ABSTRACT
of the dissertation of Kylmanbekova Zhuldyz Kairatovna
on the topic "Optimization of laboratory test systems for the diagnosis of sheep bluetongue /", submitted for the degree of Doctor of Philosophy (PhD) in the specialty 6D120200 - Veterinary Medicine

1. General characteristics of the work

Bluetongue (BT) is a seasonal disease currently registered in many settlements. Considering the epizootic situation, as well as trade and economic ties with other countries and geographical location, the territory of Kazakhstan can be classified as a zone of potential risk. In this regard, there is a possibility of introduction and spread of bluetongue in the Republic of Kazakhstan. The development and improvement of means and methods for laboratory diagnosis of bluetongue is a relevant and urgent task. One of the most important means of preventing infectious diseases is the timely use of test systems as an effective element of a preventive system.

2. Relevance of the research topic. Bluetongue (BT) is a transmissible viral disease transmitted by blood-sucking insects (Culicoides obsoletus, C. pulicaris, C. nubeculosus, C. impunctatus). The disease is characterized by inflammation of the mucous membranes of the oral cavity, especially the tongue, excessive salivation, necrotic lesions, degenerative changes in the hooves and skeletal muscles, and a febrile condition. According to the zoosanitary rules of the International Office of Epizootics (OIE), bluetongue is included in the list of diseases common to several animal species.

In natural conditions, all breeds of sheep are susceptible to the bluetongue virus. According to the classification of the International Office of Epizootics, this disease belongs to the category of especially dangerous infections.

Currently, the disease is registered in a number of countries in the Middle East and Asia, and is also widespread in European countries.

The first foci of this disease were detected in the Republic of Tajikistan in the late 1990s, which led to significant economic losses in animal husbandry. The disease diagnosis was established by employees of the Research Institute of Biological Safety Problems within the framework of contractual research.

In addition, a source of introduction of causative agents of previously unregistered particularly dangerous diseases can be imported animals, their carcasses, feed, semen, as well as products of animal origin.

The risk of introducing bluetongue into the territory of the Republic of Kazakhstan is assessed as high. The main risk factor is animals imported from abroad. In particular, a probable source of disease introduction into Kazakhstan could be breeding animals imported from certain regions of the Russian Federation, as well as from some states of the USA and Canada.

In connection with the increasing number of foci of exotic and especially dangerous diseases in the world in recent years, the development of new reliable means and methods for timely and accurate diagnosis of these infections, based on the study of samples from animals and humans, has become particularly relevant.

Currently, the problem of dangerous infectious diseases of humans and animals remains relevant for many countries of the world, including the Republic of Kazakhstan. Ensuring stable epizootic well-being against dangerous and particularly dangerous viral diseases in Kazakhstan is an important task for improving the socio-economic situation in the country.

In Kazakhstan, a technology for developing a diagnostic test system based on enzyme immunoassay (ELISA) for the detection of bluetongue in agricultural animals has not yet been developed. In this regard, the creation of a domestic ELISA diagnostic test system based on a local strain of the bluetongue virus is an important task for the veterinary science of the Republic of Kazakhstan.

Keywords: bluetongue, virus, test system, strain, serotype, diagnosis, antigen, immunoglobulin.

3. Goal and objectives of the research

The ELISA method, developed for the diagnosis of bluetongue, is widely used in many countries. However, for the first time in the Republic of Kazakhstan, the task is to prove that the developed new-generation ELISA method for detecting and indicating the bluetongue virus antigen, created on the basis of a recently isolated relevant strain, by improving it, is not inferior to foreign analogues in its characteristics.

Objectives of the work:

To achieve the goal, the following scientific research was planned:

1. Isolation of the BT virus strain from the focus of infection and study of its biological properties.
2. Optimization of BT virus cultivation conditions in cell cultures.
3. Development of effective systems for obtaining specific BT virus antigen, as well as obtaining active serum against this virus.
4. Study of methods for isolating immunoglobulin from antiviral serum.
5. Selection of effective methods for obtaining conjugate based on virus-specific immunoglobulin.
6. Optimization of ELISA conditions for detecting BT virus antigen.
7. Testing of the developed ELISA method for detecting BT virus antigen.

4. Object and subject of research

The object and subject of research was the development of effective means for detecting the antigen of the Bluetongue virus (BT).

In addition, a study of the biological properties of the BT virus strain "RT/RIBSP-07/16" was conducted. Based on this strain, specific antigen, serum, immunoglobulin, and conjugate against this virus were prepared. The final stage of the work was the development and optimization of effective conditions for performing enzyme immunoassay (ELISA).

5. Link with research work and state programs

The dissertation topic was carried out within the framework of the state program "Development and application of gene engineering and cellular technologies in medicine, agriculture, environmental protection, food and processing industry" based on the research work of the project "Development of highly effective means of prevention and diagnosis of bluetongue". (project:

"Development of highly effective means of prevention and diagnosis of bluetongue" within the program: "Development and application of gene engineering and cellular technologies in medicine, agriculture, environmental protection, food and processing industry").

