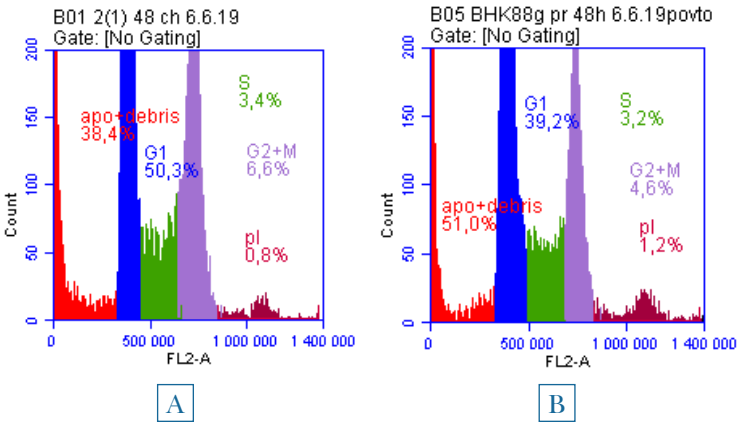


Fig. 7. DNA histogram of BHK-21/SUSP/ARRIAH (A) and BHK-21/2-17b (B) subline cells

Table 1
Comparative analysis of life cycles of BHK-21/SUSP/ARRIAH and BHK-21/2-17b subline cells based on cytometry data

Cell cycle phase	Percentage of cells undergoing the specified cell cycle phase, %		Difference in cell percentages, %
	BHK-21/SUSP/ARRIAH	BHK-21/2-17b	
Apoptosis	20.0–55.0	51.0–60.0	31.0–5.0
G1-phase	30.0–75.0	28.0–70.3	2.0–4.7
S-phase	2.0–33.0	1.5–30.0	0.5–3.0
G2+M-phase	3.0–20.0	2.6–18.0	0.4–2.0

Table 2
Evaluation of growth properties of suspension BHK-21/SUSP/ARRIAH subline cells in comparison with prototype cell sublines

Cell subline	Monolayer cultivation				Suspension cultivation		
	split ratio	monolayer formation time, days	monolayer survival time, days	number of cells per cultivation flask, mln*	growth rate	cell concentration, mln/cm^3	viable cells, %
BHK-21/13	1:3	2–3	3	40–45	maintained as suspension**		
BHK-21/2-17b	1:3	2–3	5	40–45	6.00–7.00	2.30–2.80	95–99
BHK-21/13-02	1:2–1:3	2–3	5	40–45	6.00–8.00	up to 4.00	95–99
BHK-21/13-13	1:2–1:3	2–3	5–6	40–45	6.00–8.00	2.40–4.00	95–98
BHK/SUSP/ARRIAH	low adhesion, amorphous cells with multiple cytoplasmic protrusions				6.67–11.00	4.00–5.50	96–99

* a cultivation flask with a growth surface area of 375 cm^2 is used;
** detailed information on suspension cultivation [6, 10] is not reflected.

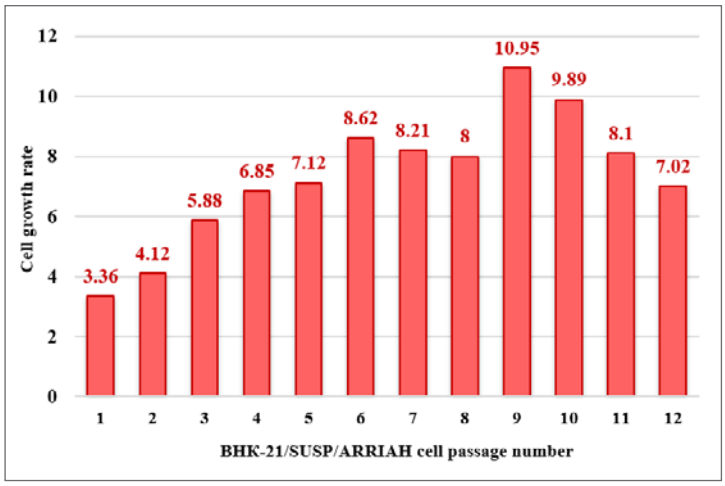


Fig. 8. Growth rate variations of BHK-21/SUSP/ARRIAH subline cells recultivated after cryopreservation (mean growth rate values are provided; n = 30, p < 0.005)

1.5–30.0% of prototype line cells, i.e. 0.5–3.0% less as compared with the cells of the new subline.

After 48 hours, cells undergoing G2-phase (post-synthesis phase) characterized by energy accumulation, protein accretion for mitosis and tetraploid DNA content attainment and cells undergoing M-phase (mitosis) account for 3.0–20% of BHK-21/SUSP/ARRIAH cells, which is 0.4–2.0% more than the percentage for BHK-21/2-17b cells (2.6–18%); this is indicative of a greater capacity of the cells of the new subline to continue division.

Thus, as a result of suspension cultivation in the growth medium with modified composition during about 100 consecutive passages, BHK-21/2-17b cells transformed into a new cell subline, BHK-21/SUSP/ARRIAH, in which, after 48 hours, cells undergoing G1-phase of the cell cycle prevail (30.0–75.0% of cells). Cells undergoing the phases of preparation for mitosis and mitosis (G2+M-phase) account for 3.0 to 20.0% of the entire population. The number of meganucleated and multinucleated cells (> 4*n*) at the beginning and at the end of the logarithmic phase increases to 2%. In other words, the basic population, apparently, underwent selection manifested as a 5.0–31.0% decrease in the number of apoptotic cells and cell debris amount and an increase in the number of cells undergoing G1-, S- and G2+M-phases that ensure cell growth by 2.0–4.7; 0.5–3.0 and 0.4–2.0%, respectively.

Table 3
Cultivation of FMD, rabies, bovine parainfluenza-3 and Aujeszky's disease viruses in suspension BHK-21/SUSP/ARRIAH cell subline ($p < 0.005$)

Cultivated viruses	Cytopathic effect, %	Virus infectivity titre	Number of tests
FMDV A/Turkey/06 strain	95–98	7.30 ± 0.13 lg $TCID_{50}/cm^3$	100
FMDV O/Saudi Arabia/08 strain	95–98	7.80 ± 0.23 lg $TCID_{50}/cm^3$	100
FMDV Asia-1/Tajikistan/11 strain	95–99	8.00 ± 0.21 lg $TCID_{50}/cm^3$	80
rabies virus "ARRIAH" strain	95–99	8.00 ± 0.13 lg $CCID_{50}/cm^3$	40
rabies virus "RV-97" strain	95–99	7.25 ± 0.20 lg $CCID_{50}/cm^3$	40
Aujeszky's disease virus "VK" strain	95–97	7.80 ± 0.15 lg $TCID_{50}/cm^3$	40
Aujeszky's disease virus "K" strain	95–97	7.50 ± 0.19 lg $TCID_{50}/cm^3$	40
bovine parainfluenza-3 virus "VGNKI-4" strain	95–98	6.00 ± 0.15 lg $TCID_{50}/cm^3$	50

Evaluation of growth properties of BHK-21/SUSP/ARRIAH subline cells in comparison with prototype sublines. BHK-21/SUSP/ARRIAH cells resulting from long-term suspension cultivation (100 consecutive passages) were tested for their growth properties in comparison with prototype BHK-21/13, BHK-21/2-17b, BHK-21/13-02 and BHK-21/13-13 sublines. The test results are presented in Table 2. These data show that BHK-21/SUSP/ARRIAH cells cultivated in suspension using semi-continuous culture technique (seeding concentration is 0.5–0.6 million cells/cm^3) reach a concentration of 4.0–5.5 million cells/cm^3 after 48 hours of cultivation under optimum conditions. Thus, they demonstrate growth rates of 6.67–11.00 and 96–99% viability. Suspension BHK-21/SUSP/ARRIAH cells grown in culture flasks under static conditions were characterized by low adhesion, amorphous form, multiple cytoplasmic protrusions (the protrusion dynamics spanned a period of several minutes).

Viability tests of BHK-21/SUSP/ARRIAH subline cells recultivated after cryoconservation. The number of living cells was determined in suspension after cryopreservation ($n = 10$). Based on the test results, it was found that the viability of cells after cryopreservation was 85–90% at passage 1 and increased to 96–99% starting from passage 2.

One-day proliferation delay was observed in depreserved cells grown in suspension during passage 1. Figure 8 shows BHK-21/SUSP/ARRIAH cell growth rates for 12 consecutive passages after cryopreservation. Proliferation rates at passages 1–3 varied from 3.36 ± 0.21 to 5.88 ± 0.15 ($p < 0.005$). Starting from passage 4, growth rates were from 6.85 ± 0.18 to 10.95 ± 0.31 ($p < 0.005$). It is considered that the normal growth rate for BHK-21 cells is 4 and above [9, 17]. In other words, BHK-21/SUSP/ARRIAH cell line attains the required cell growth rate starting from passage 2. The highest cell proliferation rate for the tested subline was observed at passage 9. It should be noted, that at passage 12 the cell growth rate was 7.02 and exceeded the lower normal value by 3.02. Thus, BHK-21/SUSP/ARRIAH cell subline is recultivated rapidly after cryopreservation and demonstrates high proliferation rates (up to 10.95 ± 0.31) and 95–99% cell viability.

Reproduction of FMD, rabies, bovine parainfluenza-3 and Aujeszky's disease viruses in BHK-21/SUSP/ARRIAH subline cells in comparison with prototype sublines (based on literature data). To prepare virus-containing material for virus vaccine production, the following virus strains were reproduced in continuous suspension BHK-21/SUSP/ARRIAH cells: FMDV A/Turkey/2006 ($n = 100$), O/Saudi Arabia/08 ($n = 100$), Asia-1/Tajikistan/2011 ($n = 80$)

strains, rabies virus "ARRIAH" (n = 40) and "RV-97" (n = 40) strains, Aujeszky's disease virus "VK" (n = 40) and "K" (n = 40) strains, bovine parainfluenza-3 virus "VGNKI-4" (n = 50) strain (Table 3).

The comparative analysis based on the obtained results and literature data for prototype cell sublines [4, 5] demonstrated that FMDV caused 95–99% cytopathic effect (CPE) and showed infectivity titres of 7.30 ± 0.13 to 8.00 ± 0.21 lg $TCID_{50}/cm^3$ (n = 280), when cultivated in BHK-21/SUSP/ARRIAH cells, and up to 7.00 lg $TCID_{50}/cm^3$ in BHK-21/2-17b, BHK-21/13-02 and BHK-21/13-13 cell cultures.

Rabies virus caused 95–99% CPE and showed infectivity titres of 7.25 ± 0.20 to 8.00 ± 0.13 lg $CCID_{50}/cm^3$ (n = 80), when reproduced in BHK-21/SUSP/ARRIAH cell subline, 6.50 lg $CCID_{50}/cm^3$ in BHK-21/13-02 cell culture, up to 7.00 lg $CCID_{50}/cm^3$ in BHK-21/2-17b and BHK-21/13-13 cell cultures. Aujeszky's disease virus cultivated in BHK-21/SUSP/ARRIAH cell culture caused 95–97% CPE and was accumulated with infectivity titres of 7.50 ± 0.19 to 7.80 ± 0.15 lg $TCID_{50}/cm^3$ (n = 80); bovine parainfluenza-3 virus caused 95–98% CPE and showed infectivity titres of up to 6.00 ± 0.15 lg $TCID_{50}/cm^3$ (n = 50).

Thus, the cells of BHK-21/SUSP/ARRIAH subline developed for production of virus vaccines and diagnostic veterinary biologicals allow for FMD virus reproduction with titres of up to 8.00 ± 0.21 lg $TCID_{50}/cm^3$, i.e. 1.00 lg $TCID_{50}/cm^3$ higher in comparison with prototype sublines (not more than 7.00 lg $TCID_{50}/cm^3$). The proposed subline ensures rabies virus accumulation with titres of up to 8.00 ± 0.13 lg $CCID_{50}/cm^3$, i.e. 0.25–1.00 lg $CCID_{50}/cm^3$ higher in comparison with prototype lines (not more than 7.00 lg $CCID_{50}/cm^3$), as well as bovine parainfluenza-3 and Aujeszky's disease virus accumulation with titres of 6.00 ± 0.15 and up to 7.80 ± 0.15 lg $TCID_{50}/cm^3$, respectively.

CONCLUSION

The biological properties of continuous suspension BHK-21/SUSP/ARRIAH subline of newborn Syrian hamster kidney cells were studied, and the possibility of using it for foot-and-mouth disease, rabies, bovine parainfluenza-3, Aujeszky's disease virus reproduction was evaluated.

It was found that BHK-21/SUSP/ARRIAH cell subline, when cultured in suspension, undergoes selection towards hypoploidy: modal class is represented by cells with 41 chromosomes (32–40% of cells); the share of cells containing 40–42 chromosomes is 78–80%; the share of polyploids averages around 1%; the range of variation in the number of chromosomes is from 36 to 54.

It was detected that BHK-21/SUSP/ARRIAH cell subline cultivated in suspension using semi-continuous culture technique demonstrates exponential growth (proliferation rate decreases after 48 hours) with growth rates of 6.67–11.00 and 96–99% cell viability.

It was established that suspension BHK-21/SUSP/ARRIAH cells grown under static conditions are characterized by low adhesion, amorphous form, multiple cytoplasmic protrusions and during mitosis accumulate in the culture medium in suspension.

It was found that, after 48 hours, G1-phase cells prevail in the cell population of BHK-21/SUSP/ARRIAH subline (30.0–75.0% of cells); cells that undergo preparation for mitosis and mitosis (G2+M-phase) account for 3.0 to 20.0% of the entire population; the number of meganucleated and multinucleated cells (> 4n) at the beginning and at the end of the logarithmic phase increases to 2%.

Suspension BHK-21/SUSP/ARRIAH cells were found to recover rapidly after cryopreservation demonstrating 95–99% viability and growth rates of 3.36–5.88 (passages 1–3) and 6.85–10.95 (passages 4–12).

The study showed that suspension BHK-21/SUSP/ARRIAH cells allow for virus reproduction with the following titres:

– FMD virus – 7.30–8.00 lg $TCID_{50}/cm^3$;
– rabies virus – 7.25–8.00 lg $CCID_{50}/cm^3$;
– bovine parainflunza-3 virus – at least 6.00 lg $TCID_{50}/cm^3$;
– Aujeszky's disease virus – 7.50–7.80 lg $TCID_{50}/cm^3$.

REFERENCES

1. Stoker M., Macpherson I. Syrian hamster fibroblast cell line BHK-21 and its Derivatives. *Nature.* 1964; 203: 1355–1357. DOI: 10.1038/2031355A0.

2. Manin B. L., Mikhalishin V. V., Korpusova T. I., Stegniy B. T., Lavrik A. A. Biological tolerance parameters of production BHK-21 cell culture [Parametry biologicheskoj tolerantnosti proizvodstvennoj kletochnoj kul'tury BHK-21]. *Veterinary Medicine: Inter-Departmental Subject Scientific Collection*. Kharkiv; 2002; 80: 404–408. (in Russian)

3. Sokova V. V., Aliyeva L. A., Bondarenko A. F., Khudyakov G. A. BHK-21/2-17b line of newborn Syrian hamster kidney cells [Kletochnaya liniya pochki novorozhdennogo sirijskogo homyachka BHK-21/2-17b]. Certificate of Authorship No. 240289 USSR. All-Union Foot-and-Mouth Disease Research Institute; 1986. (in Russian)

4. Eliseev A. K., Melnik N. V., Zenov N. I., Litenkova I. Yu., Bogach V. N., Ivanov V. S., et al. BHK-21/13-02-transplantable monolayer-suspension subline of newborn syrian hamster kidney cells, intended for FMD virus and rabies virus reproduction. Patent No. 2614074 Russian Federation, Int. C12N 5/07 (2010.01). FKP "Shchelkovskij biokombinat". No. 2005116301. Date of filing: 31.05.2005. Date of publication: 22.03.2017. Bull. No. 9. (in Russian)

5. Eliseev A. K., Zenov N. I., Krasutkin S. N., Mel'nik N. V., Khajkina L. S., Litenkova I. Yu., et al. BHK-21/13-13-finite monolayer-suspension cell subline of kidney of new-born syrian hamster, intended for reproduction of foot-and-mouth disease virus and rabies virus. Patent No. 2553552 Russian Federation, Int. C12N 5/071 (2010.01). FKP "Shchelkovskij biokombinat". No. 2014131866/10. Date of filing: 01.08.2014. Date of publication: 20.06.2015. Bull. No. 17. (in Russian)

6. Russian Cell Culture Collection (RCCC) [Rossijskaya kollekciya kletochnyh kul'tur (RKKK)]: catalogue. Ed. by G. P. Pinayev, G. G. Polyanskaya. St. Petersburg; 2004. 315 p. (in Russian)

7. Bailey J. E., Ollis D. F. Biochemical Engineering Fundamentals. 2nd ed. New Delhi: McGraw-Hill, Inc.; 1986. 984 p.

8. Cell culture techniques [Metody kul'tivirovaniya kletok]: Proceedings of the Academy of Sciences of the USSR. Executive ed. G. P. Pinayev. L.: Nauka; 1988. 319 p. (in Russian)

9. Catalogue of Russian Cell Culture Collection [Katalog Rossijskoj kollekcii kletochnyh kul'tur]. Executive ed. G. P. Pinayev, G. G. Polyanskaya. Omsk: Omsk State Pedagogical University (OSPU); 1999. 429 p. (in Russian)

10. Yurkov S. G., Zuyev V. V., Sidorov S. I., Kushnir S. D., Smyslova N. Yu., Neverovskaya N. S., et al. Catalogue of Cell Culture Collection of the All-Russian Research Institute of Veterinary Virology and Microbiology (VNIIVViM) [Katalog kollekcii kletochnyh kul'tur VNIIVViM]. Pokrov; 2000; 60–62. eLIBRARY ID: 21632428. (in Russian)

11. Lozovoy D. A., Guseva M. N., Mikhalishin D. V., Doronin M. I., Manin B. L., Shishkova A. A., et al. BHK-21/SUSP/ARRIAH – continuous suspension subline of newborn syrian hamster kidney cells, intended for reproduction of foot-and-mouth disease viruses, rabies, parainfluenza-3, Aujeszky's disease in producing antiviral vaccines, as well as for making diagnostic and preventive veterinary biopreparations. Patent No. 2722671 Russian Federation, Int. C12N 5/10 (2006.01). FGBI "ARRIAH". No. 2019131190. Date of filing: 01.10.2019. Date of publication: 02.06.2020. Bull. No. 16. (in Russian)

12. Polyanskaya G. G. Karyotype variation patterns in cell cultures during long-term cultivation in different cell lines [Zakonomernosti kariotipicheskoj izmenchivosti v kletochnyh kul'turah pri dlitel'nom kul'tivirovanii v raznyh liniyah]: author's abstract of Candidate of Science (Biology) thesis. St. Petersburg; 2000. 38 p. (in Russian)

13. 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.

