

ANNOTATION

**dissertation work of Issabekov Samat
on a theme "Development and application of bacteriophages for the
rehabilitation of livestock facilities" presented on competition of a degree of
the doctor of philosophy (PhD) on a specialty 6D120200- "Veterinary
sanitation "**

1. Relevance of the research topic.

President of the Republic of Kazakhstan from K. Tokayev's address to the people of Kazakhstan: "Agriculture is our main resource, but it is not fully utilized. We have organic and environmentally friendly products with significant production potential, which are not only in demand both in the country and abroad". This means that Kazakhstani people should consume quality products. An important national-economic task is to provide the population with food of high sanitary quality, and industry - with animal raw materials.

Successful fulfillment of this task depends on increasing the number of livestock and improving its productivity. However, the factors constraining the development of livestock and poultry farming are infectious diseases, among which the leading place in the spread of bacterial infections in the Republic of Kazakhstan is occupied by bacterial infections, in particular *Escherichia coli*, which belongs to the first group in terms of resistance.

Low efficiency of anti-epizootic measures, in particular disinfection, carried out in Kazakhstan, is due to the lack of domestic production of modern disinfectant antimicrobial preparations based on bacteriophages in our country, for example, when solving these problems. Preparation of modern disinfectants based on bacteriophages is of great importance in sterilization of objects of veterinary control and meat and dairy products, processing enterprises.

The emergence of this situation in our country is due to structural changes in bacterial foci that have occurred in recent years, as well as the fact that the infection has moved into the environment of privately owned animals. Therefore, the study of bacteriophage, a parasitic virus of bacteria capable of destroying the corresponding pathogens in the organism, has aroused great interest. Particles of bacteriophages are often found in newly isolated growths of *E. coli*, but the practical significance of sterilization of this disease remains unexplored.

Bacteriophage particles are often found in newly isolated *Escherichia coli* overgrowths, but the practical significance of sterilizing this disease remains unexplored. Bacteriophages are highly compatible with other drugs and allow their use in combination with antimicrobial agents such as other surfactants. The use of modern drugs plays an important role in the elimination of infectious diseases, preventive measures of foreign nature, and disinfection. However, disinfectants currently used in veterinary practice do not have 100% antimicrobial action. This situation requires improvement of existing disinfectants on the market and creation of fundamentally new types of biocides.

In connection with the above conditions, there is a need to conduct scientific research to obtain biopreparations based on bacteriophages and surfactants for sterilization of livestock and poultry objects and sanitary treatment of food production. In the course of realization of research work it allows to present innovative products and new technology of their development to the market of Kazakhstan. Preparation and use of sterilizing agent containing bacteriophages and surfactants is an actual problem of sterilization of livestock facilities.

2. Purpose of the thesis research: The purpose of the thesis is to prepare and apply modern disinfectants based on bacteriophages and surfactants for sterilization of livestock facilities.

3. Research Objectives:

1. Among the external environment, the first group of microorganisms are Escherichia coli, highly sensitive microorganisms, drugs and disinfectants. On the other hand, bacteriophage against brucellosis bacteria.

2. Determination of solubilizing (lytic) activity against Bolingen bacteriophage microorganisms.

3. Development of a means (substance) for disinfection of isolated bacteriophages.

4. Determination of bactericidal properties and mode of application of a disinfectant prepared in the laboratory.

5. Production tests of sterilizing biopreparation "Polyphage" developed on the basis of bacteriophages.

4. Methods of research:

The dissertation is completed out at the department of «Veterinary sanitation» of the Kazakh National Agrarian Research University, and the experimental part of the research - in the microbiological laboratory of SII "Research Institute of biological safety problems" of the Ministry of Health of the Republic of Kazakhstan. Research work was conducted using normative documents officially regulated by the veterinary legislation of the Republic of Kazakhstan. Microbiological samples were collected by standardized methods.

Production tests were conducted in farms of Almaty and Zhambyl regions.

