ABSTRACT
of the dissertation by Samat Isabekov
titled «Development and application of bacteriophages for the rehabilitation of livestock facilities»,
submitted for the degree of Doctor of Philosophy (PhD)
in the specialty 6D120200 – Veterinary Sanitation.

Relevance of the Research Topic

In his address to the people of Kazakhstan, President Kassym-Jomart Tokayev emphasized: *"Agriculture is our main resource, yet it remains underutilized. We possess organic and environmentally friendly products with significant production potential, which are in demand both domestically and internationally."* A crucial national economic priority is ensuring the population's access to food products of high sanitary quality and providing the industry with safe animal-derived raw materials.

The successful achievement of this goal depends on increasing livestock numbers and enhancing their productivity. However, the development of animal husbandry and poultry farming is constrained by infectious diseases, with bacterial infections playing a leading role-particularly *Escherichia coli*, which belongs to the highest resistance group.

The low efficiency of anti-epizootic measures, especially disinfection practices in Kazakhstan, is primarily due to the lack of modern domestically produced disinfectant agents based on bacteriophages. The development of advanced bacteriophage-based disinfectants is of paramount importance for the sanitation of veterinary-controlled facilities and enterprises involved in meat and dairy processing.

In this context, bacteriophages-parasitic viruses of bacteria capable of selectively targeting and destroying pathogenic microorganisms-present particular scientific and practical interest.

Bacteriophages exhibit high compatibility with other pharmaceutical agents and can be used in combination with antimicrobial compounds, including surfactants. The application of modern antimicrobial agents plays a crucial role in the eradication of infectious diseases, the implementation of preventive measures, and the sanitation of contaminated environments. However, currently used veterinary disinfectants do not achieve 100% antimicrobial efficacy. This limitation necessitates the improvement of existing disinfectant formulations and the development of an advanced, next-generation biological disinfectant.

Depending on the above factors, the preparation and application of a disinfectant based on bacteriophages and surfactants is an effective solution for the sanitary treatment of livestock facilities.

2. Research Aim

The aim of this dissertation is to develop and apply modern bacteriophage- and surfactant-based disinfectants for the sanitation of livestock facilities.

3. Research Objectives

To achieve this aim, the following objectives have been set:

1. Isolation of bacteriophages specific to E. coli and brucellosis bacteria from environmental samples.

2. Determination of antibiotic resistance and toxicity level of bacteriophages

3. Determination of lytic activity of isolated bacteriophages in relation to the growth of microorganisms.

4. Preparation of disinfectants based on bacteriophages and surfactants.

5. Determination of bactericidal properties and modes of application of bacteriophage biopreparations in laboratory conditions.

6. Results of industrial tests of the disinfectant "Polyphage" developed on the basis of bacteriophages.

4. Research Methods:

The study was conducted at the Department of Veterinary Sanitation of the Kazakh National Agrarian Research University, while the experimental part of the research was carried out in the Microbiology Laboratory of the Research Institute for Biological Safety Problems. The research was performed in compliance with the regulatory documents officially established by the veterinary legislation of the Republic of Kazakhstan. Microbiological samples were collected following standard methodologies, and all stages of the dissertation work were conducted with the direct involvement of the author.

Environmental samples were obtained from various livestock farms in the Almaty and Zhambyl regions. The study included 129 samples, which comprised wastewater collected near dairy farms, pig farms, and poultry farms, as well as animal manure extracts. From the collected wastewater, 30 mL samples were taken and transferred into flasks containing 120 mL of liquid nutrient medium (GRM). For manure extract, 100 g of the sample was thoroughly mixed in sterile porcelain dishes and transferred into flasks containing 150 mL of liquid GRM medium.

The materials were incubated at 37°C for 5–10 days, with 0.2–0.5 mL of a 24-hour culture of the target bacteria added daily. After the incubation period, the samples were purified from mechanical impurities through filtration, then transferred into centrifuge tubes and centrifuged at 2500 rpm for 20 minutes. The filtrates were subsequently passed through sterilizing CN filters (0.45 μm and 0.2 μm).

