

ANNOTATION

Zhanerke Muratovna Ussenova for the dissertation work “Development of manufacturing technology of an inactivated vaccine against Adenitis equorum for intranasal use” submitted by Zhanerke Muratovna Ussenova for the degree of Doctor of Philosophy (PhD) under the educational program 8D09101 – Veterinary Medicine

Relevance of the Study. Horse breeding in the Republic of Kazakhstan is one of the important sectors of the agro-industrial complex and plays a significant role in providing the population with high-quality food products. In recent years, due to the increasing horse population and the expansion of the import and export of breeding animals, the prevention of infectious diseases has become increasingly important. Among these diseases, strangles, caused by *Streptococcus equi*, is one of the widespread infectious diseases of horses that causes significant economic losses to the horse-breeding industry of the Republic.

The high contagiousness of the disease, its potential for subclinical spread, and its severe course in complicated cases complicate the epizootological situation and necessitate the improvement of effective preventive measures. Currently, the efficacy of available vaccines is assessed at varying levels, while most of them do not fully account for the biological characteristics of pathogen strains circulating in the region.

Therefore, the aim of this study was to develop a safe and effective inactivated vaccine against strangles in horses based on the study of *Streptococcus equi* strains circulating in the Republic of Kazakhstan and to evaluate its immunobiological properties.

The results of the study will contribute to the development of domestic production of veterinary biological products, improvement of the effectiveness of strangles prevention, development of import-substituting veterinary preparations, and stabilization of the epizootic situation in the Republic.

Despite the availability and use of several vaccines against strangles in horses in foreign countries, their efficacy and safety do not always demonstrate consistent results. Moreover, existing vaccines do not fully account for the specific characteristics of regional strains of the causative agent, which may reduce their epizootiological effectiveness. Therefore, conducting research aimed at developing a safe and highly effective vaccine based on a local strain of the pathogen and adapted for use under farm conditions represents a relevant and important area of scientific investigation.

Aim of the study: To develop a highly effective inactivated vaccine against strangles in horses and establish a production technology adapted for use under production conditions.

To achieve this aim, the following research objectives were established:

1. To conduct monitoring studies to determine the prevalence of strangles in horses in the Republic of Kazakhstan.

2. To isolate the causative agent from horses affected by strangles and study its biological and genetic properties.

3. To develop a technology for producing an inactivated vaccine against strangles and evaluate its biological efficacy and safety.

4. To develop and approve regulatory and technical documentation for the vaccine.

5. To conduct production trials of the vaccine and evaluate its efficacy.

Research methods: The study employed epizootological, bacteriological, serological, molecular-genetic, and biotechnological methods. The morphological, cultural, biochemical, and genetic properties of *Streptococcus equi* isolates were studied. The safety, immunogenicity, stability, and prophylactic efficacy of the vaccine were evaluated under laboratory and production conditions.

Main Provisions Submitted for Defense

- The epizootiological characteristics of strangles in the Republic of Kazakhstan were determined;

- The biological and genetic properties of the production strain *Streptococcus equi* “Kargaly-22” were investigated;

- An inactivated vaccine against equine strangles based on a local strain was developed;

- A technological production scheme for the vaccine was developed, and its quality indicators were established;

- The immunogenic and protective efficacy of the vaccine was demonstrated;

Regulatory and technical documentation for the use of the vaccine in veterinary practice was developed.

Description of the Main Research Results

Monitoring studies conducted in various regions of Kazakhstan determined the epidemiological characteristics of strangles in horses. *Streptococcus equi* strains were isolated from horses affected by strangles, and their biological, cultural, biochemical, genetic, and immunobiological properties were studied. Based on the results obtained, the promising production strain “Kargaly-22” was selected for vaccine development.

During the study, an inactivated vaccine against strangles in horses was developed based on the *Streptococcus equi* “Kargaly-22” strain. A technological scheme for vaccine production was developed, and the stages of microorganism cultivation, inactivation, concentration, and formulation with an adjuvant were optimized.

The efficacy of the inactivated vaccine developed based on the *Streptococcus equi* “Kargaly-22” strain was evaluated in horses under production conditions. Following vaccination, a gradual increase in the specific immune response was observed, with the antibody level reaching 80% on day 30. The morbidity rate in the vaccinated experimental group was approximately 5%, whereas in the unvaccinated control group, this indicator reached approximately 45%.

Throughout the observation period, the body temperature of the horses in the experimental group remained within the physiological range of 37.7–38.0 °C. In the control group, an increase in body temperature to 39.1–39.5 °C was observed on days 2–3.