14. Mamayeva S. Ye. Chromosomal analysis of cultured cells [Hromosomnyj analiz kul'tiviruemyh kletok]. *In: Cell culture techniques*. L.: Nauka; 1987; 78–98. (in Russian)

15. Kudryavtsev I. V., Khaydukov S. V., Zurochka A. V., Chereshnev V. A. Flow cytometry in experimental biology [Protochnaya citometriya v eksperimental'noj biologii]. Yekaterinburg: Ural Branch of the Russian Academy of Sciences (UB RAS); 2012. 192 p. (in Russian)

16. Ormerod M. G., Tribukait B., Giaretti W. Consensus report of the task force on standardisation of DNA flow cytometry in clinical pathology. *Analytical Cellular Pathology*. 1998; 17 (2): 103–110. DOI: 10.1155/1998/842306.

17. Rais Y., Zviran A., Geula S., Gafni O., Chomsky E., Viukov S., et al. Deterministic direct reprogramming of somatic cells to pluripotency. *Nature*. 2013; 502 (7549): 65–70. DOI: 10.1038/nature12587.

Received on 12.03.2021
Approved for publication on 05.05.2021

INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Maksim I. Doronin, Candidate of Science (Biology), Leading Researcher, Laboratory for FMD Prevention, FGBI "ARRIAH", Vladimir, Russia.

Marina N. Guseva, Candidate of Science (Biology), Senior Researcher, Laboratory for FMD Prevention, FGBI "ARRIAH", Vladimir, Russia.

Dmitry V. Mikhalishin, Candidate of Science (Veterinary Medicine), Head of Laboratory for FMD Prevention, FGBI "ARRIAH", Vladimir, Russia.

Andrey S. Sharypov, Candidate of Science (Veterinary Medicine), Researcher, Department for Research Coordination, FSBI CNMVL, Moscow, Russia.

Natalia S. Mudrak, Doctor of Science (Biology), Chief Researcher, Reference Laboratory for Avian Viral Diseases, FGBI "ARRIAH", Vladimir, Russia.

Natalia Ye. Kamalova, Doctor of Science (Veterinary Medicine), Chief Researcher, Centre for Preclinical Tests, FGBI "ARRIAH", Vladimir, Russia.

Boris L. Manin, Candidate of Science (Biology), Leading Researcher, Sector for Cell Culture, Innovation Department, FGBI "ARRIAH", Vladimir, Russia.

Доронин Максим Игоревич, кандидат биологических наук, ведущий научный сотрудник лаборатории профилактики ящура ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Гусева Марина Николаевна, кандидат биологических наук, старший научный сотрудник лаборатории профилактики ящура ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Михалишин Дмитрий Валерьевич, кандидат ветеринарных наук, заведующий лабораторией профилактики ящура ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Шарыпов Андрей Сергеевич, кандидат ветеринарных наук, научный сотрудник отдела координации научно-исследовательских работ ФГБУ ЦНМВЛ, г. Москва, Россия.

Мудрак Наталья Станиславовна, доктор биологических наук, главный научный сотрудник референтной лаборатории вирусных болезней птиц ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Камалова Наталья Евгеньевна, доктор ветеринарных наук, главный научный сотрудник центра доклинических исследований ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Манин Борис Леонидович, кандидат биологических наук, ведущий научный сотрудник сектора культуры клеток отдела инноваций ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

DOI: 10.29326/2304-196X-2021-3-38-239-242

Nutrient digestibility of fishmeal rations in primates

N. V. Gaponov[1,2], L. N. Gamko[3]

[1] FSBSI "Research Institute of Medical Primatology" (FSBSI "RIMP"), Sochi, Russia

[2] All-Russian Lupine Research Institute – Branch of the Federal Williams Research Center of Forage Production and Agroecology, Michurinsky, Bryansk Oblast, Russia

[3] FSBEI HE "Bryansk State Agrarian University" (FSBEI HE Bryansk SAU), Kokino, Bryansk Oblast, Russia

[1, 2] *ORCID 0000-0002-5086-7943, e-mail: nv.1000@bk.ru*

[3] *ORCID 0000-0002-1887-0587, e-mail: gamkol@mail.ru*

SUMMARY

The results of the study on effects of fishmeal on nutrient digestibility and intake in primates are given in the paper. Fishmeal is a feeding stuff, manufactured from fish, marine mammals, invertebrates not suitable for human consumption and by-products of their processing. Fishmeal nutrient composition includes natural substances and minerals, including phosphorus, calcium, iodine, selenium, several essential amino acids, as well as vitamins A, D and B complex. Fishmeal is known to be rich in digestible energy and proteins. One kilogram of fishmeal contains 700 grams of raw protein and up to 15 MJ of digestible energy. It should be noted that fishmeal is easily digested by animals. Nevertheless, there is a lack of data in publications on use of fishmeal as high protein feed in rations of primates. In this regard, the aim of the study was to analyze the effects of fishmeal on digestibility of mixed feed nutrients in male rhesus-macaques and to use the obtained results for understanding of prospects of fishmeal further use for feeding primates. The chemical composition and nutritional value of the total mixed ration was determined. The economic effectiveness of the fishmeal use in the rations of primates was calculated. Based on the experimental data, it was established that the inclusion of fishmeal (18% out of total) into the diet contributes to the improvement of feed intake in experimental primates and reduces the costs of complete granular feed.

Keywords: digestibility, retention, fishmeal, diet, rhesus macaque (*Macaca mulatta*), primates

For citation: Gaponov N. V., Gamko L. N. Nutrient digestibility of fishmeal rations in primates. *Veterinary Science Today*. 2021; 10 (3): 239–242. DOI: 10.29326/2304-196X-2021-3-38-239-242.

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

For correspondence: Nikolay V. Gaponov, Candidate of Science (Biology), Leading Researcher, Laboratory for Lupine Processing and Use in Food, All-Russian Lupine Scientific Research Institute – Branch of the Federal Williams Research Center of Forage Production and Agroecology, 241524, Russia, Bryansk Oblast, Michurinsky settlement, Berezovaya, 2, *e-mail: nv.1000@bk.ru*.

УДК 636.978:636.084:599.824

Переваримость питательных веществ рациона с рыбной мукой у приматов

Н. В. Гапонов[1,2], Л. Н. Гамко[3]

[1] ФГБНУ «Научно-исследовательский институт медицинской приматологии» (ФГБНУ «НИИ МП»), г. Сочи, Россия

[2] Всероссийский научно-исследовательский институт люпина – филиал ФГБНУ «Федеральный научный центр кормопроизводства и агроэкологии имени В. Р. Вильямса» (ВНИИ люпина – филиал ФНЦ «ВИК им. В. Р. Вильямса»), пос. Мичуринский, Брянская обл., Россия

[3] ФГБОУ ВО «Брянский государственный аграрный университет» (ФГБОУ ВО Брянский ГАУ), с. Кокино, Брянская обл., Россия

[1, 2] *ORCID 0000-0002-5086-7943, e-mail: nv.1000@bk.ru*

[3] *ORCID 0000-0002-1887-0587, e-mail: gamkol@mail.ru*

РЕЗЮМЕ

Представлены результаты изучения влияния рыбной муки на переваримость и усвоение питательных веществ корма у приматов. Рыбная мука – кормовой продукт, вырабатываемый из промышленных или сорных видов рыбы, морских млекопитающих, беспозвоночных, отходов их переработки. В состав рыбной муки входит комплекс природных веществ и минералов, в том числе фосфор, кальций, йод, селен, ряд незаменимых аминокислот, а также витамины A, D и группы B. Известно, что рыбная мука богата обменной энергией и протеином. В одном килограмме муки содержится до 700 г сырого протеина и до 15 МДж обменной энергии. Следует отметить, что рыбная мука легко переваривается и хорошо усваивается организмом животных. Однако в литературе имеется недостаточное количество данных по использованию рыбной муки в рационах приматов в качестве высокопротеинового корма. В этой связи целью исследований являлось изучение влияния рыбной муки на переваримость питательных веществ комбикорма у самцов

макаков-резусов и на основании полученных данных определение перспективы дальнейшего использования в кормлении приматов. Был изучен химический состав и определена питательность полученного полнорационного комбикорма. Рассчитана экономическая эффективность использования рыбной муки в рационах приматов. На основании экспериментальных данных установлено, что введение взамен высокобелковых компонентов 18% рыбной муки обеспечивает улучшение процесса усвоения питательных веществ корма у подопытных приматов и обеспечивает снижение себестоимости полнорационного гранулированного комбикорма.

Ключевые слова: переваримость, ретенция, рыбная мука, рацион, макак-резус, приматы

Для цитирования: Гапонов Н. В., Гамко Л. Н. Переваримость питательных веществ рациона с рыбной мукой у приматов. *Ветеринария сегодня.* 2021; 10 (3): 239–242. DOI: 10.29326/2304-196X-2021-3-38-239-242.

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

Для корреспонденции: Гапонов Николай Васильевич, кандидат биологических наук, ведущий научный сотрудник лаборатории переработки и пищевого использования люпина ВНИИ люпина – филиала ФНЦ «ВИК им. В. Р. Вильямса», 241524, Россия, Брянская обл., Брянский р-н, пос. Мичуринский, ул. Березовая, 2, *e-mail: nv.1000@bk.ru.*

INTRODUCTION

Quality feeding rations underpin the profitability of keeping primates and other animal species. One of the most important components of high-quality feed is fishmeal, produced by modern fish rendering plants and having valuable nutritional properties. It was found that fishmeal protein is digested by animals much better than vegetable proteins. Experience shows that fishmeal added to the diet increases the efficiency of using other feeds, poor in protein and fat, and its inclusion in the mixed feed facilitates the animal growth and weight gain and improves their general condition [1–3]. As a result of biochemical studies, the so-called albumin factor was found in fishmeal feeds, which is responsible for the absorption of vegetable protein, vitamin B12 and other water-soluble B complex vitamins by animals. For this reason, fishmeal is not only a source of a complete animal protein, but also a catalyst for biochemical processes [4, 5]. For example, studies of the Leningrad Zootechnical Institute showed that animals that absorbed 31.8% nitrogen from vegetable feed, after adding cod meal in an amount of 5% to the feed, began to absorb up to 36.3% of nitrogen, i.e. the digestibility of vegetable proteins increased by 12.5% [1, 6]. Therefore, the inclusion of fishmeal in the feed diet contributes to the growth of healthy animals. Fishmeal has a unique composition. First, it is a surprisingly wide complex of natural substances and minerals: phosphorus, calcium, a set of amino acids, iodine, selenium, as well as A, D and B complex vitamins. Thanks to these elements, the digestive processes are improved and the immune system is strengthened. By the content of digested protein, fishmeal ranks among the highest in feedstuffs. For example, 1 kg of fishmeal contains at least 535 g of digestible protein. Therefore, animals that receive fishmeal as part of their diet are less susceptible to diseases, and due to the optimal ratio of amino acids, young animals develop faster. Fishmeal is considered a valuable preventive tool against thyroid diseases, since it contains iodine in the form of organic compounds. For example, cod fat contains from 4.5 to 15.2 mg of iodine per 1 kg of fat [6, 7].

The chemical nature of fishmeal extractive substances has not yet been sufficiently studied. However, a favorable effect of premium quality fishmeal on the appetite of animals has been established. Fishmeal-receiving animals absorb a significant amount of feed nutrients well and have a high growth rate. Thanks to its components, it promotes the development of vital body systems, activation of the immune system, and increases the digestibility of nutrients [8–11]. The digestibility of high-quality fishmeal reaches 90%. In addition, the introduction of fishmeal into the diet has a positive effect on the feed conversion, and the lower the conversion rate, the more efficient the production. Therefore, fishmeal is widely used in the manufacture of mixed feeds, which have become widespread in our country.

The aim of the study was to study the effect of partial or complete replacement of the main feed ingredients with fishmeal and to determine the dietary nutrient digestibility.

For this purpose, the following tasks were set:

– to study the digestibility of nutrients in the fishmeal diet on male rhesus monkeys;

– calculate the economic efficiency of using fishmeal for feeding primates;

– to carry out mathematical analysis of the data obtained.

MATERIALS AND METHODS

The object of the study was male rhesus monkeys (*Macaca mulatta*) aged from 7 to 15 years. For the experimental purposes, two groups of five animals were formed using the method of analogous pairs by gender, origin, age and physiological state at the animal facilities of the FGBRI "Research Institute of Medical Primatology". The experiment was carried out in accordance with the generally accepted research methods developed by L. K. Ernst Federal Research Center for Animal Husbandry and other organizations [7, 12, 13].

Tests of fishmeal and other feed components were carried out at the Research Institute of Medical Primatology.

Based on the obtained laboratory data and reference publications, a feed ration for primates was formulated.

The inorganic compounds of the total mixed ration were determined using a vacuum wave-dispersive X-ray fluorescence spectrometer "SPECTROSCAN MAX-GVM" (NPO "SPEKTRON" LLC, Russia) in accordance with the "Method for measuring the mass fractions of Mg, Al, Si, Zn, P, S, Cl, K, Ca, Ba, Ti, Cr, Mn, Fe, Ni, Br, Rb, Sr in vegetable powder samples by X-ray fluorescence using X-ray spectrometers SPECTROSCAN MAX (M-049-RM/12)", FR.1.31.2014.17343. The rest of the parameters were determined using the NIRS DS2500F feed analyzer (FOSS, Denmark).

During the test period, the control group primates received a total mixed ration feed prepared at the production site of the institution using the Münch-Edelstahl GmbH feed pelletizer (Germany). The feed was composed of primary components: wheat, soy meal, skimmed milk powder, sunflower cake, corn, corn gluten, egg powder, sugar and sunflower oil. Wheat accounted for 21.4% of the ration energy value. In total, soy meal and sunflower cake (the amount of fat 10%) accounted for 17.42 and 13.83% in the mixed feed, respectively. A significant part of the ration energy value (14.39%) was skimmed milk powder. The diet of the control group animals was energetically balanced with the introduction of sunflower oil (0.8%). The remaining 32.16% of energy accounted for corn gluten (11.24%), corn (13.35%), egg powder (3.3%) and sugar (4.27%).

Test group primates received fishmeal with mixed feed, which made up 18% of the ration nutritional value. According to the experimental design, thanks to the inclusion of 60–65% of fishmeal, the content of skimmed milk powder decreased by 100%, sunflower meal – by 10%, egg powder – by 70%, corn gluten – by 2%. The diet was balanced in terms of crude protein according to generally accepted norms; the deficit of crude fiber was insignificant, but within the acceptable limits. For the remaining nutrients, the deviations were within the limits established by the regulatory requirements [14].