Bacteriophages isolated from environmental samples served as objects of research, 129 samples were studied in total. The corresponding strains were used as indicator test cultures for bacteriophages.

Samples of environmental objects brought from different farms of Almaty and Zhambyl regions: dairy products, water streams from the vicinity of pig and poultry farms, meat from the market, etc. A sample of the investigated wastewater (120 ml) was taken and poured into flasks with 30 ml (5-fold concentration) of HRM broth, which is more suitable for the development of bacteriophages. After selecting 100 g of solid material under study and mixing them thoroughly in sterile porcelain dishes, they were transferred into flasks containing 150 ml each of nutrient medium containing HRM broth medium.

Flasks with materials were placed in incubation thermostat at 37°C for 5-10 days. 0.2-0.5 ml of one-day growths of specific bacteria were added daily to the incubation material.

The flasks were stirred daily to improve aeration of the incubation mixture.

Methods of studying biological properties of bacteriophages. Titration of bacteriophages in liquid culture medium according to Appelman's method is based on the introduction of different amounts of titrated bacteriophage into nutrient broth inoculated with an equal dose of bacterial growth sensitive to a specific phage. For this purpose, 10 tubes containing 4.5 ml each of GRM or EPS (meat-peptone broth) were taken. 0.5 ml of the phage to be tested was added to the first tube and mixed thoroughly. 0.5 ml from the first tube was transferred into the second tube and so on, continued until a series of 10-fold dilutions of phage (10⁻¹-10⁻¹⁰) was obtained. To each tube of the prepared series was added daily 0.03 ml of 4-hour bacterial culture containing 10⁹ microbial cells per 1 ml sensitive to each phage. For the control, 2 tubes were taken with GRM or EPS, 0.5 ml of the phage under study (sterility control of the phagolysate filtrate) was added to one of them, and 0.03 ml of bacterial growth was added to the other. All tubes were placed in the thermostat for 18 hours at the optimal temperature for microbial growth. The result was determined by the absence of bacterial growth in the presence of bacteriophage.

5. The main points brought to the defense of the thesis:

- isolation of bacteriophages against *Escherichia coli*, hospital strain and brucellosis bacteria;
- the destructive (lytic) activity of the isolated bacteriophages was determined;
- innovative preparations based on bacteriophage were created;
- an optimal mode of wet disinfection with modern bacteriophage-based preparations was developed;
- innovative preparations based on bacteriophage were tested in production, and protocols of technological tests were drawn up.

6. Description of the main results of the research

13 different bacteriophages with high bactericidal properties were isolated from bacteriological samples taken from various surfaces and wastewater from industrial facilities in the country (1. *Escherichia coli*, 2. *Proteus vulgaris*, 3. *Proteus mirabilis*, 4. *Yersinia pseudotuberculosis*, 5. *Yersinia enterocolitica*, 6. *Salmonella enteritidis*, 7. *Salmonella typhimurium*, 8. *Salmonella infantis*, 9. *Enterococcus infantis*, 10. *Shigella sonnei*, 11. *Shigella flexneri*, 12. *Brucella abortus*, 13. *Pseudomonas aeruginosa*).

Work on the identification of virions in accordance with the types of bacteriophages taken for research was carried out using an electron microscope JEM-100 (Japan), as a result of which characteristic virions of all bacteriophages were identified, which were clearly visible. As a result of determining in laboratory conditions the bactericidal properties of a disinfectant developed on the basis of bacteriophages and surfactants, 0.1% solutions of the biological product "Polyphage" after a 5-minute exposure to *E. coli*, strains of *Staphylococcus aureus* and brucellosis range from 10.8 to 23.6% of bacteria, after 30 minutes - from 78 to 92 to, and after 60 minutes of exposure - up to 100% showed that they destroy viability.