The obtained filtrates were examined for the presence of bacteriophages on GRM agar. For this purpose, 24-hour test cultures were used as the inoculum for Petri dishes, and the filtrates were applied on top. The formation of phage lysis zones on the agar surface indicated the presence of bacteriophages.

The key biological properties of the isolated bacteriophages were examined. Lytic activity was determined using the Appelman method, the number of phage particles was assessed using the Gratia method, and the morphology of negative colonies was studied through electron microscopy. Additionally, the study evaluated the lytic spectrum of the bacteriophages, their thermal stability at both high and low temperatures, and their resistance to chloroform at a 1:10 ratio for 40 minutes.

The safety of the developed bacteriophage-based preparation was tested on laboratory animals. Three mice were injected subcutaneously with 1 mL of the preparation, and their condition was monitored for three to four days. All animals remained alive, healthy, and active, confirming the absence of toxicity.

To determine the effectiveness of the biopreparation, microbiological assessments were conducted before and after treatment. The methodology included the following steps:

1) Pre-disinfection sampling: Sterile swabs were used to collect samples from various surfaces (floors, walls, hooks, disinfection mats). In food production facilities, samples were collected from equipment and tools (knives, axes, shovels, scrapers, brushes). The collected samples were inoculated on nutrient media and incubated at optimal temperatures.

2) Biopreparation application: All test objects were treated with the bacteriophage-based disinfectant “Polifag” according to the manufacturer's instructions, ensuring adequate exposure time (e.g., 1 hour for food processing equipment). This comprehensive approach allowed for an in-depth evaluation of the antimicrobial efficacy of the developed disinfectant, supporting its potential practical application in livestock facility sanitation and food production hygiene.

3) A post-disinfection sampling procedure was conducted, where repeat swabs were taken from the same surfaces. The collected samples were then inoculated onto nutrient media and incubated.

4) The results were assessed after 24-48 hours by monitoring microbial growth. The developed biopreparation, "Polifag," will undergo commission-led evaluations for its implementation in veterinary practice, focusing on key parameters such as sterility, lytic activity, and safety.

5. The main findings to be defended in the dissertation include.

- bacteriophages active against (*Echerichia coli 14, Proteus vulgaris 4.3, Proteus mirabilis 4.2, Yersinia pseudotuberculosis 11.1, Yersinia enterocolitica 11.2, Salmonella enteretidis 14.1, Sallmonella typhimurium 14.2, Sallmonella infantis 14.3, Pseudomonas aeruginosa 11.3, Shigella sonnei 16.1, Shigella flexneri 16.2*), as well as the coccoid bacterium (*Brucella abortus 21*) and enterococcal bacterium (*Enterococcus faecalis 4.1*), were isolated;
- the lytic activity of the isolated bacteriophages was determined;
- an innovative bacteriophage-based disinfectant was developed;
- an optimal wet disinfection regimen using the bacteriophage-based preparation was established;
- industrial trials of the innovative bacteriophage-based disinfectant were conducted, and official reports on these trials were prepared.

6. Description of the main research results

From environmental and wastewater samples collected near industrial facilities, 13 bacteriophages with high lytic activity were isolated.

The study of the biological characteristics of the isolated bacteriophages confirmed their ability to selectively destroy viable target bacteria, leading to bacterial lysis. Furthermore, it was established that all isolated phages exhibited high specificity toward the test strains. Bacteriophage virions were identified using a JEM-100 electron microscope (Japan), which revealed distinct morphological features characteristic of all bacteriophages.

Industrial trials conducted at the *Kordai Invest* slaughterhouse demonstrated

the high effectiveness of the biopreparation. After disinfecting various surfaces (floors, walls, carcass hooks, disinfection mats, etc.), no microbial growth was detected on nutrient media for five days. In contrast, in the control group, where no disinfection was performed, active growth of foreign microflora was observed within two days. Trials of "Polifag" on food industry objects, including equipment and tools (knives, axes, shovels, scrapers, brushes), showed that a complete bactericidal effect was achieved within one hour after treatment.