Experiments on animals were carried out in accordance with the intergovernmental standards for accommodation and care of laboratory animals GOST 33215-2014 and GOST 33216-2014, adopted by the Intergovernmental Council for Standardization, Metrology and Certification, as well as in accordance with the Declaration of Helsinki (2000) and Directive 2010/63/EU of the European Parliament and of the Council of 22.09.2010 on the protection of animals used for scientific purposes. The study was approved by the bioethical commission of the Research Institute of Medical Primatology.

The obtained results were processed statistically and expressed as arithmetic averages and their standard errors. The statistical significance of the differences was determined using a one-factor analysis of variance with subsequent posteriori corrections for multiple comparisons using the Tukey and Sidak method. The accepted level of statistical significance is $p< 0.05$.

RESULTS AND DISCUSSION

To determine the absorption and digestibility of dietary nutrients, a physiological study was performed on ten *M. mulatta* primates. The amount of feed consumed by the animals and the amount of feces excreted by them were recorded daily. Then, the chemical composition of the feed and feces was analyzed, which allowed to determine the amount of nutrients consumed and excreted per day. Based on the obtained data, the amount of absorbed nutrients and the digestibility coefficients were determined (Table).

The analysis of the data presented in the table showed that the best results in digestibility for most of the standardized organic matter were observed in the primates of the experimental group. In this group, the digestibility of crude protein and fat was higher than in the control group by 1.66 and 21.92%, respectively ($p< 0.01$). It should be noted that nitrogen-free extracts in the structure of which starch plays an important role, were absorbed by 11.74% better in the control group than in the test group.

The research results indicate that the inclusion of fishmeal in the structure of rations for primates (18% by nutritional value) had a positive effect on the digestibility and use of basic nutrients.

Since the economic indicators of primate feeding efficiency are among the main indicators of the system regulating the profitability of their keeping, the production costs of the tested total mixed ration feed with fishmeal added were calculated in comparison with the control one.

In the structure of the control group ration, the most expensive component is skimmed milk powder, its share in the composition accounts for 14.39% (24 rubles 20 kopecks from the cost of 1 kg of mixed feed). The next most expensive component is soy meal, which accounts for 17.42% of the nutritional value, the cost of it amounted to 9 rubles 66 kopecks. The highest purchase price was set for egg powder, but due to its insignificant content in the structure of the diet (3.3%), it cost 6 rubles 98 kopecks. The costs of the remaining components of the diet are at an acceptable level, which is due to their initially low cost or low percentage content in the feed composition. Thus, the price of 1 kg of total mixed ration feed for the control group was 70 rubles 96 kopecks.

When creating a new formula of the mixed feed for primates of the experimental group, the diet was significantly

Table
Nutrient digestibility of primates' rations, % (X ± Sx)

Indicators	Crude protein	Crude fat	Crude fiber	Crude ash	NFE	Calcium	Phosphorus
Control	27.34 ± 1.04	20.09 ± 0.98	16.88 ± 1.01	49.58 ± 0.88	54.05 ± 1.12	18.37 ± 1.94	21.79 ± 2.01
Test	29.00 ± 1.14	42.01 ± 1.85**	52.13 ± 1.26	53.63 ± 1.03	42.31 ± 1.27	94.14 ± 1.18*	90.00 ± 1.84

NFE – nitrogen-free extracts.
* $p < 0.05$; ** $p < 0.01$.

changed. In particular, fishmeal replaced soy meal by 7%, sunflower cake – by 10%, egg powder – by 50% and skimmed milk powder – by 100%. In the structure of the feed ration, fishmeal (60–65%) in terms of nutritional energy value was 18%, the price was 6 rubles 84 kopecks. The cost of 1 kg of total mixed ration feed as a result amounted to 48 rubles 38 kopecks. Thus, due to the inclusion of fishmeal in the composition of the mixed feed, it became possible to reduce the cost of 1 kg of the feed by 22 rubles 58 kopecks.

The cost of feed conversion depends on the cost of feed and their nutritional value. In the control group, the cost of 1 MJ was 5 rubles 32 kopecks. The cost of digestible energy in the experimental group was lower and amounted to 3 rubles 63 kopecks per 1 MJ. A similar pattern was observed for crude protein. The low cost of 1 g of protein was observed in the experimental group, it was 14 kopecks, and in the control group – 26 kopecks.

CONCLUSION

From the analysis of the data obtained by the experiment, it can be concluded that the inclusion of fishmeal in the composition of a total mixed ration feed for primates allows to enrich the diet with nutrients, improve the digestibility of nutrients and reduce the cost of feed without losing its quality.

REFERENCES

1. Poznyakovsky V. M., Ryazanova O. A., Kalenik T. K., Datsun V. M. Testing of fish and fish products. Quality and safety [Ekspertiza ryby, ryboproduktov i nerybnyh ob"ektov vodnogo promysla. Kachestvo i bezopasnost']. Novosibirsk: Siberian University Publishing office; 2005. 311 p. (in Russian)

2. Masumura Y., Torii S., White H. J., Tanaka K. Changes with age of water-binding capacity and acid mucopolysaccharide content in the rat skin. *Journal of Gerontology*. 1971; 26 (3): 386–390. DOI: 10.1093/geronj/26.3.386.

3. Miles R. D., Chapman F. A. The benefits of fish meal in aquaculture diets. *The Fish Site*. 31 July 2006. Available at: http://www.thefishsite.com/articles/200/the-benefits-of-fish-meal-in-aquaculture-diets.

4. Vorobjov V. I., Nizhnikova E. V., Lempert O. T., Nefjedova N. P. Alternative sources of obtaining fish meal analogues. *KSTU News*. 2015; 38: 74–82. Available at: https://klgtu.ru/upload/science/magazine/news_kstu/2015_38/vorobev.pdf. (in Russian)

5. Victorov P. I., Menkin V. K. Methodology and design of zootechnical trials [Metodika i organizaciya zootekhnicheskih opytov]. M.: Agropromizdat; 1991. 112 p. Available at: https://docplayer.ru/31417368-P-i-viktorov-v-k-menkin-metodika-i-organizaciya-zootehnicheskih-opytov.html. (in Russian)

6. Lebedev E. I. Comprehensive use of raw materials in food industry. [Kompleksnoe ispol'zovanie syr'ya v pishchevoj promyshlennosti]. M.: Lyogkaya i pischevaya promyshlennost; 1982. 239 p. (in Russian)

7. Ovsyannikov A. I. Principles of experimenting in livestock production [Osnovy opytnogo dela v zhivotnovodstve]. M.: Kolos; 1976. 304 p. (in Russian)

8. Gaponov N. V., Svistunov S. V., Bondarenko N. N., Romanenko I. A. Effect of deuterium water on blood values and digestibility of nutrients of rhesus macaque. *Bulletin of the National Academy of Sciences of the Republic of Kazakhstan*. 2020; 2 (384): 22–28. DOI: 10.32014/2020.2518-1467.37.

9. Grimble G. K., Silk D. B. A. Milk Protein and Enteral and Parenteral Feeding in Disease. *In: Milk Proteins. Nutritional, Clinical, Functional and Technological Aspects*. Eds. C. A. Barth, E. Schlimme. Darmstadt: Steinkopff-Verlag; 1989; 270–282. DOI: 10.1007/978-3-642-85373-9_43.

10. Gaponov N. V., Lenkova T. N. Biotransformation of nutrients in the body of primates. *Innovative Scientific Research*. 2020; 12-1 (2): 5–14. DOI: 10.5281/zenodo.4444589.

11. Degtaryov V. N. Hydro-mechanical processing of fish [Gidromekhanicheskie processy obrabotki gidrobiontov]: monography. Petropavlovsk-Kamchatsky: KamchatGTU; 2008. 171 p. (in Russian)

12. Limits and rations in livestock feeding [Normy i raciony kormleniya sel'skohozyajstvennyh zhivotnyh]: handbook. 3rd ed., revised and amended. Ed. by A. P. Kalashnikov, V. I. Fisinin, V. V. Scheglov, N. I. Kleymenov. M.; 2003. 456 p. (in Russian)

13. Gaponov N. V. Biological value of fish flour and probiotic preparations in primate feeding. *Fodder Production*. 2021; 1: 42–46. DOI: 10.25685/KRM.2021.1.2021.006. (in Russian)

14. Gaponov N. V. Method for optimization of complete feedstuff with fish flour for cost reduction of primates keeping. Patent No. 2733291 Russian Federation, Int. A23K 10/22 (2016.01) A23K 40/10 (2016.01) A23K 50/00 (2016.01). No. 2019144389. Date of filing: 24.12.2019. Date of publication: 01.10.2020. Bull. No. 28. (in Russian)

Received on 12.05.2021
Approved for publication on 30.06.2021

INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Nikolay V. Gaponov, Candidate of Science (Biology), Leading Researcher, Laboratory for Lupine Processing and Use in Food, All-Russian Lupine Research Institute – Branch of the Federal Williams Research Center of Forage Production and Agroecology, Michurinsky, Bryansk Oblast, Russia.

Leonid N. Gamko, Doctor of Science (Agriculture), Professor, Department of Animal Feeding, Private Zootechnical Practices and Livestock Product Processing, FSBEI HE Bryansk SAU, Kokino, Bryansk Oblast, Russia.

Гапонов Николай Васильевич, кандидат биологических наук, ведущий научный сотрудник лаборатории переработки и пищевого использования люпина, ВНИИ люпина – филиал ФНЦ «ВИК им. В. Р. Вильямса», пос. Мичуринский, Брянская обл., Россия.

Гамко Леонид Никифорович, доктор сельскохозяйственных наук, профессор кафедры кормления животных, частной зоотехнии и переработки продуктов животноводства, ФГБОУ ВО «Брянский ГАУ», с. Кокино, Брянская обл., Россия.

DOI: 10.29326/2304-196X-2021-3-38-243-247

Preparation and transfer of *Burkholderia mallei* production strain 5584 in accordance with the biosafety requirements

E. A. Artemeva[1], L. A. Melnikova[2], A. P. Rodionov[3]

Federal State Budgetary Scientific Institution "Federal Center for Toxicological, Radiation, and Biological Safety" (FSBSI "FCTRBS-ARRVI"), Kazan, Russia

[1] *ORCID 0000-0002-6204-6077, e-mail: artemevaelena21@mail.ru*

[2] *ORCID 0000-0002-0159-3843, e-mail: vnivi@mail.ru*

[3] *ORCID 0000-0003-0853-5678, e-mail: alexandrvetspets@gmail.com*

SUMMARY

The Veterinary Service of the Russian Federation takes measures to ensure regular control of livestock health status, to prevent infectious diseases and their introduction into the country; and if such diseases are diagnosed, it takes measures to prevent their spread and contain outbreaks as soon as possible. Success of the taken measures depends on the use of various diagnostic, preventive and therapeutic drugs. In order to produce such medicinal products, biofactories use production and reference strains with stable biological properties, which are stored in national collections of microorganisms. The only keeper of glanders strains is the Laboratory for Collection of Strains of Microorganisms in the FSBSI «FCTRBS-ARRVI», subordinated to the Ministry of Agriculture of the Russian Federation. The following steps were taken due to the official request from FKP Kursk Biofactory – BIOK Company for the transfer of *Burkholderia mallei* production strain 5584 from the collection of the institution: the strain was passaged in golden hamsters, its viability was determined and biological properties of the culture were studied. The strain was transferred in accordance with the established procedure and in compliance with the biosafety requirements. As the work progressed, *Burkholderia mallei* strain 5584 culture was isolated and freeze-dried. Before the transfer, biological properties of the freeze-dried *Burkholderia mallei* strain 5584 were studied for their compliance with the passport data. The obtained results showed that the Laboratory for Collection of Strains of Microorganisms in the FSBSI «FCTRBS-ARRVI» provides optimal conditions to preserve the strain viability and initial biological properties after 5 years of storage. Analysis of the data obtained during the transfer of *Burkholderia mallei* strain 5584 allowed us to assess the actions taken at all stages of the procedure. It was established that the transfer procedure for the requested glanders production strain complied with the biosafety requirements and regulatory framework regulating the process.

Keywords: *Burkholderia mallei*, viability, biological properties, strain transfer

Acknowledgements: The work was carried out using the funds of the FSBSI "FCTRBS-ARRVI" within the research "Collecting, maintaining, replenishing and storing strains of highly dangerous diseases (HDD), keeping records of them, studying their biological properties and providing agro-industry companies with HDD strains".

For citation: Artemeva E. A., Melnikova L. A., Rodionov A. P. Preparation and transfer of *Burkholderia mallei* production strain 5584 in accordance with the biosafety requirements. *Veterinary Science Today*. 2021; 10 (3): 243–247. DOI: 10.29326/2304-196X-2021-3-38-243-247.

Conflict of interests: The authors declare that there is no conflict of financial/non-financial interests associated with the paper.

For correspondence: Elena A. Artemeva, Candidate of Science (Veterinary Medicine), Head of Laboratory for Collection of Strains of Microorganisms, FSBSI "FCTRBS-ARRVI", 420075, Russia, Republic of Tatarstan, Kazan, Scientific town-2, *e-mail: artemevaelena21@mail.ru*.

УДК 619:579:57.082.26:616.98:579.843.96

Особенности подготовки и выдачи производственного штамма 5584 *Burkholderia mallei* в соответствии с требованиями биологической безопасности

Е. А. Артемьева[1], Л. А. Мельникова[2], А. П. Родионов[3]

ФГБНУ «Федеральный центр токсикологической, радиационной и биологической безопасности» (ФГБНУ «ФЦТРБ-ВНИВИ»), г. Казань, Россия

[1] *ORCID 0000-0002-6204-6077, e-mail: artemevaelena21@mail.ru*

[2] *ORCID 0000-0002-0159-3843, e-mail: vnivi@mail.ru*

[3] *ORCID 0000-0003-0853-5678, e-mail: alexandrvetspets@gmail.com*

РЕЗЮМЕ

Ветеринарной службой Российской Федерации проводится комплекс мер, направленных на регулярный контроль состояния поголовья сельскохозяйственных животных, профилактику инфекционных заболеваний и недопущение их завоза в страну, а в случае диагностирования – предотвращение распространения и купирование в кратчайшие сроки. Успешная реализация данных мероприятий возможна благодаря использованию различных диагностических, профилактических и лечебных препаратов. С целью их получения предприятия биологической промышленности применяют производственные и эталонные штаммы микроорганизмов со стабильными биологическими свойствами, которые хранятся в государственных коллекциях микроорганизмов. Единственным держателем штаммов возбудителя сапа является лаборатория коллекции штаммов микроорганизмов ФГБНУ «ФЦТРБ-ВНИВИ», подведомственного Министерству сельского хозяйства Российской Федерации. В связи с официальным запросом ФКП «Курская биофабрика – фирма «БИОК» о получении производственного штамма 5584 *Burkholderia mallei* из коллекции учреждения были выполнены следующие работы: проведен пассаж штамма на золотистых хомячках, определена жизнеспособность и изучены биологические свойства культуры, в установленном порядке, с соблюдением требований биологической безопасности, произведена выдача штамма. В процессе проведенной работы выделена культура штамма 5584 *Burkholderia mallei*, которая была лиофилизирована. Перед выдачей были изучены биологические свойства лиофилизированного штамма 5584 *Burkholderia mallei* на соответствие их паспортным данным. Полученные результаты показали, что в лаборатории коллекции штаммов микроорганизмов ФГБНУ «ФЦТРБ-ВНИВИ» созданы оптимальные условия для сохранения его жизнеспособности и исходных биологических свойств по истечении 5 лет хранения. Анализ данных, полученных при выполнении работ по передаче штамма 5584 *Burkholderia mallei*, позволил дать оценку действий на всех ее этапах. Установлено, что порядок выдачи запрашиваемого производственного штамма возбудителя сапа соответствовал требованиям биологической безопасности и нормативно-правовым документам, регламентирующим проведенные мероприятия.

Ключевые слова: *Burkholderia mallei*, жизнеспособность, биологические свойства, передача штамма

Благодарность: Работа выполнена за счет средств ФГБНУ «ФЦТРБ-ВНИВИ» в рамках научно-исследовательских работ по теме «Коллекционирование, поддержание, пополнение и хранение штаммов возбудителей особо опасных болезней (ООБ), организация их учета, проведение исследований по изучению биологических свойств и обеспечения предприятий агропромышленного комплекса штаммами возбудителей ООБ».

Для цитирования: Артемьева Е. А., Мельникова Л. А., Родионов А. П. Особенности подготовки и выдачи производственного штамма 5584 *Burkholderia mallei* в соответствии с требованиями биологической безопасности. *Ветеринария сегодня*. 2021; 10 (3): 243–247. DOI: 10.29326/2304-196X-2021-3-38-243-247.

Конфликт интересов: Авторы заявляют об отсутствии конфликта финансовых/нефинансовых интересов, связанных с написанием статьи.

