ANNOTATION

to the dissertation of Abay Zhandos for the degree of Doctor of Philosophy (PhD) in the educational program "8D05110 - Virology" on the topic: "Development of technology for the production of a vector vaccine against tuberculosis in cattle"

General characteristics of the dissertation research. The primary aim of the dissertation is to develop a domestic vector vaccine for bovine tuberculosis using a recombinant avian influenza virus. This involves constructing a vector incorporating mycobacterial genes and evaluating the vaccine's safety, protective efficacy, and immunogenicity in both laboratory and target animals.

Relevance of the Dissertation. Bovine tuberculosis (bTB) is a chronic infectious disease of cattle caused by *Mycobacterium bovis (M. bovis).* The critical concern regarding bovine tuberculosis arises from its zoonotic potential, meaning it can be transmitted from animals to humans under certain conditions. Agriculture plays a pivotal role in Kazakhstan's economy, making the health and productivity of cattle a significant factor. Therefore, addressing bovine tuberculosis, with its economic, health, and social implications, is essential for the country's agricultural sector.

Despite the development of various vaccines, BCG remains the only commercially available option for both cattle and humans. However, there are increasing concerns about BCG due to post-vaccination reactions, variable immunogenicity, and difficulties in distinguishing immunized animals during diagnostic testing. A promising approach for more effective vaccines against infectious diseases is the development of attenuated recombinant vectors that deliver protective antigens to induce either prophylactic or therapeutic responses.

Advances in reverse genetics have enabled the creation of modified vector vaccines that are administered intranasally. The non-structural protein NS1 of the influenza virus serves as a promising target for genetic modification in influenza-based vectors due to its high expression in infected cells and its ability to induce

strong antibody and T-cell responses. The NS gene of the influenza virus is known for its capacity to incorporate foreign nucleotide sequences without compromising the stability of the NS1 protein.

Among mycobacterial antigens, the early secretory antigen ESAT-6 (early secretory antigen, 6 kDa) is of particular interest due to its immunodominant role. It is encoded by the RD1 genomic region, which is absent in the BCG strain after prolonged culture and is critical for early immune response in both humans and animals. The use of attenuated viral vectors to deliver and express mycobacterial antigens can enhance immunogenicity without the need for adjuvants. Viral vectors such as those based on attenuated influenza or adenoviruses are being explored for their potential to carry mycobacterial genes.

The strategic use of vaccines is vital for controlling infection. When developing candidate vaccines and vaccination strategies, it is necessary to account for the pathogenesis of *M. bovis* and compare human physiology to animal models used in preclinical trials.

Objective of the Study – To develop a technology for producing a novel vector vaccine against bovine tuberculosis using recombinant influenza viruses expressing mycobacterial proteins.

Research Objectives:

- Creation of recombinant influenza vectors expressing immunodominant *Mycobacterium bovis* proteins;
- Development of technology for the development of a vector vaccine against bovine tuberculosis;
- Conducting in-house commission tests of experimental industrial samples of bovine tuberculosis vaccine.

Objects of the Study:

- Recombinant avian influenza virus.
- Protective proteins of *Mycobacterium bovis*.
- Vector vaccine against bovine tuberculosis.

Research methods: The study utilized methods from molecular biology,

biotechnology, virology, and serology, and involved experiments conducted on animals.

Scientific novelty of the study: For the first time in the Republic of Kazakhstan, a new vector vaccine against bovine tuberculosis was developed and tested, leveraging the latest advancements in molecular biology.

Scientific and practical significance: The study holds significant value for veterinary medicine, focusing on enhancing vaccine immunogenicity, developing new vaccine formulations, and creating anti-tuberculosis vaccines through modern molecular biology and immunology approaches. This research led to the creation of a new generation vaccine for the prevention of bovine tuberculosis.

Main provisions submitted for defense:

1. Vector vaccine against bovine tuberculosis, which uses the avian influenza virus as a carrier, is an effective method for preventing the disease.

2. The technology developed for obtaining a vector vaccine against bovine tuberculosis is optimal. According to the developed technology, the vaccine complies with the organization's standard NS 405-1919-04 DP-146-2023 for quality.

3. Vector vaccine against bovine tuberculosis based on the avian influenza virus is safe for laboratory animals, has protective properties and provides immunity, which is confirmed by the results of in-hospital tests.

Main research results and conclusions:

1. Recombinant strains of the influenza virus expressing *M. bovis* Esat-6 and TB10.4 proteins were constructed using reverse genetics. The strains included genes from the *A/PR/8/34(H1N1)* strain (PB2, PB1, PA, NP, and chimeric NS1) and the *A/Astana/6/05* strain (surface antigen genes HA, NA, and M).

2. Optimal conditions for cultivating recombinant strains in chicken embryos were established: viral dose of 1,000–10,000 EID_{50}, 10-day-old embryos, 37°C temperature, and 48-hour incubation. The infective activity of the recombinant strains reached 8.87 ± 0.22 lg EID_{50}/ml.

3. Preliminary assessments of safety, immunogenicity, and protection were conducted on laboratory animals to identify promising vaccine candidates. The

selected formulations were: Influenza vector encoding ESAT-6 and TB10.4 + Montanide Gel adjuvant (1.0 ml, subcutaneous route). Influenza vector encoding ESAT-6 and TB10.4 (1.0 ml, subcutaneous route). An optimal vaccination method for calves was developed, involving two 2 ml/kg doses (6.25 EID_{50}) administered intradermally with a 21-day interval, resulting in stable immunity for 12 months.

4. A technology for the production of the influenza vector-based vaccine was established. Stabilization media were selected, and a sterile pilot series of the vaccine was produced under quality control. Regulatory documentation was created, including: “Production and control instructions for the vector vaccine”, “Standard ST 405-1919-04 GP-146-2023” and “Usage instructions for the Vector vaccine against bovine tuberculosis”.

5. Internal commission testing was conducted as per Order No. 468-O (08.12.2022) by the Director General of the Research Institute of Tuberculosis and Biotechnology, Ministry of Health of the Republic of Kazakhstan. The tests confirmed the vaccine's safety, immunogenicity, and compliance with ST 405-1919-04 GP-146-2023 standards.

6. The dissertation research resulted in nine scientific publications.

Connection with the plan of main scientific works: The dissertation research was conducted as part of the grant-funded project AP09259683, titled "Development of a technology for the production of a vector vaccine for the prevention of tuberculosis in cattle," for the period 2021–2023.

Publications: The principal findings of the dissertation are reflected in nine published scientific works, which include:

– One article in a domestic periodical recommended by the Committee for Quality Assurance in Education of the Ministry of Education of the Republic of Kazakhstan.

– One article in the journal Vaccines, indexed in the Web of Science (Q1) and Scopus (82% percentile).

– One article in the Journal of Genetic Engineering and Biotechnology, indexed in the Web of Science (Q2) and Scopus (44% percentile).

– Additionally, the research led to obtaining patents for two utility models.

Volume and structure of the dissertation: The dissertation comprises 118 pages and includes 97 references, 40 figures, and 30 tables. It is organized into the following sections: notations and abbreviations, introduction, literature review, research materials and methods, research results and their analysis, conclusion, and bibliography.