

## ANNOTATION

of the dissertation work of Bazarbayev Ryskeldi on the topic "Characteristics of virus strains used for the manufacture of polyvalent inactivated vaccine against infectious poultry bronchitis and Newcastle disease" submitted for the degree of Doctor of Philosophy (PhD) in the specialty 6D120100 - "Veterinary Medicine"

### 1. Relevance of the research topic

Poultry farming is the most intensively developing branch of animal husbandry not only in Kazakhstan but also throughout the world. It is capable of providing the population with inexpensive and dietary protein food products. This is due to the high growth rate of young agricultural bird species, which is accompanied by high feed conversion. This is what makes poultry farming the most profitable branch of agricultural animal husbandry.

Such features of breeding meat poultry contribute to the increase in the number of poultry enterprises in the country and the growth of their livestock. However, there are factors that reduce the profitability of poultry farming in Kazakhstan. The restraining factors include high prices for imported feed additives for growing chickens, high cost of grains - as the main feed for birds, as well as other feed and technological problems. All these factors can only partially reduce the level of profit, while infectious diseases accompanied by high mortality can completely destroy poultry farming as an industry in the country.

Thus, the greatest threat to poultry farming is of a veterinary nature and requires constant monitoring of the spread of highly contagious infectious diseases and their timely prevention.

One of the primary aspects of biosecurity in industrial poultry farming is continuous monitoring studies aimed at identifying pathogens of viral diseases among poultry by determining the titers of specific antibodies in the blood using serological methods.

An analysis of literary sources shows that viral bird diseases such as Newcastle disease, infectious bronchitis and avian influenza are widespread in Kazakhstan. The most likely routes of introduction and spread of infection among the agricultural poultry population are the active import of breeding material from abroad, which is necessary for the stable development of the industry, as well as the influence of wild birds, which serve as natural reservoirs of viral infection.

High variability of viruses, constant emergence of new variants and genotypes require development of fast and at the same time sensitive methods of diagnostics and differentiation of viral virions. This will allow effective control of genetic and phenotypic variability of pathogens of infectious diseases of birds in the local population. All listed factors emphasize the relevance of the chosen topic and its practical significance for development of poultry industry in Kazakhstan.

**2. The purpose of the dissertation research:** Monitoring the spread of Newcastle disease and infectious bronchitis of chickens in Kazakhstan, isolating pathogens using virological and serological methods, determining their antigenic properties in order to select effective strains for the development of a polyvalent inactivated vaccine, and differentiating these diseases using molecular genetic methods.

### 3. Research objectives:

To achieve the goals, the following tasks were set:

- Study of the epizootic situation regarding the incidence of Newcastle disease and infectious bronchitis in poultry farms in the North Kazakhstan, East Kazakhstan, Akmola, Zhambyl and Almaty regions of our country;
- Determination of the characteristics of clinical manifestations of Newcastle disease and infectious bronchitis in chickens in poultry farms in Kazakhstan;
- Isolation and identification of field strains of Newcastle disease and infectious bronchitis of chickens obtained from foci of infection in various regions of the Republic of Kazakhstan;



- Determination of immunogenic properties of newly isolated strains for the purpose of preparing a polyvalent inactivated vaccine;
- Simultaneous diagnostics of Newcastle disease and infectious bronchitis in chickens using a molecular genetic method.

#### **4. Research methods:**

The main research work was carried out at the Department of «Biological Safety» of the Kazakh National Agrarian University, in the laboratory of the «Expert Group of Animals» of the UNIVET Scientific and Production Center LLC.

Research and development work was carried out between 2018 and 2024.

Monitoring studies in 2019–2021 were conducted at poultry farms in the North Kazakhstan and Almaty regions. Serological screening of antibody titers to infectious bronchitis and Newcastle disease viruses was conducted at farms in Northern Kazakhstan and among poultry in private farms in the Almaty region. The choice of farms was due to the different intensity of veterinary and preventive measures. Clinical and pathological studies of infectious bronchitis were conducted in the outbreak area in Northern Kazakhstan, and the study of the Newcastle disease virus was conducted in farms in the Almaty region.

In monitoring studies of the spread of viral diseases in productive poultry, the main method was serological analysis of specific antibody titers in immunized and non-vaccinated chickens. For this purpose, blood samples from 10 to 25 birds of different ages were randomly selected in a number of farms. Groups for collecting blood samples were formed taking into account the technological map for growing replacement young stock or the productivity phase of adult birds. Blood was collected from the axillary vein in sterile AVATUBE vacuum tubes (without additives) in a volume of 1-2 ml and left at room temperature to form a clot and separate the serum, further storage and transportation of samples was carried out at a temperature of +4 °C.