Для корреспонденции: Артемьева Елена Александровна, кандидат ветеринарных наук, заведующий лабораторией коллекции штаммов микроорганизмов ФГБНУ «ФЦТРБ-ВНИВИ», 420075, Россия, Республика Татарстан, г. Казань, Научный городок-2, *e-mail: artemevaelena21@mail.ru*.

INTRODUCTION

Freedom of the Russian Federation from infectious diseases depends on a set of anti-epizootic measures that includes systematic monitoring of livestock health, prevention of infectious disease and spread; prevention of its introduction into the country, and if it is diagnosed – prevention of its spread and containment of the outbreak in the shortest possible time [1].

Due to the impact of globalization on spread of infections, regular emergence of new and old infectious diseases, total urbanization and new economic conditions, not a single country is now able to completely protect its territory from introduction of highly dangerous diseases. Such infections pose a threat not only to animals, but also to people and can damage national economies, break economic and cultural links [2].

Therefore, it is of top priority to develop and produce reliable, highly-specific diagnostic and preventive drugs, therapeutic agents and modern indication systems at the country's biofactories; such production is regulated by Instruction of the Chief Veterinary Officer of the Russian Federation No. 22-7/443 of 08.05.92 "Issuing a permit for production of veterinary medicinal products and their certification". In order to produce diagnostic preparations and vaccines, biofactories use production and reference strains from the national collections of pathogenic microorganisms, where optimal conditions are ensured to preserve them in the original state, excluding any transformation in biological, serological, toxicological properties and sensitivity to antibiotics [3–8]. Strains of highly dangerous microorganisms stored in the national collections are handled in accordance with the regulatory framework and the Federal laws and resolutions of the Government of the Russian Federation [8, 9].

Glanders is a dangerous bacterial infection from the group of zoonoses – a highly dangerous, highly contagious disease of animals and humans; presently, there is no vaccine or specific treatment available for glanders. As scientific publications demonstrate, there are cases of professional infection with the *Burkholderia mallei* bacterium among microbiologists working with this pathogen. As a result, burkholderia is equated according to its pathogenicity with such pathogens as *Yersinia pestis* and *Francisella tularensis*. Recently, several cases of *B. mallei* infection in humans have been reported in the laboratories of Russia and the USA, including one fatal outcome [10]. Currently, the Russian Federation is free from the disease, but the risk of introduction from other countries remains [2].

Taking into account the above, there is still a need to produce high-quality products for glanders diagnosis. FKP "Kursk Biofactory – BIOK Company" produces "Glanders serum for the complement fixation test", "Glanders antigen for the complement fixation test", "Stained glanders antigen for the plate agglutination test", as well as "Mallein". These medicinal products are made on the basis of *B. mallei* production strain 5584, received in accordance with the established procedure from the National (official) Laboratory for Collection of Strains of Microorganisms in the FSBSI "FCTRBS-ARRVI", which is the only keeper of glanders strains among other institutions subordinate to the Ministry of Agriculture of the Russian Federation [2].

Following the official request from FKP "Kursk Biofactory – BIOK Company" to receive *Burkholderia mallei* production strain 5584 from the Laboratory for Collection of Strains of Microorganisms in the FSBSI "FCTRBS-ARRVI", the purpose of this work was to conduct a passage, determine viability, study biological properties and transfer the pathogen in accordance with the established procedure and in compliance with the biosafety requirements.

MATERIALS AND METHODS

The work was carried out in accordance with SP 1.3.3118-13 "Safety guide for work with Pathogenicity Groups I–II microorganisms". The freeze-dried *B. mallei* production strain 5584 was used. The strain was stored in the Laboratory for Collection of Strains of Microorganisms in the FSBSI "FCTRBS-ARRVI" at a temperature of +4 °C and standardized according to the international Master seed standard.

B. mallei strain 5584 was passaged in golden hamsters, when the pathogen suspension was administered subcutaneously into the occipital region at a dose of 5 and 10 IU according to the turbidity standard (GISK named after L. A. Tarasevich), in the volume of 0.2 and 0.4 ml, respectively. The dead hamsters, as well as those ones in agony, were euthanized by ether and subjected to autopsy. The work with laboratory animals was carried out in accordance with the Helsinki Convention on the Humane Treatment of Experimental Animals (1975) and the European Convention for the Protection of Vertebrate Animals Used for Experiments or for Other Scientific Purposes (1986).

Bacteria culture tests were carried out in liver, spleen, lungs, injection site, heart blood; the tested materials were inoculated using a Pasteur pipette on meat-peptone agar and broth with 4% glycerin (MPGA, MPGB) and incubated at 37 °C for 3–10 days. Virological properties of the isolated culture were tested to match the glanders pathogen. If the observed signs matched the passport data, the strain was freeze-dried.

Table
Biological properties of *Burkholderia mallei* strain 5584

Name of the strain	5584 *Burkholderia mallei*
Formation of hydrogen sulfide, mm	0.2
Curdling of skimmed milk	Milk curdling
Growth in potatoes according to Pavlovsky	In the first days, the colonies are in the form of honey droplets, then merge into a slimy plaque, on Day 7–8 the color turns to brown
Growth in MPGB	Turbidity of the medium, a delicate film on the surface, over time a slimy precipitate forms at the bottom of the test tube, which rises in the form of a corkscrew when shaken
Growth in MPGA	Growth in the form of translucent colonies with smooth edges and a mother-of-pearl hue
Gram Staining	Gram-negative rods with pronounced granular layer
Loeffler Staining	Pale blue rods with red granular layer
Motility	Non-motile
Proteolytic activity on 12% gelatin	Does not break down

Fig. 1. Materials required for packing vials with glanders production strain 5584

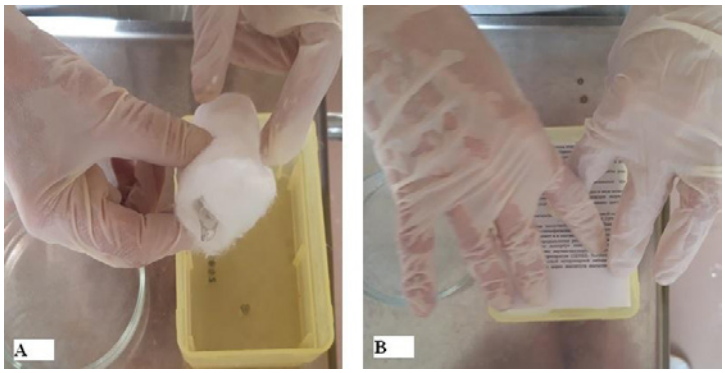


Fig. 2. Placing vials into containers and enclosing the first copies of the accompanying documents

Before FKP "Kursk Biofactory – BIOK Company" received the strain requested, viability of the culture was determined and its biological properties were controlled. For these purposes, a vial with a strain was taken from the collection, then appropriately registered in the "Biological agent movements records" (Form No. 514/u) and the "Individual registration card for the biological agent in the collection" (Form No. 517/u). The vial was opened, the freeze-dried mass was suspended in a 0.85% sodium chloride solution, and the resulting bacteria suspension was inoculated onto nutrient media containing 4% glycerin. The vial and the culture remains in it were destroyed, a report was made on opening a vial with biological agents of Pathogenicity Groups I–II for the purpose of inoculation or destruction (Form No. 521/u) and a mark was made in the "Journal of disinfection for biological agents" (Form No. 520/u). The cultures were incubated at 37 °C in a thermostat for 3–10 days. As soon as the second generation of the strain culture was obtained, its biological properties were tested to match the passport data. For this purpose, the following properties were controlled: cultural, microscopic (tinctorial-morphological and mobility), biochemical (saccharolytic, proteolytic).

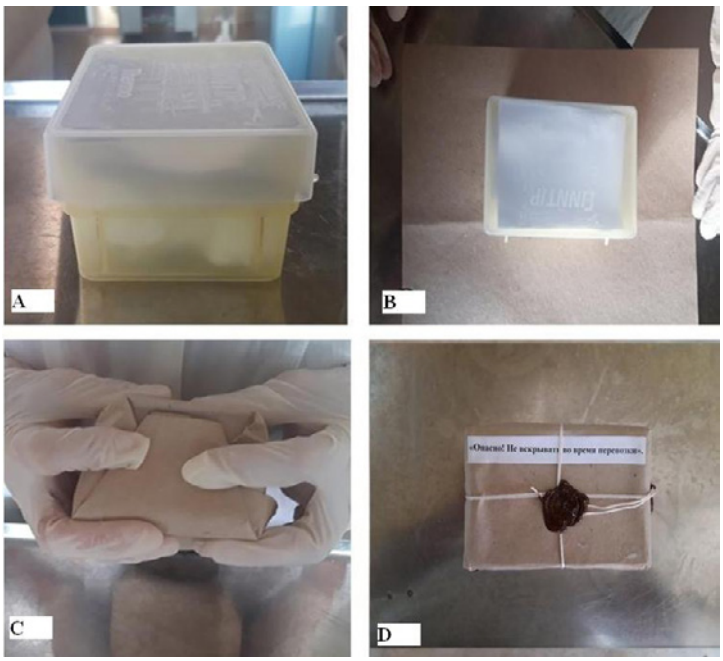


Fig. 3. Packing a container with a vial with B. mallei production strain 5584

RESULTS AND DISCUSSION

B. mallei production strain 5584 transfer procedure started after receipt of the following documents from FKP "Kursk Biofactory – BIOK Company": an official request signed by the director; photocopies of the license and hygiene certificate; bank details certifying safe working conditions are in place and the company is eligible to receive it.

B. mallei strain 5584 was passaged in golden hamsters and freeze-dried. Before FKP "Kursk Biofactory – BIOK Company" received the requested strain, its viability was tested according to the biological properties (Table), given in the passport and corresponding to the indicators given in the Bergey's Manual of Determinative Bacteriology (1980).

The conducted studies show that strain 5584, stored for 5 years in a freeze-dried form at a temperature of +4 °C, is viable. Biological properties were tested on differential diagnostic media and their compliance with the passport data was established. It suggests that the Laboratory for Collection of Strains of Microorganisms provides optimal storage and working conditions for the microorganisms.

The transfer of the glanders production strain is associated with potential contamination risks (during studies of viability and basic properties of the cultures, due to careless handling of vials when packing them, due to mistakes made while processing documents and due to other unauthorized actions, etc.), which is confirmed by recorded glanders cases among veterinary and medical staff working with pathogen cultures. Glanders aerosol cultures are especially hazardous due to the risk of inhalation [10]. As mentioned above, glanders production strain 5584 was transferred to FKP "Kursk Biofactory – BIOK Company" strictly in accordance with the requirements of SP 1.2.036-95 and SP 1.3._-17.

Based on the written permission of the FSBSI "FCTRBS-ARRVI" director *B. mallei* production strain 5584 (biological properties checked) was transferred to representatives of FKP "Kursk Biofactory – BIOK Company", following presentation of their ID documents and a power of attorney in accordance with a transfer certificate indicating the number of vials (Form No. 525/u) and a record made in the "Journal of biological agents transfer" (Form No. 516/u). In order to pack the vial with the strain, the following materials were prepared: a waterproof container with a hermetically sealed lid, hygroscopic cotton wool, a twine, wrapping paper, sealing wax (Fig. 1).

The following accompanying documents are issued for the contents of the container (in two copies): a letter (on the official letterhead) for the contents of the container and a package certificate; a photocopy of the strain passport with its complete characteristics; a special cargo transportation certificate with information about the consignee, date of dispatch and type of transport. The vial with the freeze-dried culture was wrapped with hygroscopic cotton, placed into the container, the first copies of the listed documents were put inside (Fig. 2A, B).

The container was hermetically sealed with a lid, wrapped in paper, laced and stamped with a wax seal bearing special signs "Caution! Do not open during transportation!" (Fig. 3A, B, C, D).

CONCLUSION

Analysis of the data obtained during the transfer of *B. mallei* strain 5584 from the Laboratory for Collection of Strains of Microorganisms in the FSBSI "FCTRBS-ARRVI" to FKP "Kursk Biofactory – BIOK Company" allowed us to assess the actions taken in compliance with Rules of SP 1.2.036-95 and SP 1.3._-17. Study of viability and biological properties of production strain 5584 showed that the Laboratory for Collection of Strains of Microorganisms of the Institution provides optimal conditions to preserve the strain viability and original biological properties after 5 years of storage.

REFERENCES

1. Melnikova L. A., Bukova N. K., Makaev Kh. N., Ivanova S. V., Mustafina I. N., Savkova M. G. Glanders – particularly dangerous disease: characterization, epizootology, and detection. *Veterinarian*. 2016; 4: 22–25. eLIBRARY ID: 26492766. (in Russian)

2. Ivanov A. V., Melnikova L. A., Bukova N. K., Makaev Kh. N., Ivanova S. V., Chernov A. N., et al. Standardization of 5584/24-8^{X} *Burkholderia mallei* field strain. *Veterinarian*. 2014; 1: 21–24. eLIBRARY ID: 21256563. (in Russian)

3. Onishenko G. G., Kutyrev V. V., Toporkov A. V., Osin A. V. Current state of collection activity relative to the use of infectious agents of I–II pathogenicity groups. *Problems of Particularly Dangerous Infections*. 2010; 1 (103): 5–10. DOI: 10.21055/0370-1069-2010-1(103)-5-10. (in Russian)

4. Grishkina T. A., Timofeyeva E. V., Spiridonov V. A. Assessment of the effects of long storage on glanders pathogen museum collection stains. *Problems of Particularly Dangerous Infections*. 2004; 1 (87): 40–42. eLIBRARY ID: 9269679. (in Russian)

5. Kostyukova T. A., Smolensky V. Yu., Lyapin M. N. Development of instruction and methodical data basis of the institution as an element of biosafety provision as regards works with pathogenic biological agents. *Problems of Particularly Dangerous Infections*. 2014; 3: 25–29. DOI: 10.21055/0370-1069-2014-3-25-29. (in Russian)

6. Kamalov I. G., Konstantinov A. V., Diev V. I. About depositing of microorganism strains into the collection of the FGBI "ARRIAH". *Proceedings of the Federal Centre for Animal Health*. 2013; 11: 111–116. eLIBRARY ID: 21056921. (in Russian)

7. Dyatlov I. A. Problems of pathogenic microorganisms collections. *Bacteriology*. 2018; 3 (1): 5–6. eLIBRARY ID: 35598142. (in Russian)

8. Onishchenko G. G., Kutyrev V. V., Osin A. V. Collection activities in the sphere of pathogenic microorganisms usage for the provision of biological safety in the Russian Federation. *Infectious diseases: News, Opinions, Training*. 2016; 1 (14): 37–46. eLIBRARY ID: 25736102. (in Russian)

9. Onishchenko G. G., Kutyrev V. V., Toporkov A. V., Osin A. V. Current state of collection activity relative to the use of infectious agents of I–II pathogenicity groups. *Problems of Particularly Dangerous Infections*. 2010; 1 (103): 5–10. DOI: 10.21055/0370-1069-2010-1(103)-5-10. (in Russian)

10. Laboratory for glanders diagnostics. Methodological Guidelines MG 4.2.2831-11 [Laboratornaya diagnostika sapa. Metodicheskie ukazaniya MU 4.2.2831-11]. M.: Federal Center for Hygiene and Epidemiology of Rospotrebnadzor; 2011. 22 p. Available at: https://www.rospotrebnadzor.ru/documents/details.php?ELEMENT_ID=4855. (in Russian)

Received on 29.04.2021
Approved for publication on 24.06.2021

INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Elena A. Artemeva, Candidate of Science (Veterinary Medicine), Head of Laboratory for Collection of Strains of Microorganisms, FSBSI "FCTRBS-ARRVI", Kazan, Russia.

Lilia A. Melnikova, Candidate of Science (Veterinary Medicine), Leading Researcher, Laboratory for Collection of Strains of Microorganisms, FSBSI "FCTRBS-ARRVI", Kazan, Russia.

Alexander. P. Rodionov, Junior Researcher, Laboratory for Collection of Strains of Microorganisms, FSBSI "FCTRBS-ARRVI", Kazan, Russia.

Артемьева Елена Александровна, кандидат ветеринарных наук, заведующий лабораторией коллекции штаммов микроорганизмов ФГБНУ «ФЦТРБ-ВНИВИ», г. Казань, Россия.

Мельникова Лилия Арсентьевна, кандидат ветеринарных наук, ведущий научный сотрудник лаборатории коллекции штаммов микроорганизмов ФГБНУ «ФЦТРБ-ВНИВИ», г. Казань, Россия.

Родионов Александр Павлович, младший научный сотрудник лаборатории коллекции штаммов микроорганизмов ФГБНУ «ФЦТРБ-ВНИВИ», г. Казань, Россия.