Scientific and technical program BR249927852 "Organization and implementation of complex research to ensure sustainable development of the agro-industrial complex of the Kostanay region with the creation of a research and technological center" 2024-2026.

6. Methodology and research methods

The research work for the doctoral dissertation was carried out in the "Diagnostics of Infectious Diseases" laboratory of the Research Institute of Biological Safety Problems, part of the QazBioPharm JSC holding of the Ministry of Health of the Republic of Kazakhstan. The study used 5 goats and 5 sheep, as well as various reagents and research methods. During the work, virological, immunological, and serological methods were used.

Planned scientific research during the work:

- Isolation of the BT virus strain from biological samples delivered from the Republic of Tajikistan;
- Revitalization (updating) of the BT virus strain "RT/RIBSP-07/16";
- Isolation of BT virus from processed chick embryo;
- Isolation of the BT virus causative agent in cell cultures;
- Study of the biological properties of the BT virus strain "RT/RIBSP-07/16";
- Obtaining BT virus antigen;
- Obtaining specific antiserum against BT virus;
- Selection of methods for obtaining immunoglobulins;
- Selection of methods for preparing conjugates;
- Development of an ELISA method for detecting BT virus antigen;
- Investigation of the specificity and sensitivity of the developed ELISA method;
- Application of enzyme immunoassay in carrying out epizootological monitoring of BT.

7. Scientific novelty

For the first time, a strain of the BT virus from small ruminants was isolated from epizootic foci in the Republic of Tajikistan. The new strain **"RT/RIBSP-07/16"**, isolated from sick animals, was isolated on treated chicken embryos and in cell cultures.

The biological properties of the strain **"RT/RIBSP-07/16"** were studied. Based on this strain, diagnostic preparations (specific antigen, specific antiserum, specific immunoglobulin and conjugate) were developed.

Based on these diagnostic preparations, optimal conditions for performing enzyme immunoassay (ELISA) for the detection of BT virus antigen in clinical samples in the Republic of Kazakhstan were selected.

The specificity and sensitivity of the developed ELISA test system were studied. In addition, the developed ELISA test system was used in carrying out epizootological monitoring of BT in the Republic of Kazakhstan.

8. Practical significance

In laboratory conditions, a set of preparations for the diagnosis of BT and the detection of the causative agent's antigen by enzyme immunoassay (ELISA) was developed. The developed ELISA kit can be used in farms for making a full diagnosis of BT.

For prompt detection of the BT virus antigen, an enzyme immunoassay method was developed. For the implementation and practical application of this ELISA method in veterinary practice, the following normative and technical documents were proposed:

- Set of preparations for laboratory diagnosis of 16 serotypes of infectious bluetongue and detection of the causative agent's antigen by enzyme immunoassay, ST MEK 3893436-018-2009.
- Provisional instructions for the manufacture and control of preparations intended for laboratory diagnosis of 16 serotypes of infectious bluetongue and detection of the causative agent's antigen by enzyme immunoassay.
- Provisional instructions for the use of a set of preparations for laboratory diagnosis of 16 serotypes of infectious bluetongue and detection of the causative agent's antigen by enzyme immunoassay.

Acts of implementation in production: No. 1 dated 12.08.2025 – KH "Adil"; No. 2 dated 08.10.2025 – KH "Ilyas".

9. Main provisions submitted for defense

The following provisions are submitted for defense:

The BT virus strain was isolated from an epizootic focus, and its biological and pathogenic properties were studied.

The main factors influencing the effectiveness of BT virus cultivation in cell cultures (cell type, infectious dose, incubation regime) have been scientifically substantiated, and optimal technological parameters have been proposed.

An improved method for obtaining and purifying BT virus antigen with high diagnostic significance has been developed.

A technology for obtaining and purifying highly specific immunoglobulins against the BT virus has been proposed.

Based on immunoglobulins specific to the BT virus, an enzyme conjugate with high stability has been obtained for the first time, and its diagnostic properties have been evaluated.

A new modification of enzyme immunoassay (ELISA) for the detection of BT virus antigen has been developed, and its analytical and diagnostic characteristics have been experimentally proven.

10. Main results of research in the form of conclusions

1. From biological samples delivered from the Republic of Tajikistan, the BT virus strain "RT/RIBSP-07/16" was isolated.
2. Effective conditions for the cultivation of the BT virus strain "RT/RIBSP-07/16" in processed chick embryos and cell cultures were optimized.
3. The biological properties of the BT virus strain "RT/RIBSP-07/16" were studied.

4. For the detection of BT virus antigen by ELISA, specific antigen, specific antiserum, specific immunoglobulin, and conjugates were prepared.

5. Optimal conditions for detecting BT virus antigen using the ELISA test system have been developed, and its specificity and sensitivity have been studied.