During the production trials, no allergic reactions, signs of systemic intoxication, severe complications, or pronounced local inflammatory reactions were recorded in the vaccinated horses. The results obtained confirm the safety of the developed inactivated vaccine under production conditions and demonstrate its high prophylactic efficacy against strangles in horses.

Justification of the Novelty and Significance of the Obtained Results

For the first time in the Republic of Kazakhstan, an inactivated vaccine against strangles in horses was developed based on the local production strain *Streptococcus equi* “Kargaly-22”. A technological scheme for vaccine production was developed, and its quality parameters, sterility, safety, immunogenicity, and stability were determined. The prophylactic efficacy of the developed vaccine was demonstrated under laboratory and production conditions.

Production trials of the vaccine fully confirmed its practical efficacy. A reduction in the morbidity rate was observed among vaccinated horses. In addition, the severity of clinical signs characteristic of strangles was lower in vaccinated animals. The general physiological condition of the vaccinated horses remained stable, and no decrease in productivity parameters was observed. These results demonstrate the suitability of the developed preparation for use under production conditions.

During the production trials, particular attention was paid to assessing the safety of the vaccine. No allergic reactions, signs of systemic intoxication, or severe complications were recorded in the vaccinated horses. Furthermore, no local inflammatory reactions were observed in the vaccinated animals. These findings confirm the safety of the developed vaccine for use in veterinary practice.

As a result of the studies conducted, regulatory and technical documentation for the vaccine was developed. An organizational standard, as well as instructions for the manufacture, quality control, and use of the vaccine, were prepared.

The documentation provides a detailed description of the quality parameters of the preparation, safety requirements, storage conditions, and administration regimens. This documentation provides the basis for the production of the developed vaccine under production conditions and its application in veterinary practice.

Relationship of the Research to Scientific Program. The study was carried out within the framework of the Ministry of Agriculture of the Republic of Kazakhstan Program BR10764975, “Program-Targeted Financing of Scientific Research and Activities for 2021–2023”.

Description of the Doctoral Student’s Contribution to the Publications.

The main concept, scientific direction, aim, and objectives of the research presented in the dissertation were defined with the direct participation of the author. The author conducted a comprehensive analysis of the scientific literature, systematized

relevant national and international scientific sources, and summarized current scientific data on the epizootiological characteristics of strangles in horses, the biological properties of the causative agent, *Streptococcus equi*, as well as contemporary approaches to the development of prophylactic biological products against this infection.

The author directly carried out the isolation and identification of *Streptococcus equi* strains and investigated their cultural, morphological, biochemical, and biological properties. In addition, the author actively participated in the assessment of the virulence properties of the pathogen, preparation of culture media, performance of bacteriological studies, and analysis of the obtained results.

Within the framework of the dissertation research, a technological scheme for the production of an inactivated vaccine against strangles in horses based on the *Streptococcus equi* “Kargaly-22” production strain was developed, and its main stages were optimized with the direct participation of the author. The author carried out the preparation of the vaccine, inactivation and concentration of the bacterial biomass, incorporation of the adjuvant, and determination of the quality parameters of the final vaccine product.

Laboratory studies were conducted to assess the sterility, safety, immunogenicity, and stability of the developed vaccine, and the obtained results were subjected to statistical analysis. The systematization of experimental data, preparation of tables and figures, formulation of scientific conclusions, and substantiation of the practical significance of the results were performed with the direct participation of the author.

The author directly participated in the preparation of scientific articles, conference proceedings, and patent documentation based on the materials of the dissertation research. The analysis and generalization of the research findings, formulation of conclusions, and preparation of the dissertation were carried out with the author’s direct personal involvement.

Based on the research findings, scientific papers were published and a utility model patent was obtained. A total of four scientific publications were published based on the dissertation research, including one article in a peer-reviewed scientific journal indexed in the international Scopus database.

In addition, two articles were published in 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, and one paper was published in the proceedings of an international scientific and practical conference.

Based on the materials of the dissertation research, a utility model patent entitled “Method for Producing a Vaccine Against Strangles in Horses for Intranasal Administration” was granted by the National Institute of Intellectual Property of the Republic of Kazakhstan (Kazpatent).

Structure and Scope of the Dissertation. The dissertation consists of an introduction, literature review, materials and methods, research results, discussion of

results, conclusions, practical recommendations, and appendices⁷. The dissertation comprises 103 pages and includes 16 tables, 21 figures, and 9 appendices. The reference list contains 177 sources.