DOI: 10.29326/2304-196X-2021-3-38-248-253

Reparative histogenesis of bone tissue in femoral fractures in rats using biocomposite material along with immunocorrection

V. V. Reshetnyak[1], V. V. Burdeyniy[2], V. V. Pronin[3], Ye. A. Iskaliev[4]

[1,2] FSBEI HE "Kostroma State Agricultural Academy" (FSBEI HE Kostroma SAA), Karavaevo, Kostroma Oblast, Russia

[3] FGBI "Federal Center for Animal Health" (FGBI "ARRIAH"), Vladimir, Russia

[4] Veterinary Clinic "AiBolit", Kostroma, Russia

[1] *e-mail: resh_vv76@mail.ru*

[2] *e-mail: van@ksaa.edu.ru*

[3] *ORCID 0000-0002-6240-3062, e-mail: pronin@arriah.ru*

SUMMARY

The paper studies the effect of the RVI biocomposite material belonging to the group of osteoplastic biocomposite materials, the RV-2 immunomodulator – a synthetic dipeptide inducing an immunocorrective effect, and combinations of these drugs on the reparative histogenesis of bone tissue in femoral fractures in rats. It was found that the remodeling of the primary bone callus into the secondary one in the fracture of the studied animals was of a diverse nature. This process was the most pronounced in the group where the components were used in complex, i.e. the bone defect was filled with RVI during the surgery, as well as RV-2 was injected intramuscularly to rats at a dose of 10 mcg per 1 kg of live weight for five days, starting immediately after the surgery. Well-formed coarse-fibrous connective tissue callus was recorded in animals of this group. The connective tissue was stained more intensely which indicates a denser arrangement of fibers in the callus. Focal cartilage tissue spanning bone fragments was observed within the callus. At the periphery of the site the cartilaginous callus was subjected to endochondral ossification with replacement by coarse-fibrous trabeculae with elements of lamellar bone tissue having haversian canals in the center. The inter-girdle spaces were filled with elements of the myeloid bone marrow in the forming bone tissue. Markedly proliferated osteoblasts were visible in the cambial layer of the periosteum. The bone tissue ratio increased up to (60.21 ± 2.62)%, which significantly exceeded the same indicator in the control group and in all experimental groups. The low content of connective tissue and the high ratio of bone tissue indicated more active osteogenesis processes and reparative regeneration in comparison with other groups.

Keywords: rats, immunomodulator, biocomposite material, bone callus, bone, cartilage and connective tissues, chondrocytes

For citation: Reshetnyak V. V., Burdeyniy V. V., Pronin V. V., Iskaliev Ye. A. Reparative histogenesis of bone tissue in femoral fractures in rats using biocomposite material along with immunocorrection. *Veterinary Science Today*. 2021; 10 (3): 248–253. DOI: 10.29326/2304-196X-2021-3-38-248-253.

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

For correspondence: Vladimir V. Reshetnyak, Candidate of Science (Veterinary Medicine), Associate Professor, Head of the Department of Internal Non-Infectious Diseases, Surgery and Obstetrics, FSBEI HE Kostroma SAA, 156530, Russia, Kostroma Oblast, Kostroma Raion, Karavaevo, *e-mail: resh_vv76@mail.ru.*

УДК 619:636.028:611.018:616-001.5:615.37

Репаративный гистогенез костной ткани при переломах бедренной кости у крыс при использовании биокомпозиционного материала на фоне иммунокоррекции

В. В. Решетняк[1], В. В. Бурдейный[2], В. В. Пронин[3], Е. А. Искалиев[4]

[1,2] ФГБОУ ВО «Костромская государственная сельскохозяйственная академия» (ФГБОУ ВО «Костромская ГСХА»), пос. Караваево, Костромская обл., Россия

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

[4] Ветеринарная клиника «АйБолит», г. Кострома, Россия

[1] *e-mail: resh_vv76@mail.ru*

[2] *e-mail: van@ksaa.edu.ru*

[3] *ORCID 0000-0002-6240-3062, e-mail: pronin@arriah.ru*

РЕЗЮМЕ

Проведено изучение влияния биокомпозиционного материала РВИ из группы остеопластических биокомпозиционных материалов, иммуномодулятора РВ-2 – синтетического дипептида, обладающего иммунокорригирующим действием, и комбинаций этих препаратов на репаративный гистогенез костной ткани при переломах бедренной кости у крыс. Установлено, что ремоделирование первичной костной мозоли во вторичную в области перелома у исследуемых животных носило разноплановый характер. Наиболее выраженно данный процесс протекал в группе с комплексным использованием компонентов, когда костный дефект во время операции заполняли препаратом РВИ и внутримышечно в течение пяти дней, начиная сразу после операции, инъецировали РВ-2 в дозе 10 мкг на 1 кг живой массы крыс. У животных этой группы регистрировали картину хорошо сформированной грубоволокнистой соединительнотканной мозоли. Соединительная ткань была окрашена более интенсивно, что свидетельствует о более плотном расположении волокон в костной мозоли. В ее толще отмечено наличие очагово расположенной хрящевой ткани, которая коммутировала между собой костные отломки. На периферии хрящевая мозоль подвергалась энхондральной оссификации с замещением грубоволокнистыми костными трабекулами с элементами появления пластинчатой костной ткани с гаверсовыми каналами в центре. В толще формирующейся костной ткани межбалочные пространства заполнены элементами миелоидного костного мозга. В камбиальном слое надкостницы видна выраженная пролиферация остеобластов. Доля костной ткани увеличена до (60,21 ± 2,62)%, что достоверно превышает аналогичный показатель как в контрольной, так и во всех опытных группах. Низкое содержание соединительной ткани и высокая доля костной ткани свидетельствуют о более активно протекающих процессах остеогенеза и репаративной регенерации в сравнении с другими группами.

Ключевые слова: крысы, иммуномодулятор, биокомпозиционный материал, костная мозоль, костная, хрящевая и соединительная ткани, хондроциты

Для цитирования: Решетняк В. В., Бурдейный В. В., Пронин В. В., Искалиев Е. А. Репаративный гистогенез костной ткани при переломах бедренной кости у крыс при использовании биокомпозиционного материала на фоне иммунокоррекции. *Ветеринария сегодня*. 2021; 10 (3): 248–253. DOI: 10.29326/2304-196X-2021-3-38-248-253.

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

Для корреспонденции: Решетняк Владимир Вячеславович, кандидат ветеринарных наук, доцент, заведующий кафедрой внутренних незаразных болезней, хирургии и акушерства ФГБОУ ВО «Костромская ГСХА», 156530, Россия, Костромская область, Костромской район, пос. Караваево, *e-mail: resh_vv76@mail.ru.*

INTRODUCTION

The steady increase in the intensity of living process due to the rapid development of technological progress inevitably leads to an increase in the number and severity of skeletal traumatic injuries [1]. They account for up to 52.1% of all non-infectious disease cases. At the same time, mechanical injuries are most often recorded in animals (within the range of 32.7–44.5%) and are frequently complicated by fractures of tubular bones [2, 3].

Many morphological aspects of bone fracture healing are still poorly understood. Thus, questions remain regarding mechanisms of growth inhibition, maturation and remodeling of bone callus, as well as the link between inflammation, regeneration and fibrosis in case of slow healing and non-healing fractures. Traditional means and methods of treatment for this pathology do not always prevent the development of various complications [4]. In this regard, the need for further study of the mechanisms of bone fracture healing, the search for new materials and methods of treatment aimed at activating the reparative processes during healing can be justified [5].

At present a large number of drugs and biologically active substances have been developed and used to correct osteogenesis in both human and veterinary surgery and orthopedics. In this regard the use of biocomposite materials and drugs that have an immunomodulatory effect is very promising. Most publications reflect the results of medical studies, while scientific data in the veterinary field are limited to individual reports [2, 5–8]. In all cases, the authors provide data on effectiveness of separate use of drugs, while the possibility of their combined use presents both scientific and practical interest.

Based on the above, the aim of the paper was to study the effect of the RVI biocomposite material, the RV-2 immunomodulator and their combinations on the reparative histogenesis of bone tissue in femoral fractures in rats.

MATERIALS AND METHODS

The experiments were carried out at the Academic Department of Internal Non-Infectious Diseases, Surgery and Obstetrics and the Clinical and Diagnostic Center of the FSBEI HE "Kostroma State Agricultural Academy" (Kostroma), and the histological studies were conducted on the basis of the Preclinical Testing Center of the FGBI "ARRIAH' (Vladimir).

The study was performed using 30 mongrel white rats of 5–6 months of age weighing 200–250 g, which were kept in the animal facility under equal conditions and received a standard food diet in accordance with international regulatory documentation (Directive 2010/63/EU of the European Union on the protection of animals used for scientific purposes, and the interstate standard GOST 33215-2014 "Guidelines for accommodation and care of laboratory animals. Rules for equipment of premises and organization of procedures").

Two products were used for the study: RVI – preparation of the group of osteoplastic biocomposite materials

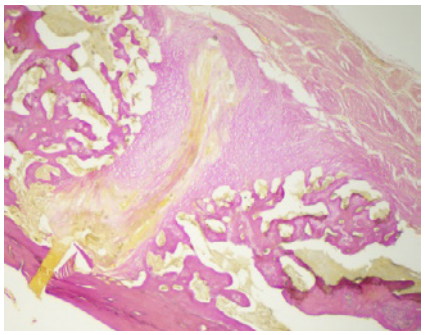


Fig. 1. Bone callus formation, control group (Van Gieson staining, magnification 40×)

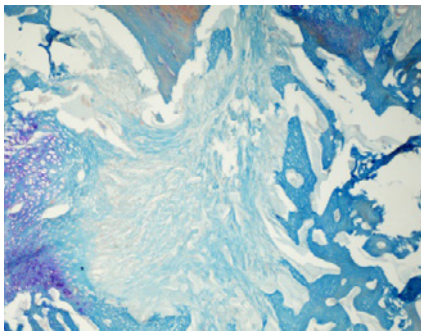


Fig. 2. Formation of cartilage islets, control group (Mallory staining, magnification 40×)

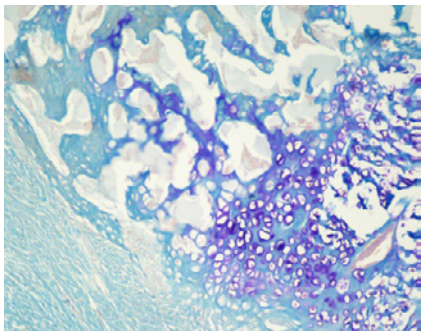


Fig. 3. Formation of bone trabeculae, control group (Mallory staining, magnification 100×)

in the form of granules, which is a synthetic hydroxyapatite with added collagen and an antibiotic (LLC company "Intermedapatit", Russia) and RV-2 – a synthetic dipeptide, which is an analogue of the active center of one of the native thymic hormones, having an immunomodulatory effect and stimulating the regeneration processes in case of their disturbance (DEKO Company Ltd., Russia).

All animals were divided into six groups ($n = 5$ in each group): one control group and five experimental groups. A common diaphyseal femoral fracture was modeled in animals in operating room conditions using adequate anesthesia, followed by intragrade osteosynthesis to fix the bone fragments.

No additional therapeutic measures were performed in the control group. During reposition between the ends of the fracture in rats of experimental group 1 the bone defect was loosely (at two-thirds) filled with RVI granules pre-moistened with isotonic sodium chloride solution; the rats of groups 2, 3 were intramuscularly injected with RV-2 at a dose of 10 mg per 1 kg of body weight once a day for five days immediately after surgery (group 2) or on day 5 (group 3) after surgery. In the other two groups both drugs were used: in group 4 – according to the schemes of groups 1 and 2, in group 5 – according to the schemes of experimental groups 1 and 3.

The animals were removed from the experiment and euthanized using carbon dioxide on day 45 after surgery. The whole femoral bones taken for the surgery were fixed in a 10%-neutral formalin solution; the callus (the fusion) was separated at the dorsal and ventral sides, and the pin was removed using a sharp scalpel (scissors, forceps).

Decalcifying fluid and histology equipment manufactured by Kreonika Ltd. (Russia) were used in the study.

After decalcification the biomaterial was placed in TLP720 tissue processor and was poured in paraffin using ESD-2800 filling station. Histological sections 5–8 microns thick were obtained using a semi-automatic rotary microtome RMD-3000. The sections were stained with hematoxylin and eosin in an ALS-96 automatic linear steiner, and Van Gieson and Mallory staining was additionally conducted to detect connective tissue.

The sections were studied using MICMED-6 microscope (LOMO, Russia). E31S PM video camera and ToupView Software (Hangzhou ToupTek Photonics Co., Ltd., China) were applied for measurements and photographic documentation. A measuring scale of the camera was calibrated using the object micrometre plate for transmitted light OMP (LOMO, Russia).

The ratio of tissue components (bone, cartilage, and connective tissues) in the area of bone fragment alignment was determined in the five fields of view for each of the five sections from all groups.

The measurement results were processed using the variation and statistical analysis and Statistica 7.0 Software as well as the calculation of the mean values (M) and errors (m). The differences were considered reliable if $P \leq 0.05$.

Table
Morphometric parameters of structures in the area of bone callus formation in rats of the control and experimental groups, % ($n = 5$)

Group number	Bone tissue	Cartilage tissue	Connective tissue
control	55.31 ± 2.80	14.43 ± 1.26	30.35 ± 2.46
Experiment 1	42.43 ± 3.62*	15.77 ± 1.41	41.91 ± 3.67*
Experiment 2	44.60 ± 2.96*	16.51 ± 1.54	38.94 ± 2.92*
Experiment 3	40.64 ± 3.38*	23.06 ± 1.87*	36.45 ± 2.87*
Experiment 4	60.21 ± 2.62*	23.58 ± 1.93*	16.37 ± 1.33*
Experiment 5	33.11 ± 2.14*	18.42 ± 1.76*	48.52 ± 3.28*

*$P < 0.05$ as compared to the control group.

RESULTS AND DISCUSSION

Histological examination revealed that a bone callus as an apparent coarse-fibrous connective tissue was formed in the contact zone between two bone fragments in animals of the control group (Fig. 1). Islands of cartilage tissue with well-distinguishable single chondrocytes, as well as isogenic cell groups were observed in the callus (Fig. 2). Randomly oriented bone plates with a large number of fibroblasts on their surface were found at the contact region of bone fragments. A large number of osteoblasts were found deep in the newly formed bone trabeculae, their periosteal surface was represented by fairly densely arranged connective tissue fibers, intensely colored according to Mallory (Fig. 3). Ratio of bone, cartilage and connective tissue was 55.31 ± 2.80; 14.43 ± 1.26; 30.35 ± 2.46%, respectively (Table).

At the same time, the bone callus in rats of experimental group 1, as compared with the control one, differed in a less pronounced morphological structure (Fig. 4). The bone intermediary zone was composed of bundles of large-cell spongy bone tissue. The bone marrow cavity in the fusion zone was filled with myeloid bone marrow with arteries of different calibers (Fig. 5). In addition, less intensive formation of young bone plates was found in this group, and the ratio of bone tissue at the site of the defect was significantly lower and amounted to 42.43 ± 3.62%, with no significant differences in the content of cartilage tissue between the groups. At the same time, the ratio of connective tissue increased to 41.91 ± 3.67% ($P \leq 0.05$, Table).