6. The developed ELISA test system for detecting BT virus antigen showed high efficiency during monitoring in the southern regions of the Republic of Kazakhstan.

11. Reliability and validity of the obtained results

The authenticity and reliability of the obtained scientific results are ensured by the use of modern scientific approaches and methods widely used in the field of veterinary virology, serological diagnostics, and biotechnology. The research work was planned in accordance with generally accepted scientific principles and methodological requirements, and experimental studies were conducted systematically and consistently.

All laboratory studies were performed in the infectious disease diagnostics laboratory of the Research Institute of Biological Safety Problems. During the work, methodological protocols for conducting virological and immunological analyses were used.

Experimental studies were conducted using a sufficient volume of biological material, and to increase the accuracy of the results, experiments were repeated multiple times. The collected data were analyzed using modern statistical processing methods, which ensured the scientific validity and representativeness of the results. The conclusions obtained during the study were compared with scientific data published in the works of domestic and foreign scientists in the field of veterinary virology, which confirmed their mutual correspondence and scientific validity.

12. Information on publications on the main results.

Dissertation materials: 10 articles have been published on the dissertation topic, including 4 articles in publications recommended by the Committee for Education and Science Supervision of the Ministry of Education and Science of the Republic of Kazakhstan. Also, 1 article on general veterinary medicine with a percentile of 65, quartile - Q2, has been published in scientific publications included in the international information resources Web of Science (Clarivate Analytics) and Scopus (Elsevier): https://doi.org/10.47278/journal.ijvs/2022.198. 4 articles have been published in the materials of international conferences. There are 2 acts of implementation in production.

13. Approbation of research results

The main results were reported at 4 International Scientific and Practical Conferences:

- Серомониторниг животных на блутанг на территории Республики Таджикистан // VI Международная конференция молодых ученых: Биофизиков, Биотехнологов, Молекулярных Биологов и Вирусологов-2019 г.Сб. тез/АНО «Иннов.центр Кольцово». - Новосибирск: ИНЦ НГУ, 2019. - С.349-353.
- Получение и сравнительная оценка специфических сывороток к серотипам вируса блутанга // VI Международная конференция молодых

ученых: Биофизиков, Биотехнологов, Молекулярных Биологов и Вирусологов-2019 г. Сб. тез/АНО «Иннов.центр Кольцово». - Новосибирск: ИНЦ НГУ, 2019. - С.353-357.

- Блутанг вирусына тәнді антигенін дайындау // С.Сейфуллин атындағы Қазақ агротехникалық университетінің 125 жылдығына арналған «Сейфуллин оқулары-15: Жастар, ғылым, технологиялар: Жаңа идеялар мен перспективалар» атты халықаралық ғылыми-теориялық конференция, Нұр-Сұлтан, 2019, Б.-38-40
- Эпизоотическая ситуация по блютангу в мире и приграничных регионах страны Казахстана // Материалы международной научно-практической конференции «применение инноваций в области развития ветеринарной науки» Баку-2019 г.- Б.-98-102.

14. Personal contribution of the doctoral student

The doctoral dissertation is a completed research work carried out by the author independently and meets the requirements of the Committee for Education and Science Supervision of the Ministry of Education and Science of the Republic of Kazakhstan. The reliability of the research results and the validity of the practical work are confirmed by photomaterials, tables, scientific articles, and the normative-technical document (NTD) for the test system (kit) for diagnosing BT ELISA in agricultural animals.

15. Volume and structure of the dissertation

The dissertation is presented in printed form with 122 pages in computer format and consists of the following sections: introduction, literature review, research results, discussion of research results, conclusion, practical recommendations, list of sources, and appendices. In addition, the work contains 39 tables, 10 figures, and 140 references.

16. Main results of research

In the course of scientific work, the following results were obtained:

1. For the first time, the bluetongue virus strain "RT/RIBSP-07/16" of small ruminants, widespread in the territory of the Republic of Tajikistan, was isolated and fully identified by molecular-biological and virological methods. This strain has been included in the collection of microorganisms of the Research Institute of Biological Safety Problems and transferred for long-term storage.

2. The main biological properties, reproductive activity, and pathogenic characteristics of the isolated viral strain were comprehensively studied. As a result of studies conducted on experimental models, typical clinical signs of BT in sheep and goats were identified.

3. Optimal cultivation parameters (infection dose, incubation regime, and cultivation time) ensuring effective virus reproduction in intact chicken embryos and cell cultures were determined. These conditions allowed for the accumulation of viral material in high titers and its use for further laboratory research.

4. A scientifically based method for obtaining diagnostic preparations of the bluetongue virus (monospecific antigen, serum, immunoglobulin, conjugate) was developed. Based on these diagnostic preparations, ELISA parameters for detecting

the BT antigen were optimized, and their diagnostic specificity, sensitivity, and efficacy were investigated.

5. High diagnostic specificity, sensitivity, and efficacy of the developed ELISA test system were confirmed during monitoring in the southern regions of the Republic of Kazakhstan.