

Name of the program: BR10764944 "Development of methods for analytical control and monitoring of food safety"

Relevance: Ensuring the safety of livestock products is one of the important state strategic goals aimed at preserving the health of the nation. In addition, one of the key conditions for the participation of the exporting country in the food trade is its ability to ensure the quality and safety of products in accordance with the regulations of the importing countries, WHO, FAO and OIE. Therefore, the improvement of methods for determining contaminants hazardous to human health in livestock products is always a priority for both domestic and world veterinary science.

Purpose: Development of methods for monitoring the safety of livestock products.

Expected results:

Upon completion of the program:

Methods for analytical control and monitoring of the safety of meat and milk for the content of antibiotics will be developed; fish - for the content of antibiotics, salts of heavy metals, radionuclides and infection with helminthiases and bacterioses.

An analysis of the risks of the emergence of resistance to antibiotics in pathogenic microflora isolated from animals and from raw materials and products of animal origin will be carried out.

A multiplex RT-PCR test system will be developed for accelerated monitoring of food safety of milk and determination of antibiotic resistance loci in pathogenic staphylococci and streptococci.

A method will be developed for the study of cows' milk in bacterial infections.

An immunochromatographic assay (LFT) will be developed for rapid detection of the causative agent of campylobacteriosis in livestock products.

A rapid method (LFT) will be developed to assess the safety of livestock products (residue of antibiotics in meat and milk).

Will be developed and published:

- methodological recommendations on methods of analytical control and monitoring of fish safety for the content of antibiotics, salts of heavy metals, radionuclides and contamination with helminthiases and bacterioses;

- methodological guidelines for risk analysis of the emergence of resistance to antibiotics in pathogenic microflora isolated from animals and from raw materials and products of animal origin.

Applications will be submitted for obtaining 4 patents of the Republic of Kazakhstan:

- for a multiplex PCR-RT test system to detect pathogenic cocci and determine the loci of antibiotic resistance of pathogens;

- for methods of research of milk of cows at bacterial infections;

- for immunochromatographic analysis (LFT) for express detection of the causative agent of campylobacteriosis in livestock products;

- for the express method (LFT) for assessing the safety of livestock products

(the residual amount of antibiotics in meat and milk).

At least 3 articles will be published in peer-reviewed foreign scientific journals with a non-zero impact factor and at least 20 publications in foreign and domestic journals recommended by COXON, 1 (one) monograph is prepared for publication.

Achieved results for 2021.

The synthesis of guide RNAs using in vitro transcription was carried out. The ribonucleoprotein complex of CRISPR-Cas endonuclease systems with guide RNAs was assembled. The ability of the Cas12a enzyme to form a complex with guide RNAs was determined by the presence of hydrolysis of the target sequences. Evaluated endonuclease and collateral activity against target sequences, obtained a set of target sequences, consisting of primers for isothermal amplification of specific regions of pathogen genomic DNA.

A collection of bacterial strains of *Bacillus* sp., which are sensitive to broad-spectrum antibiotics, has been created. The optimal conditions for cultivation of the *Bacillus* sp.T2 strain, which has the maximum sensitivity to the minimum concentration of antibiotics, have been selected. The parameters for visual detection of the growth of the *Bacillus* sp.T2 strain in liquid and solid nutrient media were determined.

Conjugates of antibiotics with carrier proteins have been prepared, schemes for immunization of laboratory animals have been worked out, which make it possible to obtain specific antibodies in high titers.

Genetic constructs containing genes for two diagnostic proteins *Campylobacter jejuni* Omp18 and MOMP were obtained. The Omp18 and MOMP genes of *C. jejuni* were synthesized under de novo conditions. The expression activity of the pET28/Omp18, pET28/MOMP, pET32/Omp18, and pET32/MOMP plasmids in the *E. coli* BL21 strain was rather high. Recombinant *C. jejuni* Omp18 and MOMP proteins showed antigenic activity in ELISA against specific antibodies.

In the reservoirs of Northern and Western Kazakhstan, infection of fish with eggs of *Capillaria* spp., metacercariae of *Diplostomum spathaceum*, *Opisthorchidae*, *Pseudamphistomum tuncatum* and the larval stage of *Posthodiplostomum cuticola* and *Ligula intestinalis* was established.

On the territory of Kostanay, North Kazakhstan and Akmola regions, the circulation of pathogens of zoonotic enteropathogenic infections was established: *Salmonella* spp., *S. aureus*, *E. coli*, *Campylobacter* spp., *L. monocytogenes*, which have a high level of resistance to individual antibiotics.

The level of prevalence of antibiotic-resistant pathogens and antibiotic resistance loci of *Staphylococcus aureus* and *Streptococcus agalactiae* were determined. The *mecA*, *TEM*, *ermC*, *mef(A)* and other genes were selected to determine the sensitivity to antimicrobials.