In experimental group 2 the callus was represented by a pronounced, dense, formed fibrous connective tissue. Single, randomly oriented bone plates were recorded in the area of bone fragment alignment (Fig. 6). Closer to the periosteum, the formed cartilage tissue with single

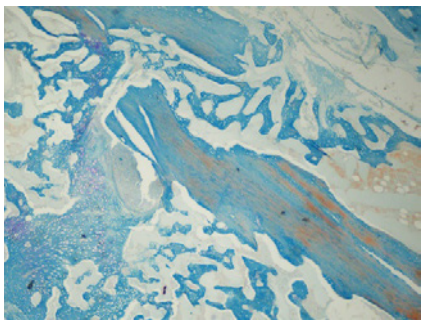


Fig. 4. Formation of bone callus, experimental group 1 (Mallory staining, magnification 40×)

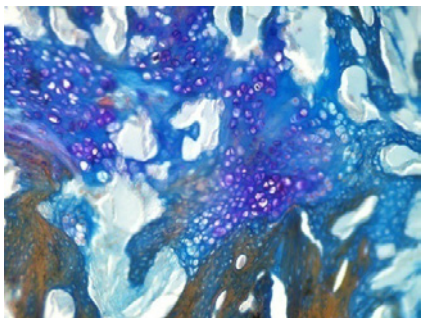


Fig. 5. Formation of bone trabeculae, experimental group 1 (Mallory staining, magnification 100×)

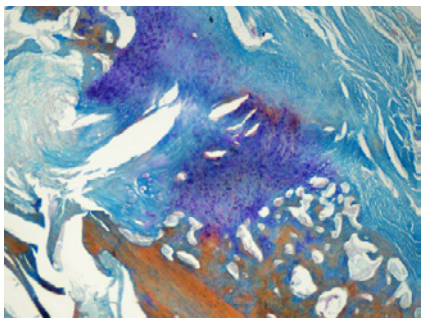


Fig. 6. Bone callus structures, experimental group 2 (Mallory staining, magnification 40×)

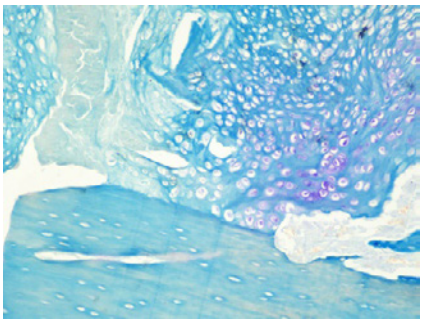


Fig. 7. Cartilaginous tissue of the bone callus, experimental group 2 (Mallory staining, magnification 100×)

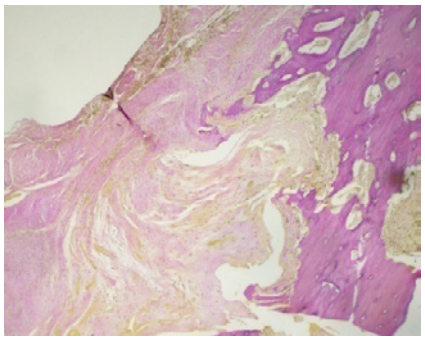


Fig. 8. Bone callus, experimental group 3 (Van Gieson staining, magnification 40×)

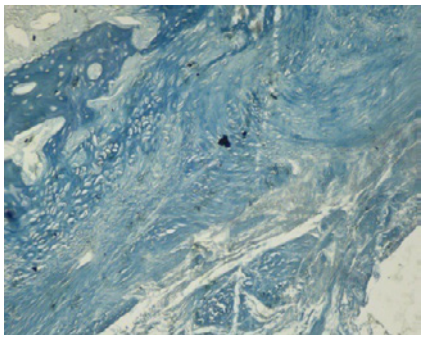


Fig. 9. Bone, experimental group 3 (Mallory staining, magnification 100×)

chondrocytes was visualized (Fig. 7). The specific parameters of bone and cartilage tissue did not have reliably significant differences as compared with experimental group 1. However, in comparison with the control group, a lower ratio of bone tissue along with a higher connective tissue content ($P < 0.05$, Table) and insignificantly increased cartilage tissue content was observed.

Morphometric parameters of bone callus structures in rats of experimental group 3 were not subjected to reliably significant changes as compared with experimental groups 1 and 2. It should be noted that there was a trend for decrease in the proportion of bone tissue and reliably significant differences in the content of cartilaginous tissue in comparison with experimental groups 1 and 2 (Table). The callus was well pronounced, with a predominance of coarse fibrous connective tissue, showing less intense fuchsinophilic properties when stained according to Van Gieson protocol, which indicates a looser arrangement of fibers (Fig. 8). Chondrocytes were widely spaced and basophilic intercellular matter was predominant in the cartilaginous callus. The formation of immature bone plates was noted on the periphery of the cartilaginous callus (Fig. 9).

A well-formed coarse-fibrous connective tissue callus was recorded in animals of experimental group 4. The connective tissue was stained more intensely, indicating a denser arrangement of fibers in the callus. Focally located cartilage tissue which commuted between bone fragments was found in it (Fig. 10). At the periphery of the site the cartilaginous callus was subjected to endochondral ossification with replacement by coarse-fibrous trabeculae with elements of lamellar bone tissue having haversian canals in the center. In the forming bone tissue, the inter-girdle spaces were filled with elements of the myeloid bone marrow (Fig. 11). In the cambial layer of the periosteum, a pronounced proliferation of osteoblasts was visible (Fig. 12). The bone tissue ratio increased up to 60.21 ± 2.62%, which reliably exceeds the same indicator in the control group, as well as in all experimental groups. The low content of connective tissue and the high ratio of bone tissue indicate more active processes of osteogenesis and reparative regeneration in comparison with other groups.

The bone callus was insignificantly pronounced in experimental group 5, on the periphery it was represented by loosely arranged bundles of coarse-fibrous connective tissue with deeply visible cartilagious islands (Fig. 13). The defect area was filled with myeloid substance and islands of weakly fuchsinophilic, coarse-fibrous, connective tissue (Fig. 14). In the periosteal zone, the rudiments of bone trabeculae formed by the replacement of reticulofibrous tissue were visualized (Fig. 15). The bone volume ratio was the lowest (33.11 ± 2.14%) in all the groups presented, along with the highest indicator (48.52 ± 3.28%) of the connective tissue ratio (Table).

CONCLUSION

The presented data indicate that the most stimulating effect on reparative osteogenesis was produced when using a combination of the RVI biocomposite material based on synthetic hydroxyapatite with added collagen and an antibiotic along with a five-day immunocorrection course with RV-2 preparation from the group of synthetic dipeptides, starting from day 1 after surgery. This

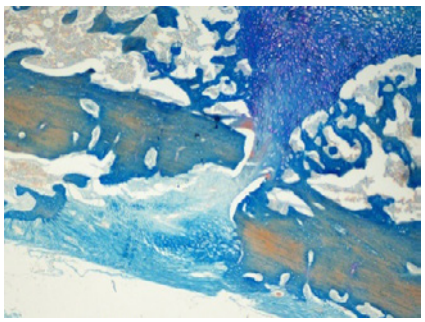


Fig. 10. Bone, experimental group 4 (Mallory staining, magnification 40×)

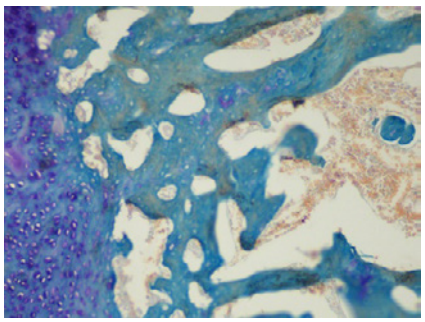


Fig. 11. Bone callus, experimental group 4 (Mallory staining, magnification 40×)

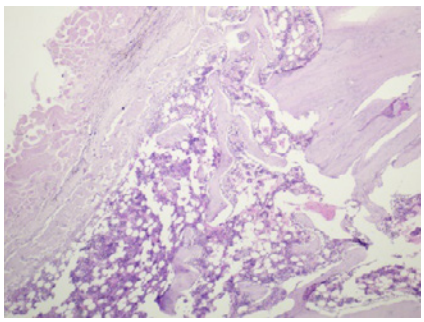


Fig. 12. Bone tissue, experimental group 4 (hematoxylin and eosin staining, magnification 40×)

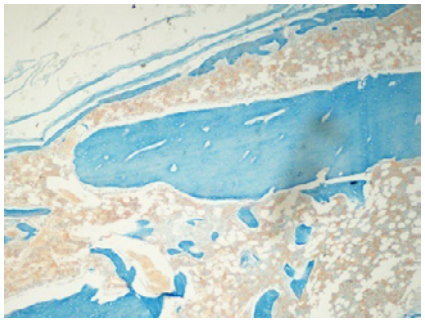


Fig. 13. Bone callus, experimental group 5 (Mallory staining, magnification 40×)

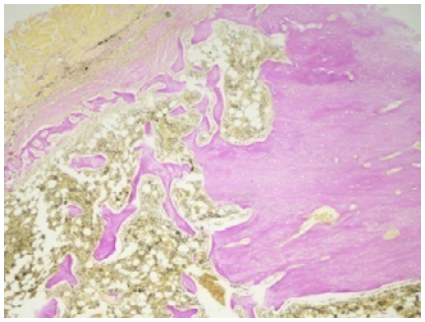


Fig. 14. Bone callus, experimental group 5 (Van Gieson staining, magnification 40×)

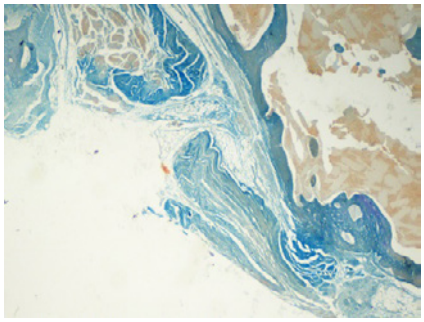


Fig. 15. Bone callus (formation of bone trabeculae), experimental group 5 (Mallory staining, magnification 40×)

is confirmed by a higher ratio of bone tissue and a low content of connective tissue at the sites where fragments fit together, as well as active ossification processes and appearance of elements of lamellar bone tissue.

REFERENCES

1. Marchenkova L. O., Gorbach E. N., Kononovich N. A., Stepanov M. A. Comminuted tibial fracture healing by the use of the mineral complex micellate (experimental study). *Genij Ortopedii*. 2017; 23 (3): 292–296. DOI: 10.18019/1028-4427-2017-23-3-292-296.

2. Bochkarev V. V., Videnin V. N., Druzhinina T. V. Application of material on the basis of hydroxyapatite for bone tissue replacement in surgery of toy breed dogs with fractures of the antebtachium bones. *Issues of Legal Regulation in Veterinary Medicine*. 2015; 3: 118–121. eLIBRARY ID: 24283115. (in Russian)

3. Derevyanchenko V. V. Clinical and morphological substantiation of the effectiveness of the use of osteofixers made of nano-modified titanium dioxide in traumatology [Kliniko-morfologicheskoe obosnovanie effektivnosti primeneniya v travmatologii osteofiksatorov iz nanomodificirovannogo dioksida titana]: author's abstract of Candidate of Science thesis (Veterinary Medicine). Saratov; 2015. 22 p. Available at: https://dlib.rsl.ru/viewer/01005560511#?page=1. (in Russian)

4. Berchenko G. N. The biology of fracture healing and influence biocomposite nanostructured material COLLAPAN on activation of repair of bone fractures. *Medical alphabet*. 2011; 1 (2): 14–19. eLIBRARY ID: 17392726. Available at: https://www.elibrary.ru/download/elibrary_17392726_50033852.pdf. (in Russian)

5. Stekolnikov A. A., Reshetnyak V. V., Burdeyniy V. V., Iskaliyev E. A. Dynamics of white blood under femoral bone fractures in rats on the background of application of RV-2 immunomodulator and RVI bio-compositional material. *International Bulletin of Veterinary Medicine*. 2019; 4: 147–152. eLIBRARY ID: 41559298. (in Russian)

6. Berchenko G. N., Kesyan G. A., Urazgil'deyev R. Z., Arsen'ev I. G., Mikelaishvili D. S., Bolbut M. V. Comparative experimental-morphologic study of the influence of calcium-phosphate materials on reparative osteogenesis activization in traumatology and orthopedics. *Acta Biomedica Scientifica*. 2006; 4 (50): 327–332. eLIBRARY ID:13026681. (in Russian)

7. Mikhanov V. A., Polyakova V. S., Kopylov V. A., Mkhitaryan E. E., Meshcheryakov K. N., Bakaeva N. R., Shurygina E. I. Reparative histogenesis of one tissue in an open shaft fractures of long bones in rats with preparations "Vinfar". *Modern Problems of Science and Education*. 2015; 3. eLIBRARY ID: 23703590. Available at: http://www.science-education.ru/ru/article/view?id=19689. (in Russian)

8. Sakhno N. V. Optimization of reparative ostogenesis in bone trauma of pets [Optimizaciya reparativnogo osteogeneza pri kostnyh travmah u melkih domashnih zhivotnyh]: author's abstract of Doctor of Science thesis (Veterinary Medicine). M.; 2012. 47 p. Available at: https://dlib.rsl.ru/viewer/01005009913#?page=1. (in Russian)

Received on 29.03.2021
Approved for publication on 19.05.2021

INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Vladimir V. Reshetnyak, Candidate of Science (Veterinary Medicine), Associate Professor, Head of Department of Internal Non-Infectious Diseases, Surgery and Obstetrics, FSBEI HE Kostroma SAA, Karavaevo, Kostroma Oblast, Russia.

Vasiliy V. Burdeyniy, Doctor of Science (Veterinary Medicine), Professor, Department of Epizootology, Parasitology and Microbiology, FSBEI HE Kostroma SAA, Karavaevo, Kostroma Oblast, Russia.

Valery V. Pronin, Doctor of Science (Biology), Professor, Head of the Centre for Preclinical Tests FGBI "ARRIAH", Vladimir, Russia.

Yevgeniy A. Iskaliyev, Chief Veterinary Practitioner of "AiBolit" Veterinary Clinic, Kostroma, Russia.

Решетняк Владимир Вячеславович, кандидат ветеринарных наук, доцент, заведующий кафедрой внутренних незаразных болезней, хирургии и акушерства ФГБОУ ВО «Костромская ГСХА», г. Кострома, Россия.

Бурдейный Василий Владимирович, доктор ветеринарных наук, профессор кафедры эпизоотологии, паразитологии и микробиологии ФГБОУ ВО «Костромская ГСХА», г. Кострома, Россия.

Пронин Валерий Васильевич, доктор биологических наук, профессор, руководитель центра доклинических исследований ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Искалиев Евгений Айдарханович, главный врач ветеринарной клиники «АйБолит», г. Кострома, Россия.

DOI: 10.29326/2304-196X-2021-3-38-254-260

Analysis of veterinary and sanitary inspection in Russian Federation Subjects

A. M. Selyanin[1], M. A. Shibayev[2], A. V. Belchikhina[3], A. K. Karaulov[4]
FGBI "Federal Centre for Animal Health" (FGBI "ARRIAH"), Vladimir, Russia
[1] *ORCID 0000-0003-1200-4597, e-mail: selyanin@arriah.ru*
[2] *ORCID 0000-0002-9382-0109, e-mail: shibaev@arriah.ru*
[3] *ORCID 0000-0003-1442-2469, e-mail: belchihina@arriah.ru*
[4] *ORCID 0000-0002-5731-5762, e-mail: karaulov@arriah.ru*

SUMMARY

The paper represents the analysis of the key parameters specifying the food safety system in the Russian Federation as of January 1, 2020. Such issues as competence of the veterinary experts and laboratories in the field of veterinary and sanitary inspection as well as availability of the necessary equipment including equipment for radiometric tests were particularly considered. Implementation of the internal audit procedure by the veterinary and sanitary testing laboratories was assessed. Data on the availability of the slaughterhouses and slaughter units/facilities for emergency slaughter as well as their staffing with the veterinarians are demonstrated. The study results indicate that 39% of the laboratories are not equipped with the necessary laboratory equipment; 8% of the laboratories perform tests using non-calibrated laboratory equipment, and only 2/3 of the laboratories are covered by the regular internal audits. Evidence of insufficient control over the attestation of the veterinarians involved in the veterinary and sanitary expertise was identified. Moreover, insufficient number of slaughter facilities and veterinary and sanitary testing laboratories in the regions of the country was highlighted as well as inadequate staffing of the laboratories with the veterinarians responsible for the official control of the compliance with the veterinary rules and technical regulations and for the veterinary and sanitary inspections. Therefore, in some regions of the country the national veterinary services lack any capacities necessary to perform the emergency slaughter of the diseased and suspect animals in the isolated and controlled environment with the subsequent on-site storage and decontamination of the slaughter products or their disposal or destruction. The study results demonstrate a number of gaps in the veterinary and sanitary inspection system thus indicating the need for corrective actions to be taken both on the federal and local levels.

Keywords: food safety, veterinary and sanitary testing laboratory, veterinarians, slaughter facilities

Acknowledgements: The study was funded by the FGBI "ARRIAH" within the framework of "Veterinary Welfare" research work.

For citation: Selyanin A. M., Shibayev M. A., Belchikhina A. V., Karaulov A. K. Analysis of veterinary and sanitary inspection in Russian Federation Subjects. *Veterinary Science Today*. 2021; 10 (3): 254–260. DOI: 10.29326/2304-196X-2021-3-38-254-260.

