ABSTRACT

of the dissertation by Sarmykova Makhpal Kenzheevna titled "Development of a technology of biologics based on a bacteriophage and its application in the therapy of strangles horses" submitted for the degree of Doctor of Philosophy (PhD) under the educational program 8D09101 - "Veterinary Medicine"

Relevance of the research topic

Strangles in horses is an acute, highly contagious bacterial infection caused by *Streptococcus equi*, characterized by inflammation of the upper respiratory tract, formation of purulent exudate in the nasopharyngeal region, and suppuration of regional lymph nodes. An epizootiological feature of the disease is that it occurs both as isolated sporadic cases and in the form of persistent enzootic foci and large-scale epizootics. In the Republic of Kazakhstan, strangles is one of the most relevant infectious diseases in horse breeding.

Strangles most frequently occurs in young horses under five years of age, which is associated with the incomplete functional maturation of their immune system. Clinically, the disease manifests as elevated body temperature, mucopurulent discharge from the nasopharynx, and the formation of abscesses in the lymph nodes of the head and neck.

The spread of infection occurs through direct contact with infectious exudate, as well as indirectly via contaminated water, feed, equipment, and husbandry items. An important role as the main reservoir of the pathogen is played not only by clinically diseased animals, but also by convalescents and subclinical carriers, which remain externally healthy but continue to shed *Streptococcus equi*.

Transmission of *Streptococcus equi* occurs via aerosol, alimentary, and contact routes, and its relative resistance to environmental factors contributes to the prolonged persistence of the pathogen in premises and on farm territories, creating conditions for recurrent outbreaks of infection.

Strangles causes significant biological, economic, and epizootiological damage to horse breeding. The disease leads to increased morbidity within herds, delayed growth and development of foals, increased mortality, and a reduction in the breeding value of livestock. In addition, expenditures on therapeutic, preventive, and quarantine measures impose substantial economic losses on farms.

At present, the widespread circulation of *Streptococcus equi* strains, the development of antibiotic resistance, and the tightening of international and national regulations aimed at restricting the use of antimicrobial agents in veterinary practice increase the relevance of developing alternative therapeutic approaches to the treatment of strangles.

In this context, biological preparations based on bacteriophages are of particular scientific and practical interest. Bacteriophages, as natural viruses possessing high specificity toward bacteria, are considered a modern and safe means of biological control of *Streptococcus equi*.

Phage therapy allows for the selective elimination of pathogenic microorganisms while preserving the normal microbiota and is regarded as an alternative to antibiotic therapy or as a complementary method.

In Kazakhstan, systematic and comprehensive studies on strangles in horses, particularly those focused on the development of therapeutic bacteriophage-based biopreparations specific to *Streptococcus equi*, remain insufficient. Therefore, the development of a biological preparation based on a specific bacteriophage against *Streptococcus equi* and its application in the treatment of strangles in horses represent a relevant scientific and practical task.

The results of the conducted patent search revealed the absence, within the territory of the Republic of Kazakhstan, of registered patent solutions related to bacteriophage-based biopreparations specific to *Streptococcus equi* intended for the treatment of strangles in horses. This further emphasizes the scientific novelty and practical significance of this research direction.

Aim of the study: The aim of this study was to isolate a lytic bacteriophage against *Streptococcus equi*, characterize its phenotypic and genotypic properties, develop a bacteriophage-based biopreparation, and evaluate its therapeutic application for the treatment of strangles in horses.

Research objectives:

1. To collect biological samples from foals affected by strangles for the isolation of lytic bacteriophages specific to *Streptococcus equi*.
2. To isolate lytic bacteriophages active against pathogenic *S. equi* strains.
3. To investigate the biological and molecular-genetic properties of the isolated bacteriophages (virion morphology, lytic activity and host range, genomic features, resistance to temperature and chloroform, etc.).
4. To select an effective bacteriophage and develop a biopreparation based on it, including optimization of production technology.
5. To conduct in vivo evaluation of the phage biopreparation for the treatment of strangles and to prepare regulatory and technical documentation.

Research Methods

The study employed bacteriological, virological, molecular-genetic, and biological methods. A bacteriophage specific to *Streptococcus equi* was isolated from environmental sources, and its lytic activity was assessed in vitro.

Therapeutic efficacy and safety of the developed phage biopreparation were evaluated in vivo using clinical, bacteriological, and comparative analytical approaches.

Main provisions to be defended:

- Biological, morphological, and molecular-genetic characteristics of the bacteriophage active against *Streptococcus equi*;
- Technology for the production of the bacteriophage-based biopreparation Streptophagum equi;
- Results of in vitro and in vivo studies of the biopreparation;
- Evaluation of the therapeutic efficacy of Streptophagum equi in the treatment of equine strangles.

Summary of main results

The study resulted in the development of a scientifically substantiated laboratory prototype of a bacteriophage-based biopreparation, Streptophagum equi, specifically targeting *Streptococcus equi*.