Achieved results for 2022.

An experimental kit for the isolation of pathogens' genomic DNA from milk with a sensitivity of 10^3 CFU/ml was prepared, and the optimal parameters and specificity of microorganisms' DNA detection in milk were determined. The

conditions for the detection of broad-spectrum antibiotics in milk have been optimized, and a protocol for analytical control has been developed. Hybridomas clones producing monoclonal antibodies specific to oxytetracycline, streptomycin, and chloramphenicol, as well as to *Campylobacter jejuni* recombinant proteins were obtained. It has been established that pathogens of zoonotic enteropathogenic infections, such as *Salmonella* spp., *Staphylococcus aureus*, *Escherichia coli*, *Campylobacter* spp., and *Listeria monocytogenes* circulate on the territory of the northern regions of the Republic of Kazakhstan (RK). The study of the genotypic profiles of isolated microorganisms' cultures showed the presence of resistance genes to β -lactams, aminoglycosides, tetracyclines, sulfonamides, and macrolides. Primers and fluorescently labeled probes were selected for specific regions of the genes of *S. aureus* and *Str. agalactiae*; a highly specific PCR was developed with diagnostic sensitivity for the target genes of nuclease (*nuc*) and glucose kinase (*glck*) equal to 13 and 12 copies/ μ l, respectively. The ecological safety of fish in terms of food contaminants has been studied and the main fish parasitosis in the western, northern and central regions of the RK have been described.

During the reporting period, 13 original papers were published, among them: 11 (ten) - in journals recommended by the Committee for Control in Education and Science of the Ministry of Education and Science of the Republic of Kazakhstan for the publication of the main results of scientific research, 1 (one) - in a journal included in the Russian Scientific Index citations, and 1 (one) - in the journal included in the Scopus database. In addition, the results of research were published in the proceedings of conferences (Appendix E,F,G,H). All the papers have references to the received earmarked funding, indicating the Individual Registration Number of the Program (No.BR10764944) and the source of funding.

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**List of publications published as part of the project in 2021:
*in national publications:***

1 Bulashev A.K. Immunobiotechnological methods for the determination of antibiotics in food. // Bulletin of ENU named after L.N. Gumilyov. – 2021. - №3(136). – P. 35-50. DOI: 10.32523/2616-7034-2021-136-3-35-50

2 Nurgaliev B.E., Kadralieva B.T., Usenov Zh.T., Zhumabaev A.K., Safety and quality of fish affected by invasion in the West Kazakhstan region // Journal of Science and Education. - V.1, Issue 65. – P. 42–49.

DOI: 10.52578/2305-9397-2021-1-4-42-49.

in foreign publications:

1. Bulashev AK, Ingirbay BK, Mukantayev KN, Syzdykova AS (2021) Evaluation of chimeric proteins for serological diagnosis of brucellosis in cattle, *Veterinary World*, 14(8): 2187-2196.

DOI: www.doi.org/10.14202/vetworld.2021.2187-2196;

2. Bulashev A.K., Kuibagarov M.A., Akanova Zh.Zh., Zhagipar F.S. Immunoassay of food for antibiotics // Integration of Education, Science and Business in Modern Environment: Summer Debates: abstracts of the 3rd International Scientific and Practical Internet Conference, August 11-12, 2021. – Dnipro, Ukraine, 2021. – P.48-50;

3. Tuleuov A.M., Kadralieva B.T., Nurgaliev B.E., Usenov Zh.T. The level of fish infection with opisthorchiasis and anisakidosis on the ural river in the West Kazakhstan region. Integration of Education, Science and Business in Modern Environment: Summer Debates: abstracts of the 3rd International Scientific and Practical Internet Conference, August 11-12, 2021. – Dnipro, Ukraine, 2021. – P.188-190.

4. Tuleuov A.M., Kadralieva B.T., Nurgaliev B.E., Usenov Zh.T. The study of healthy and infected fish // Proceedings of the International Scientific Internet Conference "Trends and Prospects for the Development of Science and Education in the Context of Globalization". - Pereyaslav, 2021. - Issue. 74. - P.160-164.