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

For correspondence: Arkady M. Selyanin, Leading Veterinarian, Information and Analysis Centre, FGBI "ARRIAH", 600901, Russia, Vladimir, Yur'evets, *e-mail: selyanin@arriah.ru.*

УДК 919:614.31(470):637:633/635-021.66

Анализ организации ветеринарно-санитарной экспертизы в субъектах Российской Федерации

А. М. Селянин[1], М. А. Шибаев[2], А. В. Бельчихина[3], А. К. Караулов[4]
ФГБУ «Федеральный центр охраны здоровья животных» (ФГБУ «ВНИИЗЖ»), г. Владимир, Россия
[1] *ORCID 0000-0003-1200-4597, e-mail: selyanin@arriah.ru*
[2] *ORCID 0000-0002-9382-0109, e-mail: shibaev@arriah.ru*
[3] *ORCID 0000-0003-1442-2469, e-mail: belchihina@arriah.ru*
[4] *ORCID 0000-0002-5731-5762, e-mail: karaulov@arriah.ru*

РЕЗЮМЕ

В статье представлен анализ наиболее важных показателей, характеризующих организацию системы обеспечения безопасности пищевых продуктов в Российской Федерации по состоянию на 1 января 2020 г. В частности, рассмотрены вопросы компетентности ветеринарных специалистов и лабораторий ветеринарно-санитарной экспертизы, обеспеченность лабораторий необходимым оборудованием, в том числе и радиометрическим, проведена оценка

реализации процедуры внутреннего аудита лабораторий ветеринарно-санитарной экспертизы. Представлены данные об обеспеченности субъектов Российской Федерации убойными и убойно-санитарными пунктами/площадками, а также их укомплектованность ветеринарными специалистами. Результаты исследования свидетельствуют о том, что 39% лабораторий ветеринарно-санитарной экспертизы не укомплектованы необходимым лабораторным оборудованием, 8% лабораторий применяли для проведения исследований неповеренное лабораторное оборудование и только 2/3 лабораторий были охвачены периодическими внутренними аудитами. Выявлены факты недостаточного контроля за аттестацией ветеринарных специалистов, осуществляющих ветеринарно-санитарную экспертизу. Кроме того, отмечена недостаточная обеспеченность регионов страны местами убоя животных и лабораториями ветеринарно-санитарной экспертизы, а также неполная укомплектованность их ветеринарными специалистами, в задачи которых входит проведение государственного надзора за соблюдением требований ветеринарных правил и технических регламентов, проведение ветсанэкспертизы. Таким образом, в некоторых регионах страны у государственной ветеринарной службы отсутствует возможность убоя больных и подозреваемых в заболевании животных в изолированных контролируемых условиях с последующим хранением и обеззараживанием продуктов убоя, или их утилизацией, или уничтожением на месте. Полученные результаты исследования показывают наличие ряда пробелов в организации системы ветеринарно-санитарной экспертизы, что свидетельствует о необходимости введения корректирующих мер как на федеральном, так и на региональном уровне.

Ключевые слова: безопасность пищевой продукции, лаборатория ветеринарно-санитарной экспертизы, ветеринарные специалисты, места убоя животных

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

Для цитирования: Селянин А. М., Шибаев М. А., Бельчихина А. В., Караулов А. К. Анализ организации ветеринарно-санитарной экспертизы в субъектах Российской Федерации. *Ветеринария сегодня*. 2021; 10 (3): 254–260. DOI: 10.29326/2304-196X-2021-3-38-254-260.

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

Для корреспонденции: Селянин Аркадий Михайлович, ведущий ветеринарный врач информационно-аналитического центра ФГБУ «ВНИИЗЖ», 600901, Россия, г. Владимир, мкр. Юрьевец, *e-mail: selyanin@arriah.ru*.

INTRODUCTION

Food product safety – is the state of food products, indicating the absence of unacceptable risk associated with harmful effects on humans and future generations. A harmful effect on humans is caused by factors associated with the presence of contaminants in food products: radionuclides, toxins, pathogens that threaten human life or health [1]. According to the World Health Organization, food safety should be a priority public health issue [2].

The safety of food products of animal and plant origin can't be ensured without establishing the system of veterinary and sanitary inspection (VSI system) the basis of which are veterinary specialists and veterinary and sanitary laboratories fit with equipment.

Assessment of the organization and effectiveness of the VSI system in the country is part of the assessment of the organization of the national veterinary service, carried out within the framework of international trade, as well as within the framework of regionalization [3, 4].

Today official information on the state of the veterinary and sanitary inspection system and its assessment is unavailable in the Russian Federation that is why it is impossible to objectively reflect the actual situation in the regions of the country.

In this regard, the investigation was aimed at collection of information regarding the most significant parameters (availability of slaughterhouses and slaughter units/facilities for emergency slaughter, staffing with veterinary specialists, material and technical equipment of laboratories for veterinary and sanitary inspection, etc.) and a comprehensive analytical assessment of VSI system in the Russian Federation regions.

MATERIALS AND METHODS

The practical basis for the analysis of how the RF National Veterinary Service ensures veterinary and sanitary safety of food products was the information entered into the 'Assol.Express' operational reporting system by the executive veterinary authorities of the country's Subjects as of January 1, 2020, according to the primary data collection form developed by the FGBI "ARRIAH".

The study used generally accepted methods and techniques for data analysis: generalization and formalization of information, the method of comparative analysis.

RESULTS AND DISCUSSION

Food safety processes should address the entire food chain, from production to consumption. One of the official regulation measures aimed at ensuring food quality and safety is the adoption of Customs Union technical regulations that establish safety requirements (including sanitary and epidemiological, hygienic, and veterinary) for regulated products, as well as forms and procedures or assessing (confirming) the conformity of the regulated products to the requirements of technical regulations.

According to the current legislation, the VSI is one of the variants confirming compliance of the food products with the CU Technical Regulations and is the only and final way to confirm compliance and safety of non-processed food products of animal origin [5].

Fig. 1. Diagram of the food safety system

As can be seen from the presented diagram (Fig. 1), food safety is ensured by a set of various systems interacting with each other.

This paper addresses the VSI system in terms of parameters having a key impact on food safety, affecting all stages of the food product supply chain from 'farm to fork':

– availability of slaughterhouses and slaughter units/facilities in the Subjects of the Russian Federation;

– sufficient number of veterinary specialists performing VSI of products of animal and plant origin;

– sufficient number of veterinary service specialists at livestock farms;

– VSI equipment and facilities, availability of a quality management system;

– the competence of the veterinary specialists of the VSI service and the availability of a medical certificate of admission to work with food products.

Veterinary and sanitary inspection at slaughterhouses. Meat and meat products shall be produced in compliance with hygienic as well as veterinary and sanitary requirements as well as considering the risk arising at all the stages of the technological process.

The necessary level of slaughter hygiene can be achieved by proper veterinary assistance, lairaging, antemortem veterinary inspection, the slaughter process itself, and other factors influencing the quality and safety of the finished products [6]. The statutory regulated requirements for meat and meat product manufacturing can be complied with only at special slaughterhouses controlled by the National Veterinary Service and having passed the official registration procedure.

To assess the effectiveness of the VSI at slaughterhouses, the availability of slaughterhouses and slaughter units/facilities in the RF Subjects as well as the sufficient number of qualified veterinary specialists was taken into account.

As a result of the analysis, it was found that only 28 RF Subjects are provided with slaughter sites (slaughter units/facilities) designated for the needs of the residents in full, in 9 Subjects no slaughter sites are available, in other Subjects the situation appears to be diverse (Fig. 2).

The business operators in the RF Subjects are not provided with a sufficient number of slaughter units/facilities: only 41 Subject is completely provided with them, in 2 Subjects no slaughter units/facilities are available and in the rest of the Subjects the need in them is satisfied 1–99% (Fig. 2).

Slaughter units/facilities in 68 RF Subjects are staffed by veterinary specialists engaged in official control for compliance with veterinary rules and technical regulations and VSI. In three RF Subjects the slaughter sites are not staffed by veterinary specialists at all. In the rest of the Subjects this parameter varies a lot – from 1–99%.

Only 35 RF Subjects demonstrate a sufficient number of slaughter units/facilities, in 24 Subjects such sites are not available and in the rest of the Subjects their amount varies from 1–99%. So, in most of the RF Subjects the National Veterinary Service lack isolated facilities for slaughtering diseased and disease suspected animals under controlled conditions with subsequent storage and decontamination of slaughter products or their disposal or on-site destruction.

It should be noted that proper veterinary services rendered to the animal farm involving daily comprehensive control predetermine the quality and safety of the products. However, the results of the analysis performed show that more than half (51%) of animal farms in the country, not including small-scale farms, don't have their own veterinary service.

The absence of available slaughter units/facilities results in uncontrolled slaughter on small-scale farms without

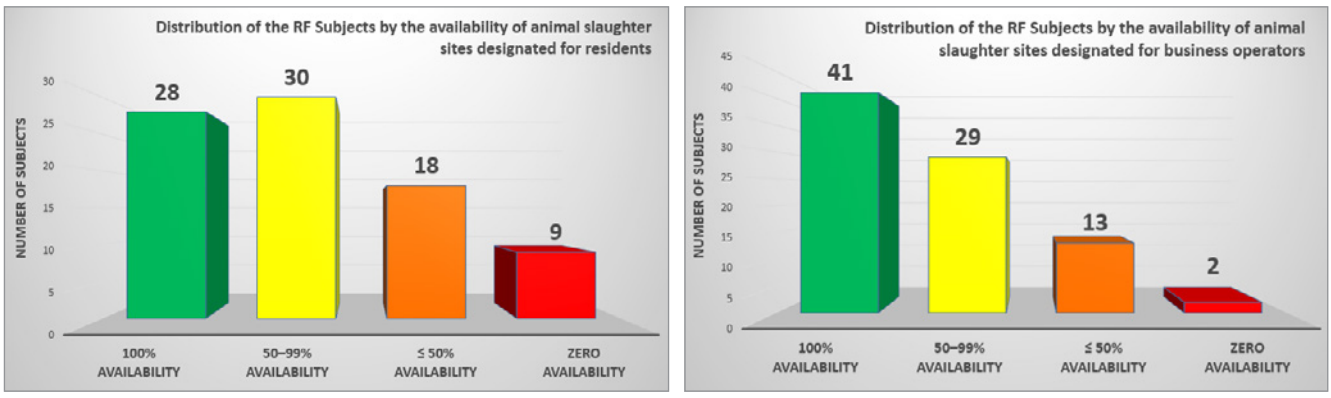


Fig. 2. Distribution of the RF Subjects by the availability of the slaughter facilities

veterinary and sanitary inspection of animal slaughter products. Besides, there's a possibility of slaughtering animals with unknown animal health status due to the absence of proper veterinary control and lack of VSI specialists on animal farms. This circumstance creates a high risk of consuming unsafe food products and infection of the staff with zooanthroponoses.

VSI laboratories. It is known that veterinary and sanitary inspection of animal products is performed at slaughterhouses and VSI laboratories located at the institutions of the National Veterinary Service and trade enterprises, including food markets. 40 RF Subjects are self-sufficient in VSI laboratories, 35 Subjects are 50–99% self-sufficient and 10 Subjects are 50% self-sufficient (Fig. 3).

Even though the VSI laboratories shall be organized in markets selling food products of animal and/or plant origin according to the law [7], 102 out of 1,703 food markets in the RF Subjects lack such laboratories, and this situation has persisted over the past few years. The specified facts are considered as a violation of the law and contribute to the threat of the emergence and spread of infectious and invasive diseases transmitted to humans from animals since the safety of food products sold in these markets has not been confirmed. The situation is aggravated by the fact that trade enterprises that are not legally regulated by the above legislation (for example, shopping centers) are not Subject to the obligation to organize VSI laboratories. However, such outlets often sell non-commercial food products and unprocessed products of animal origin, the safety of which in veterinary and sanitary terms has not been confirmed due to the absence of VSI laboratories.

The Customs Union (CU) legislation establishes that both unprocessed food products of animal origin and non-commercial products shall comply with the requirements of the CU technical regulations which is to be confirmed through VSI. In this case, the VSI laboratory (or veterinary expert) is the only link in confirming the safety of regulated food products, and in fact, these laboratories perform the functions of testing laboratories. Based on their reports food products are eligible for free marketing. Under the current legislation, VSI laboratories are not Subject to compulsory accreditation, therefore, competency assessment was not carried out for any of the laboratories in the country.

In this research, the competence of VSI laboratories was determined according to the following criteria:

– availability of necessary measuring instruments and their technical condition, verification of measuring instruments;

– availability of normative and methodological documents regulating testing;

– internal audit of VSI laboratories conducted by institutions subordinate to the veterinary executive authorities.

The results of the analysis based on these criteria demonstrate that:

– 1,097 out of 2,795 (39%) VSI laboratories cannot fully carry out mandatory veterinary sanitary inspection of all food products (meat, milk, eggs, feed, etc.), which contradicts to the current legislation of the Russian Federation [8], and in 5 Subjects none of the VSI laboratories has a full range of necessary laboratory equipment. Therefore this service is not fully available for the residents. Due to this fact, the products not having undergone this procedure are likely to enter the food production chain;

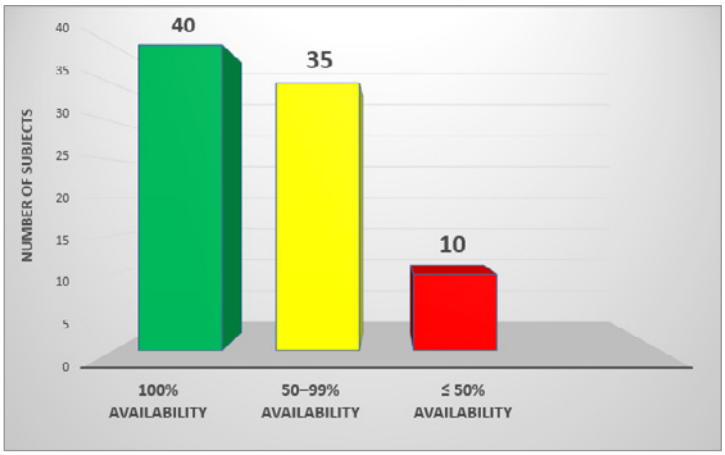


Fig. 3. Distribution of the RF Subjects by the availability of the veterinary and sanitary testing laboratories

– in the reporting period (2019), 247 out of 2,795 (9%) VSI laboratories conducted research using non-calibrated laboratory equipment which casts doubt on the objectivity of the measurement results obtained using this equipment, and, therefore, conclusions on compliance of food products with current regulatory requirements;

– 125 out of 2,795 (4.5%) VSI laboratories are not fully provided with regulatory and methodological documents for veterinary and sanitary inspection;

– in 1,763 out of 2,795 (63%) VSI laboratories in 29 RF Subjects, an internal audit procedure has been introduced and is being implemented. It includes periodic internal inspections for assessing the performance of laboratories and their technical condition. The introduction of such audits into the practice can increase the effectiveness of control over the activities of VSI laboratories under the current legislation, including research.

Since the VSI laboratories function as testing laboratories and ensure the veterinary and sanitary safety of food products, all tests must be carried out properly and ensure the reliability of the results. In testing laboratories, this is confirmed by accreditation, which is the evidence of competence and ability to obtain reliable results [9]. Since the accreditation procedure for VSI laboratories is not regulated at the legislative level, it is necessary to apply this practice in respect of these laboratories. One of the possible ways to implement this may be the inclusion of VSI laboratories in the structure of veterinary diagnostic laboratories, after which they will be included in the scope of accreditation of the head institution. This practice is currently partially implemented in the reference centers and interregional veterinary laboratories of the Russian Federal Service for Veterinary and Phytosanitary Surveillance (Rosselkhoznadzor), and previously it was used for a long time in the USSR.

Radiation control of food products. The activities of VSI laboratories for conducting radiation control of products of animal and plant origin are worth noting. Under the current legislation of the Russian Federation, the National Veterinary Service shall perform dosimetric control of all products to be sold in marketplaces

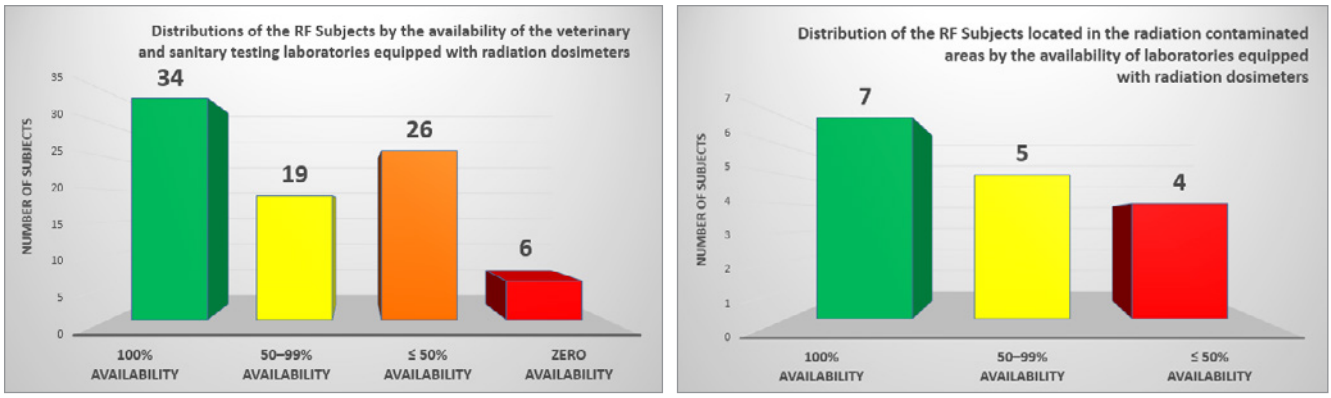


Fig. 4. Distributions of the RF Subjects by the availability of the veterinary and sanitary testing laboratories equipped with radiation dosimeters

and twice a year radiometric control of each product type [10]. Despite this, the VSI laboratories are fully equipped with dosimeters-radiometers in only 34 Subjects, in 6 Subjects none of them are provided with this equipment, and in other RF Subjects, 1–99% of laboratories are provided with such equipment. In total, only 1,787 out of 2,795 (64%) VSI laboratories are equipped with the specified radiometric equipment. Only in 7 out of 16 RF Subjects, the territories of which are included in the list of areas affected by radioactive contamination as a result of the disaster at the Chernobyl nuclear power plant and the accident at FSUE "Mayak Production Association", the VSI laboratories are fully equipped with dosimeters-radiometers, in 5 Subjects only 50 to 99% laboratories are self-sufficient in such equipment and 4 Subjects – less than 50% (Fig. 4) [11, 12].