The antibody titer was determined using the enzyme-linked immunosorbent assay (ELISA) using commercial diagnostic kits "Avian Infectious Bronchitis Virus Antibody Test Kit" and "Newcastle disease Virus Antibody Test Kit" from the Dutch manufacturer BioChek. In this case, a complete set of equipment for ELISA laboratories from the same manufacturer was used to conduct the analysis, which included a washer and incubator for microplates, as well as an enzyme-linked immunosorbent assay reader. The studies were carried out in accordance with the manufacturer's recommendations for diagnostic kits. The optical density of the solution was determined on an ELISA ELX 800 reader (BioChek, Winooski, VT, USA). The results were recorded and analyzed using BioChek Monitoring Software.

Pathological material from sick birds was taken during the period of manifestation of clinical signs of the disease. Selection and preparation of pathological material was carried out according to established methods. Sections of lungs and trachea were taken from chickens, and kidneys and ovaries from adult birds. Samples were stored frozen at -20°C until the end of research work.

For virus isolation, 9-12-day old chicken embryos grown by JSC Alel-Agro were used. The methods for culturing Newcastle disease and infectious bronchitis viruses in chickens, as well as the hemagglutination reaction (HAR), hemagglutination inhibition reaction (HIR), and neutralization reaction (NR) were carried out according to the approved instructions.

During the dissertation work, molecular genetic research methods were applied using information on the nucleotide sequence of strains of chicken infectious bronchitis and Newcastle disease viruses from the GenBank database, as well as by testing methods on isolated strains of these viruses in the population of agricultural birds in the North Kazakhstan and Almaty regions.

Specific primers were developed for the joint identification of the viral genomes of infectious bronchitis and Newcastle disease in the reaction mixture. In the development of primer systems for the detection of Newcastle disease virus, F gene sequences were used, while for the infectious bronchitis virus of chickens, S1 gene sequences were used. In the development of specific oligonucleotides, a local database of nucleotide sequences of the pathogen genes obtained



from GenBank was created. Analysis of their conservatism was carried out using the BioEdit program.

#### **5. The main provisions submitted for the defense of the dissertation:**

- Results of monitoring studies on the spread of respiratory diseases of birds in production enterprises of the North Kazakhstan and Almaty regions;
- Dynamics of antibody formation after vaccination against Newcastle disease and infectious bronchitis in chickens, as well as the features of the clinical and pathological manifestations of these diseases in birds;
- Diagnosis by serological testing and isolation of the causative agent of Newcastle disease and infectious bronchitis of chickens at poultry production facilities;
- Characterization of antigenic properties of local candidate strains for the preparation of a polyvalent inactivated vaccine against infectious bronchitis of chickens and Newcastle disease;
- Molecular genetic characteristics of Newcastle disease and infectious bronchitis viruses circulating in Kazakhstan and their differentiation by reverse transcription polymerase chain reaction (RT-PCR).

#### **6. Description of the main results of the study.**

As a result of monitoring the epizootic situation in poultry farms in the northern, eastern and southeastern regions of the Republic of Kazakhstan, a stable increase in titers of specific antibodies to Newcastle disease and infectious bronchitis viruses of chickens was established, exceeding the expected post-vaccination indicators. The identified trend indicates the circulation in the poultry population of field strains of viruses that have the ability to cause epizootic outbreaks.

Complex diagnostic and laboratory studies have confirmed the incidence of infectious bronchitis and Newcastle disease among broiler and egg-laying hens, despite preventive measures. It has been established that these infectious processes are caused by the impact of the chicken infectious bronchitis virus and low-pathogenic strains of the Newcastle disease virus. The results of the study indicate the development of a complex viral infection.

Conducted virological studies made it possible to isolate the ЯК-Сев-ИБК2024 strain of infectious bronchitis virus of chickens. According to the results of PCR diagnostics and genome sequencing, it was established that the isolated strain has a genetic relationship with such known variants of infectious bronchitis virus as Israel 02, Israel 1494 and the QX variant. Serotype analysis confirmed a high degree of genomic proximity of the isolated strain to the specified foreign analogues.

The isolated strain ЯК-Сев-ИБК2024 is characterized by a wide range of antigenic properties, promoting the production of antibodies of various types and possessing high immunogenicity. This is confirmed by the results of experimental infection, during which high titers of specific antibodies were recorded in chickens on the 21st day. The obtained data indicate the need to use the ЯК-Сев-ИБК2024 strain in the development of prophylactic biopreparations for vaccination of poultry in the northern regions of Kazakhstan, since this variant of the virus is widespread and poses a current threat to poultry farms in this region.