List of publications published as part of the project in 2022:

1 Bulashev A.K., Kuibagarov M.A., Akanova Zh.Zh., Zhagipar F.S., Dzhangulova A.N. Immunoassay of antibiotics in animal products // Bulletin of Science, S. Seifullin Kazakh Agrotechnical University. - 2022. – Vol. 1 (112). - P. 289-300 (in Russian, CQASHE)

2 Bulashev A.K., Ingirbay B.K., Mukantayev K.N., Syzdykova A.S. Evaluation of chimeric proteins for serological diagnosis of brucellosis in cattle // *Veterinary World* – 2021. – Vol.14(8). – P.2187-2196 (Quartile Q2, Web of Science), percentile 79 (Scopus)

3 Mukantayev K.N., Borovikov S.N., Syzdykova A.S., Zhakhina A.A. The use of recombinant antigens of *Campilobacter jejuni* to obtain specific polyclonal antibodies // Bulletin of Science, S. Seifullin Kazakh Agro Technical University. - 2022. – Vol.2 (113). - P. 146-155. (in Russian, CQASHE)

4 Aktayeva S., Kiribayeva A., Makasheva D., Astrakhanov M., Tursunbekova A., Baltin K., Khassenov B. Isolation, identification and usage of *Bacillus* strains in microbial inhibition test in milk // *Eurasian Journal of Applied Biotechnology*. – 2022. – № 4. – P.49–57. DOI: 10.11134/btp.4.2022.6 (CQASHE)

5 Maikanov B.S., Adilbekov Zh.Sh., Lider L.A., Aubakirova G.A., Auteleeva L.T. Monitoring of fish safety in water bodies of Akmola region //

Bulletin of Science, S. Seifullin Kazakh Agrotechnical University.- 2022. – Vol.1 (112). - P. 311-323 (in Russian, CQASHE)

6 Adilbekov Zh.Sh., Lider L.A., Bainiyazov A.A., Mussagieva D.K. Veterinary and sanitary examination of commercial fish in reservoirs of the Karaganda region //J. Science and Education.- 2022. – Vol. 2–1 (67). – P. 96-104 (in Russian, CQASHE)

7 Nurgaliev B.E., Kadralieva B.T., Usenov Zh.T., Zhumabaev A.K., Tuleuov A.M. Results of the parasitological study of fish of «Bol'shikh» and «Maly'kh» rivers in West Kazakhstan region // J. Science and Education.-2022- Vol.3-1 (68) - P. 3-12 (in Russian, CQASHE)

8 Mendybayeva A.M., Modestas Ruzauskas, Aleshina Yu.E., Alieva G.K., Mukanov G.B., Ryshchanova R.M.. Assessment of the risk of emergence of resistance to antibiotics of opportunistic and pathogenic microflora isolated from animal products // Bulletin of the Krasnoyarsk State Agrarian University.–2022.– №2–P.147–156.–URL: <https://doi.org/10.36718/1819-4036-2022-2-147-156> (in Russian, Russian Science Citation Index)

9 Mendybayeva A.M., Ryshchanova R.M. Antibiotic resistance of *Salmonella spp.* strains isolated from animals and birds in the territory of Northern Kazakhstan // Bulletin of Science, S. Seifullin Kazakh Agrotechnical University.– 2022.–Vol.1(112).–P.324-334.–URL: [https://doi.org/10.51452/kazatu.2022.1\(112\).937](https://doi.org/10.51452/kazatu.2022.1(112).937) (in Russian, CQASHE)

10 Kuzeubaeva A.S., Usenbaev A.E., Ryshchanova R.M., Akanova Zh.Zh. Antibiotic resistance of *Escherichia coli* isolates contaminating cheese // Bulletin of Science, S.Seifullin Kazakh Agrotechnical University.–2022.–Vol.2(113).– P.123-132.–URL: [https://doi.org/10.51452/kazatu.2022.2\(113\).1028](https://doi.org/10.51452/kazatu.2022.2(113).1028) (in Kazakh, CQASHE).

11 Chuzhebaeva G., Alieva G., Baimenov B. and Malikzada K. Basic biological properties and resistance to antibiotics of *Staphylococcus aureus* and *Streptococcus agalactiae* isolates isolated from milk of cows of the Kostanay region of Kazakhstan // J. Education and Science.–2022.–Vol.1(66).–P.3–11. DOI: <https://doi.org/10.52578/2305-9397-2022-1-1-3-12>: [in Russian, CQASHE].

12 Chuzhebaeva G.D., Baimenov B.M., Alieva G.K., Mukanov T.M. Evaluation of primers and fluorescently labeled probes for the identification of *Staphylococcus aureus* and *Streptococcus agalactiae* and their antibacterial drug resistance genes // J. Education and Science. – 2022.- Vol.3-1 (68).-P.105-114. DOI 10.56339/2305-9397-2022-3-1-105-114. [in Russian, CQASHE].

13 Amanzholova M.Zh., Shayzadinova A.M., Abeldenov S.K. Expression and purification of recombinant DNA endonuclease CRISPR/Cas system // Bulletin of Karaganda University, Series “Biology. Medicine. Geography”. - 2022. – Vol. 4 (107). – P. 1-7. (in Russian, CQASHE).