Thus, only 319 of 439 VSI laboratories (73%) located on the territory of 16 Subjects in areas affected by radioactive contamination are provided with appropriate radiometric equipment. The current situation hinders the performance of radiation control the framework of veterinary and sanitary inspection and creates a risk of marketing potentially radiation-contaminated products which is critical for the specified above areas affected by radioactive contamination.

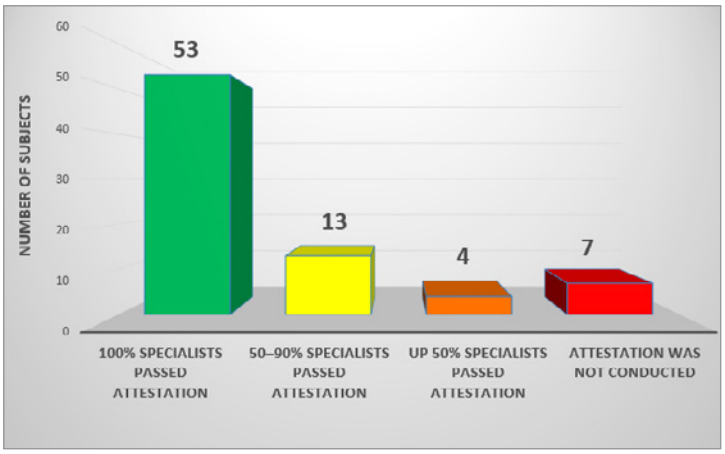


Fig. 5. Distribution of the RF Subjects by the level of attestation of the veterinarians involved in veterinary and sanitary inspections

Competence of veterinary specialists engaged in the veterinary and sanitary inspection. The leading role in performing veterinary and sanitary inspection lies with veterinary specialists having professional skills and knowledge in this field of activity. Special attention shall be given to the competence of the veterinary specialists at slaughterhouses where they ensure the safety of food products on their way to the customer. Under the current legislation, the qualification level of the VSI specialists is checked during periodical attestation [13–15]. The analysis demonstrated that only 3,704 out of 4,301 VSI specialists passed attestation (Fig. 5).

Herewith in 24 Subjects of the country, 597 veterinarians haven't been subjected to attestation to check the conformity of food products with the established requirements. Therefore, the results of the veterinary and sanitary inspection performed by these VSI specialists cannot be considered 100% reliable and objective which can affect the safety of food products.

Of little significance in ensuring food safety is control of the specialist's health status in regards to food-borne diseases. The health status is examined during periodical health checks the results of which are recorded in their medical cards. The study performed demonstrates that only 7,875 out of 11,666 veterinarians and laboratory technicians (67.5%) contacting with food products undergo health checks and have individual medical cards. In 7 Subjects of the country, none of the specified above specialists has undergone health checks which are indicative of the systemic RF law violation [16]. Thus, 3,791 veterinarians ensuring food safety have not passed the medical examination and shall not contact food products. Similar situations contribute to favourable conditions for food product contamination and increase the risk of toxicoinfections.

CONCLUSION

The analytical study performed allows concluding that as of January 1, 2020, there are certain gaps in the food safety management system due to imperfect legislation as well as VSI system functioning at the regional level. In particular, lack of the slaughter sites designated for public needs, as well as slaughter units/facilities; insufficient number of VSI laboratories and their competence; in most cases improper management of VSI laboratory activities as

well as insufficient control for the competence of the veterinarian performing the veterinary and sanitary inspection.

The current state of the system of VSI-based food product safety confirmation arises the necessity to implement corrective actions both on the federal and regional levels. These corrective actions include:

– legislative recognition of the VSI laboratory competence through accreditation;

– legislative recognition of the ban to issue accompanying veterinary documents for slaughter products by slaughterhouses not serviced by veterinarians (meat inspectors) and having no certificate confirming compliance of the facility and production processes with technical regulations, subsequent updating of the existing FGIS "VetIS" registers;

– creating a list of the veterinarians certified for performing veterinary and sanitary inspection using FGIS "VetIS", thus blocking access of non-certified veterinarians to the option of issuing accompanying veterinary documents in FGIS "VetIS" 'MERCURY' for slaughter products;

– inclusion of the List of the accredited VSI Laboratories to FGIS "VetIS" 'CERBERUS'.

We suppose that the implementation of the specified measures will contribute to more effective control of food product safety within the entire production cycle and prevent the marketing of dangerous and poor quality food products.

REFERENCES

1. Control-oriented classification of the marketed food products by their potential risk of harm to human health and property losses [Klassifikaciya pishchevoj produkcii, obrashchaemoj na rynke, po risku prichineniya vreda zdorov'yu i imushchestvennyh poter' potrebitelej dlya organizacii planovyh kontrol'no-nadzornyh meropriyatij]: Methodical Guidelines (approved by the Order of the Federal Service for Consumer Rights Protection and Human Welfare of 18.01.2016 No. 16). Available at: https://www.garant.ru/products/ipo/prime/doc/71213192. (in Russian)

2. World Health Organization. Food safety. Available at: https://www.who.int/news-room/fact-sheets/detail/food-safety (date of access: 02.12.2020).

3. OIE. Terrestrial Animal Health Code. Vol. 1. General provisions. Paris: OIE; 2019. Available at: https://www.oie.int/en/what-we-do/standards/codes-and-manuals/terrestrial-code-online-access.

4. OIE Tool for the Evaluation of Performance of Veterinary Services. Paris: OIE; 2019. 51 p. Available at: https://www.oie.int/fileadmin/Home/eng/Support_to_OIE_Members/docs/pdf/2019_PVS_Tool_FINAL.pdf.

5. Technical Regulations of the Customs Union TR CU 021/2011. On Food Safety: Approved by the decision of the Commission of the Customs Union on December 9, 2011 No. 880. Available at: https://www.eurexcert.com/TRCUpdf/TRCU-0021-On-food-safety.pdf.

6. WHO/FAO. Codex Alimentarius. Animal food production. Rome; 2007. 192 p.

7. On retail markets and amendments to the Labour Code of the Russian Federation [O roznichnyh rynkah i o vnesenii izmenenij v Trudovoj kodeks Rossijskoj Federacii]: Federal Law of 30.12.2006 No. 271-FZ. *ConsultantPlus*. Available at: http://www.consultant.ru/document/cons_doc_LAW_64936. (in Russian)

8. Veterinary Law [O veterinarii]: Law of the Russian Federation No. 4979-1 of 14.05.1993. *ConsultantPlus*. Available at: http://www.consultant.ru/document/cons_doc_LAW_4438. (in Russian)

9. GOST ISO/IEC 17025-2019. General requirements for the competence of testing and calibration laboratories. Available at: https://docs.cntd.ru/document/1200166732. (in Russian)

10. Regulation on the state veterinary control of the radioactive contamination of the veterinary regulated objects in the Russian Federation [Polozhenie o sisteme gosudarstvennogo veterinarnogo kontrolya radioaktivnogo zagryazneniya ob"ektov veterinarnogo nadzora v Rossijskoj Federacii]: approved by the RF Minselkhoz on 20.02.1998. *ConsultantPlus*. Available at: http://www.consultant.ru/cons/cgi/online.cgi?req=doc&base=EXP&n=314178#TUAAbdS2Tc9bVoxU1. (in Russian)

11. List of settlements located in the zones of radioactive contamination due to the disaster at the Chernobyl NPP [Perechen' naselennyh punktov, nahodyashchihsya v granicah zon radioaktivnogo zagryazneniya vsledstvie katastrofy na Chernobyl'skoj AES]: approved by Russian Federation Governmental Ordinance of 08.10.2015 No. 1074. Available at: https://base.garant.ru/71216726. (in Russian)

12. On measures to be taken for implementation of the Russian Federation Law on social protection of citizens exposed to radiation caused by the disaster in the industrial group "Majak" in 1957 and due to the discharge of radioactive wastes in Techa River [O merah po realizacii Zakona Rossijskoj Federacii «O social'noj zashchite grazhdan, podvergshihsya vozdejstviyu radiacii vsledstvie avarii v 1957 godu na proizvodstvennom ob"edinenii "Mayak" i sbrosov radioaktivnyh othodov v reku Techa»]: Russian Federation Governmental Ordinance of 08.10.1993 No. 1005 (edited on 20.11.1999). Available at: https://docs.cntd.ru/document/9004672. (in Russian)

13. Guidance on health marks applied to meat [Instrukciya po veterinarnomu klejmeniyu myasa]: approved by Minselkhozprod of Russia on 28.04.1994. Available at: https://docs.cntd.ru/document/9005941. (in Russian)

14. On introduction of attestation of managing, engineering and other specialized employees of the industrial, construction, agricultural, transport and communication establishments and institutions [O vvedenii attestacii rukovodyashchih, inzhenerno-tekhnicheskih rabotnikov i drugih specialistov predpriyatij i organizacij promyshlennosti, stroitel'stva, sel'skogo hozyajstva, transporta i svyazi]: approved by the Ordinance of the Council of Ministers of the USSR on 26.07.1973 No. 531. Available at: https://docs.cntd.ru/document/9035213?marker=7D20K3. (in Russian)

15. Regulation on the procedure of attestation of managing, engineering and other specialized employees of the industrial, construction, agricultural, transport and communication establishments and institutions [Polozhenie o poryadke provedeniya attestacii rukovodyashchih, inzhenerno-tekhnicheskih rabotnikov i drugih specialistov predpriyatij i organizacij promyshlennosti, stroitel'stva, sel'skogo hozyajstva, transporta i svyazi]: approved by the Ordinance of the Labor and Salary State Committee of the Council of Ministers of the USSR on 05.10.1973 No. 267. Available at: https://docs.cntd.ru/document/9035214. (in Russian)

16. List of activities requiring preliminary and regular medical checks (examination) of the employees [Perechen' rabot, pri vypolnenii kotoryh provodyatsya obyazatel'nye predvaritel'nye i periodicheskie medicinskie osmotry (obsledovaniya) rabotnikov]: Annex 2 to the Order of the Russian Federation Ministry of Health and Social Development of 12.04.2011 No. 302n. Available at: http://www.consultant.ru/document/cons_doc_LAW_120902/ade2f21aef1dcbb633ff5de4fa0a5cb2c5a40613. (in Russian)

Received on 29.04.2021
Approved for publication on 19.06.2021

INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Arkady M. Selyanin, Leading Veterinarian, Information and Analysis Centre, FGBI "ARRIAH", Vladimir, Russia.

Mikhail A. Shibayev, Candidate of Science (Veterinary Medicine), Head of Sector, Information and Analysis Centre, FGBI "ARRIAH", Vladimir, Russia.

Anastasia V. Belchikhina, Junior Researcher, Information and Analysis Centre, FGBI "ARRIAH", Vladimir, Russia.

Anton K. Karaulov, Candidate of Science (Veterinary Medicine), Head of Information and Analysis Centre, FGBI "ARRIAH", Vladimir, Russia.

Селянин Аркадий Михайлович, ведущий ветеринарный врач информационно-аналитического центра ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Шибаев Михаил Александрович, кандидат ветеринарных наук, заведующий сектором информационно-аналитического центра ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Бельчихина Анастасия Владимировна, младший научный сотрудник информационно-аналитического центра ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Караулов Антон Константинович, кандидат ветеринарных наук, руководитель информационно-аналитического центра ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

VETERINARY SCIENCE TODAY JOURNAL INVITES AUTHORS TO SUBMIT THEIR PAPERS FOR PUBLICATION

Photo: Alexander Plonskiy

- 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

Facebook.com/arriah.ru/ @fgbi_arriah

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.

FGBI "FEDERAL CENTRE FOR ANIMAL HEALTH"
(FGBI "ARRIAH")
ФГБУ «ФЕДЕРАЛЬНЫЙ ЦЕНТР ОХРАНЫ ЗДОРОВЬЯ ЖИВОТНЫХ»

OIE REGIONAL REFERENCE LABORATORY FOR FOOT AND MOUTH DISEASE
РЕГИОНАЛЬНАЯ РЕФЕРЕНТНАЯ ЛАБОРАТОРИЯ МЭБ ПО ЯЩУРУ

OIE REFERENCE LABORATORY FOR HIGHLY PATHOGENIC AVIAN INFLUENZA AND LOW PATHOGENIC AVIAN INFLUENZA (POULTRY) AND NEWCASTLE DISEASE
РЕФЕРЕНТНАЯ ЛАБОРАТОРИЯ МЭБ ПО ВЫСОКОПАТОГЕННОМУ И НИЗКОПАТОГЕННОМУ ГРИППУ ПТИЦ И НЬЮКАСЛСКОЙ БОЛЕЗНИ

FGBI "Federal Centre for Animal Health" (FGBI "ARRIAH")

ANNOUNCES

admission open for 2021 under two postgraduate training programmes:

36.06.01 Veterinary Medicine and Zootechnics, researchers' speciality *06.02.02 Veterinary Microbiology, Virology, Epizootology, Mycology and Mycotoxicology, Immunology* – 6 openings;

06.06.01 Biological Sciences, researchers' speciality *03.02.02 Virology* – 4 openings.

DOCUMENTS REQUIRED:

– an application addressed to the Director of the FGBI "ARRIAH";

– a document (documents) to certify the identity and citizenship of the applicant;

– specialist's or master's original diploma;

– the list of published papers, inventions and research activity reports signed as appropriate. Those who have no published papers or inventions are required to submit a library research paper on a topic related to the chosen training programme;

– a document certifying the applicant's personal accomplishments taken into account for admission to training in accordance with admission rules (contest, festival, exhibition winner or laureate certificates, etc.);

– a health certificate (form 086/u);

– 2 photos (4 × 6 cm).

Postgraduate training admission documents are accepted from 1 June till 31 August 2021

Postgraduate training applicants are required to take competitive admission examinations in the chosen speciality, philosophy, a foreign language in accordance with the state educational standards for higher professional education.

Address of the Admission Board:
600901, Russia, Vladimir, Yur'evets,
FGBI "Federal Centre for Animal Health"
(FGBI "ARRIAH"), www.arriah.ru

For enquiries, please call: +7 (4922) 52-99-62;
26-15-12 (ext. 25-20, 20-20, 22-20)

about the conference to be held on 2–3 December 2021

The Council of Early Career Researchers of the FGBI "Federal Center for Animal Health" invites young professionals, postgraduates and researchers under 35 years of age to participate in the VI International Scientific Conference "Achievements of Early Career Scientists for Veterinary Practice" dedicated to the 60th anniversary of the FGBI "ARRIAH" Postgraduate Department, which will be held on December 2–3, 2021 at: 600901, Vladimir, Yur'evets, FGBI "ARRIAH".

FOCUS AREAS OF THE CONFERENCE:

– epizootology (monitoring of infectious diseases of livestock, wild and domestic animals, vaccination, immunology);

– biotechnology (virus and cell cultivation, vaccine production);

– virus diagnostics and molecular biology.

Types of conference participation: in praesentia, in absentia, via remote communication.

The papers will be published in the conference proceedings and then put into the database of the Russian Science Citation Index (RSCI). All scientific papers will take part in the competition, and the winning papers in each section of the conference will be published in the *Veterinary Science Today* journal, which is included in the List of Higher Attestation Commission.

In order to work out the program and prepare the conference materials for publication in a timely manner, please submit the following materials via e-mail by September 15, 2021 to *nikeshina@arriah.ru:* a completed application form for conference participation; a scientific paper for publication (requirements for the paper format are published on our website); an expert opinion of the submitting organization allowing open-access publication.

Information on the conference is available on the FGBI "ARRIAH" website www.arriah.ru in the 'Seminars and Conferences' section.

Contact information:

tel. +7(4922) 26-15-12 (ext. 25-20), *e-mail: romenskaya@arriah.ru*

tel. +7(4922) 52-99-62, *e-mail: elnikova@arriah.ru*

tel.+7 (4922) 26-15-12 (ext. 23-10), *e-mail: komova@arriah.ru*

tel: +7 (4922) 26-15-12 (ext. 22-27), *e-mail: nikeshina@arriah.ru*

tel: +7 (4922) 26-15-12 (ext. 25-01), *e-mail: guseva_ey@arriah.ru*