Several natural bacteriophage isolates active against *S. equi* were obtained from clinical material collected in epizootic foci. Among them, one isolate demonstrating high and stable lytic activity was selected as the production strain.

The selected bacteriophage was comprehensively characterized in terms of morphology, biology, and molecular genetics. It exhibited strong lytic activity, a broad host range, and biological safety. Genomic analysis confirmed the absence of virulence and antibiotic resistance genes, supporting its suitability for therapeutic use.

A production technology for the biopreparation was developed, and key quality parameters-including sterility, lytic activity, and pH-were shown to meet regulatory standards. Stability studies demonstrated preservation of biological activity under defined storage conditions.

In vivo trials confirmed the clinical efficacy of Streptophagum equi in both experimental and field conditions. Regulatory and technical documentation was developed, and the product was patented and registered in Kazakhstan as a veterinary medicinal product (Registration Certificate No. RK–VP–4–5745–2).

Justification of the originality and practical significance of the obtained results

During the course of this study, a bacteriophage exhibiting high specificity and stable lytic activity against the pathogen *Streptococcus equi* was isolated for the first time in the Republic of Kazakhstan, and its comprehensive characterization was carried out in terms of biological, morphological, and molecular-genetic properties. Determination of the complete nucleotide sequence of the isolated phage genome and subsequent bioinformatic analysis expanded current scientific understanding of the structural and functional organization of lytic bacteriophages of streptococcal origin and established a theoretical basis for the development of phage-based biopreparations targeting *S. equi*.

The developed bacteriophage-based biopreparation “Streptophagum equi”, intended for the treatment of strangles in horses, provides an effective and rapid means of restoring the health status of farms affected by this disease. The proposed phage therapy approach can be considered as an alternative to antibiotic therapy, contributing to the reduction of the risk of antimicrobial resistance dissemination. The results of this scientific work create the prerequisites for the implementation of phage therapy in veterinary practice in the Republic of Kazakhstan, the expansion of scientific knowledge and technological expertise in this field, and the improvement of resilience and productivity in the horse breeding industry.

Compliance with the priorities of scientific development and state programs

The dissertation research was carried out in accordance with the priority directions of development of veterinary science, microbiology, biotechnology, and biological safety of the Republic of Kazakhstan. The study corresponds to state objectives aimed at ensuring epizootic welfare, developing domestic biological products for veterinary use, and reducing the use of antimicrobial agents through the implementation of alternative, environmentally safe, and highly specific methods for the treatment of infectious animal diseases.

The research was conducted within the framework of the grant project of the Ministry of Education and Science of the Republic of Kazakhstan No. AR08855635,

“Obtaining a bacteriophage for the treatment of equine strangles” (2020–2022). The study was carried out at the Research Institute for Biological Safety Problems.

The results obtained during the implementation of the aforementioned project served as the scientific and experimental basis for the dissertation research devoted to the isolation of a lytic bacteriophage specific to *Streptococcus equi*, the study of its phenotypic and genotypic properties, the development of a biological preparation technology based on it, and the evaluation of its effectiveness in the treatment of strangles in horses.

Practical significance of the study

The practical significance of the work lies in the development of the bacteriophage-based biopreparation “Streptophagum equi” for the treatment of strangles in horses, the substantiation of the technological parameters of its production, and the confirmation of its therapeutic efficacy under in vivo conditions. The developed preparation can be applied in veterinary practice as an alternative or an adjunct to antibiotic therapy, ensuring a reduction in antibiotic load and expanding the possibilities for treating animals affected by strangles. The obtained results are important for improving therapeutic and preventive measures in farms unfavorable for strangles in horses.

Contribution of the doctoral candidate to publications

The doctoral candidate actively participated in discussions at various scientific conferences and made a significant contribution to the dissemination of research findings.

Based on the dissertation materials, 17 scientific publications have been published, including 2 articles in peer-reviewed scientific journals indexed in the Scopus international database, namely: Veterinaria Italiana (2023, Q2, percentile – 50) and International Journal of Veterinary Science (2024, Q1, percentile – 76).

In addition, the dissertation results have been published as follows: 1 article in journals recommended by the Committee for Quality Assurance in Science and Higher Education of the Republic of Kazakhstan, 4 articles in peer-reviewed journals recommended by the Higher Attestation Commission of the Russian Federation, 5 articles in Russian and national scientific journals, and 4 articles in the proceedings of international scientific and practical conferences.

The practical significance of the research results is confirmed by one patent for an invention obtained based on the dissertation materials.

Volume and Structure of the Dissertation

The dissertation consists of an introduction, main chapters, conclusion, and appendices. The main text comprises 109 pages, while the total volume including appendices is 156 pages. The dissertation includes 17 tables and 30 figures. The list of references contains 160 sources by domestic and international authors.