As a result of the research carried out at the slaughterhouse of Kordai Invest LLP, where the disinfectant biological product "Polifag" was used, bacteriological samples from the territories grown on a nutrient medium, when observed for five days, no growth of shoots was observed. In the samples from the control group, after two days, the growth of foreign microorganisms was detected. As a result of the study, it was proven that the prepared biological product "Polyphage" destroys microorganisms 100% when used as a disinfectant.

7. Rationale for the novelty and importance of the findings.

Veterinary practice includes objects of veterinary and sanitary supervision, veterinary and livestock, poultry, fish, food production and transportation, technological equipment, as well as unfavorable points, danger zones, slaughterhouses, food production associated with the processing of meat, milk, eggs, raw materials..., from microflora causing brucellosis, pseudotuberculosis, diphtheria, salmonellosis, colibacillosis and diarrheal diseases of young animals from sick animals (Proteus, Klebsiella, pseudomonosis, enterococci, yersinia and other infections belonging to the first group of resistance) and other foci resistant to the first group, a disinfectant based on developed new bacteriophages is presented. The formulation includes various bacteriophages against bacteria:

Brucella abortus, Enterococcus faecalis, Proteus mirabilis, Proteus vulgaris, Yersinia pseudotuberculosis, Yersinia enterocolitica, Pseudomonas aeruginosa, Echerichia Coli, Salmonella enteritidis, Salmonella typhimurium, Salmonella infantis, Shigella sonne, Shigella flexneri.

8. Correspondence to the directions of science development or state programs:

Scientific and research work of the Committee of Science of the Ministry of Higher Education and Science of the Republic of Kazakhstan in the course of commercialization of the results of scientific and scientific-technical activity of JSC "Fund of Science" № 230-16-GK "New Polyphages biopreparations for sanitary treatment of medical premises, food production and residential premises" "Commercialization" was carried out under the budget program.

9. Description of doctoral student's contribution to the preparation of each publication.

The main results of scientific and experimental research were presented at international scientific conferences, symposia and congresses.

11 scientific papers on the materials of dissertation works, including 1 article published in the journal "Veterinary World", included in Scopus Q1, percentile 79), 2 patents for utility model, 3 articles - in the republican scientific journals recommended by the Committee for control of education and science of the Ministry of Higher Education and Science of the Republic of Kazakhstan, 5 articles - in the materials of international conferences.

IV - International Conference "Bacteriophages: theoretical and practical aspects of use in medicine, veterinary medicine and food industry" held in Ulyanovsk, Russian Federation in 2017, September 24-26, 2018 in Nizhny Novgorod, Russian Federation. G.N. At the Fourth Scientific and Practical Conference "Bacteriophages: theoretical and practical aspects of use in medicine,

veterinary medicine and food industry" organized by the Gabrichevsky Research Institute of Epidemiology and Microbiology. Gabrichevsky;

In 2018 at the II All-Russian international scientific-practical conference held in Vologda - Molochnoye, the article "Research of disinfectant activity of preparations based on surfactants"; In 2019 in Ulyanovsk, Russian Federation, P.A. At the republican scientific-practical conference organized by the Ulyanovsk State Agrarian University named after Stolypin on the theme "Agrarian science and education in the modern period: experience, problems and ways of their solution", the article "Disinfection mode in approbation studies of preparations was presented

June 18, 2021 in Kostanai A. The article "The use of bacteriophages as a disinfectant" was published in the collection of the international scientific-practical conference "Actual problems and trends in the development of modern agrarian science and veterinary science", dedicated to the memory of Dr. veterinary sciences, Professor Valentin Ivanovich Piontkovsky organized by Kostanay Regional University named after Baitursynov.

10. Scope and structure of the thesis.

The dissertation is typed on the computer on 93 pages and its structure consists of an introduction, defining the direction of the study, independent research, research results, conclusion, practical recommendations, list of used literature and appendices. The work is decorated with 12 tables and 16 figures. The list of literature includes works of 225 domestic and foreign scientists.