The results of this study suggest that "Polifag" can be recommended for incubator egg treatment and the disinfection of eggs before distribution to the retail market. The conducted research confirmed that the biopreparation "Polifag" exhibits high bactericidal activity (100%) and can be effectively used as a disinfectant in various veterinary and sanitary control facilities, livestock and poultry farms, and for disinfecting equipment used in the slaughter of animals originating from farms affected by *brucellosis, pseudotuberculosis, salmonellosis, colibacillosis*, and diarrheal diseases in young animals (*Proteus, Klebsiella, Enterococcus, Yersinia*).

7. Justification of Novelty and Importance of the Obtained Data

In veterinary practice, a disinfectant based on new bacteriophages has been developed for disinfection of veterinary and sanitary supervision facilities, livestock and poultry farming, food production, technological equipment, as well as unfavorable territories: slaughterhouses and enterprises processing raw materials (meat, eggs, etc.).

The application of the Polifag biopreparation effectively halts bacterial infections, contributing to the production of food products with high sanitary quality standards.

8. Alignment with Scientific Development Directions or Government Programs

The research was conducted as part of the project for the commercialization of scientific and scientific-technical research results under the JSC "Science Fund" on behalf of the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan, project No. 230-16-GK, titled "Commercialization of New Polyphage Biopreparations for the Sanitation of Medical Facilities, Food Production Enterprises, and Residential Premises."

9. Description of the Doctoral Candidate’s Contribution to Each Publication

The doctoral candidate actively participated in discussions at various conferences and contributed significantly to the publication of research findings.

A total of 11 scientific papers have been published based on the dissertation work, including: 1 article in *Veterinary World*, indexed in Scopus Q1 (79th percentile), 3 articles in national scientific journals recommended by the Committee for Quality Assurance in Science and Higher Education of the Ministry of Science and Higher Education of the Republic of Kazakhstan, 5 articles in the proceedings of domestic and international conferences, congresses, and symposia Additionally, 2 patents have been obtained.

In the proceedings of the Fourth Scientific and Practical Conference "Bacteriophages: Theoretical and Practical Aspects of Application in Medicine,

Veterinary Medicine, and the Food Industry", organized by the G. N. Gabrichevsky Research Institute of Epidemiology and Microbiology and held in Nizhny Novgorod (Russian Federation) on September 24-26, 2018, the article was published.

In 2019, in Ulyanovsk (Russian Federation), at the National Scientific and Practical Conference "Agricultural Science and Education at the Present Stage: Experience, Problems, and Ways to Solve Them," organized by the P. A. Stolypin Ulyanovsk State Agrarian University, 1 article was published.

In 2021, in Kostanay, in the proceedings of the International Scientific and Practical Conference "Current Problems and Trends in the Development of Modern Agricultural Science and Veterinary Medicine," organized by A. Baitursynov Kostanay Regional University and dedicated to the memory of Doctor of Veterinary Sciences, Professor Valentin Ivanovich Piontkovsky, 1 article was published.

In 2021, in the scientific journal "Biosafety and Biotechnology," organized by the Research Institute for Biological Safety Problems, 1 article was published.

In 2022, in the scientific journal "Research, Results" at the Kazakh National Agrarian Research University in Almaty, 1 article was published.

In the scientific publication "Science and Education", a scientific-practical journal of the Zhangir Khan West Kazakhstan Agrarian-Technical University, recommended by the Committee for Quality Assurance in Science and Higher Education of the Ministry of Science and Higher Education of the Republic of Kazakhstan, three articles were published: in 2020 - 2 articles, in 2024 - 1 article.

10. Scope and structure of the thesis

The dissertation was typed on a computer and consists of 112 pages. Its structure includes an introduction, definition of the research direction, original research, research results, conclusions, practical recommendations, references, and appendices.

The dissertation is formatted with 14 tables and 26 illustrations. The reference list comprises 215 works by both domestic and foreign scholars.