In addition to the infectious bronchitis virus, high titers of antibodies against the Newcastle virus were found in the blood of the controlled birds, which exceeded their post-vaccination level and indicated the circulation of "wild" type viruses in poultry farms. In order to determine the strains of the Newcastle disease pathogen, a number of monitoring, diagnostic, virological and other studies were carried out to identify the most common strains of the Newcastle disease virus. As a result, the ПМБ-1 / chicken / Almaty 66/2020 strain was selected, which was most often detected in poultry and which had fairly high immune properties. This strain of the pathogen has a moderate impact on the economic damage to the poultry industry in Kazakhstan, which makes it a good candidate as a genetic basis for the preparation of a regional vaccine for Kazakhstan in further biotechnological research

The isolated strains ЯК-Сев-ИБК-2024 of the chicken infectious bronchitis virus



The results of the studies showed the possibility of using the indirect agglutination reaction to detect the titer of paramyxovirus antibodies not only in the blood serum of vaccinated and sick birds, but also in the contents of chicken eggs, while the level of antibodies was almost at the same level. In addition, when conducting these serological studies, the method for preparing antigen ED was improved to study the intensity of population immunity to the Newcastle disease virus in poultry using a chemical mediator. The highest sensitivity in determining antibody titers to the ПМВ-1 / chicken / Almaty / 2020 strain, at the level of 1:4096-1:8192, was obtained using reagents obtained by the menthol method and detecting antibodies that exceeded the indicators in the hemagglutination inhibition reaction by 4-8 times.

Primers have been developed for the simultaneous detection of the presence of pathogens causing Newcastle disease and infectious bronchitis in chickens using real-time reverse transcription polymerase chain reaction, which allows for faster diagnosis of the diseases.

#### **7. Justification of the novelty and importance of the obtained data**

As a result of monitoring studies conducted in the outbreak of the disease in productive poultry in the North Kazakhstan and Almaty regions, "wild" strains of Newcastle disease and infectious bronchitis viruses were isolated, which caused the outbreak of the disease.

Based on the results of serological studies in all poultry farm houses, it was established that the average titer of specific antibodies was in the expected range - from 12904 to 17097. At the same time, the variation coefficient did not exceed 5%, although according to the regulatory documentation preceding the use of the commercial kit, the expected range of variability should have been from 20% to 60%. Such low variability, as shown by the analysis of index vaccination, confirms the hypothesis about the effect on the immune system of the bird of an antigen stronger than the vaccine strains.

Immunological, virological and clinical studies conducted through artificial infection with isolated strains have shown that these strains have low virulence and at the same time high immunogenicity, which makes them promising candidates for the creation of vaccines.

The conducted studies have shown that the use of the indirect hemagglutination reaction, carried out using an improved method for preparing an antigen erythrocyte diagnosticum, gave good results in determining the level of specific antibodies not only in the blood serum of vaccinated and sick birds, but also in the composition of chicken eggs.

In the course of the conducted reverse transcription polymerase chain reaction (RT-PCR) as a result of electrophoresis in polyacrylamide gel, two amplification products of 100 and 230 nucleotides in length were obtained, which corresponds to the target amplicons of the genes of the vaccine strains of the chicken infectious bronchitis virus and the Newcastle disease virus. These results allow us to determine the type of the viral target gene in duplex RT-PCR. This indicates that the proposed primers can be used for differential diagnostics in case of simultaneous infection of birds with two respiratory viruses - the Newcastle disease virus and the chicken infectious bronchitis virus.

The constructed phylogenetic trees of the viruses of infectious bronchitis of chickens behind the spike gene S1 and the Newcastle disease virus behind the gene F will allow to accelerate the typing of the isolated nucleotide sequences and to relate them to the known strains of these viruses. It will also help to organize the intraspecific classification and avoid further confusion in the nomenclature of genetic groups in the future.

**8. Compliance with scientific development directions or state programs:** The conducted studies were an integral part of the research program "Characterization of virus strains used for the preparation of a polyvalent inactivated vaccine against avian infectious bronchitis and Newcastle disease" for 2019-2021, state registration № 0119PKH0320, carried out by the Department of Biological Safety of the Kazakh National Agrarian Research University, as well as part of the research programs of a number of diagnostic institutions (the laboratory "Animal Expert Group" of UNIVET-Scientific and Production Center LLP).

#### **9. Description of the 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.

6 scientific papers based on dissertation materials, including 1 article in the International Open Veterinary Journal included in the Scopus database; 5 scientific articles (2019-2025) in Kazakh periodicals and some materials of a scientific conference.

In the materials of the International scientific and practical conference "Modern challenges of biotechnology, veterinary science and medicine". - Gvardeisky, 2020.

Utility model patent No. 7256, 2022/0221.2. Asanov, N., Bazarbayev, R. K., Musoev, A. M., Asanova, S. E., Otarbayev, B. K., Khusainov, D. M., Islamov E. I., Tolymbekova A. B., Kurbanbayeva N. M., Kenzheev Sh. T., Makhashov E. Method for diagnosing Newcastle disease

Utility model patent No. 7257, 2022/0222.2. Asanov, N., Bazarbayev, R. K., Musoev, A. M., Asanova, S. E., Otarbayev, B. K., Khusainov, D. M., Islamov, E. I., Musina, G. Sh., Kurbanbaeva, N. M., Kenzheev, Sh. T., Makhashov, E., Akhaeva, D. N. Method for serological diagnosis of infectious bronchitis chickens

Author's certificate No. 8228, 14.02.2020. Nurkhodjaev, N. O., Bazarbaev R. K. Results of the study of birds for infectious bronchitis of chickens in certain regions of the Republic of Kazakhstan.

## **10. Volume and structure of the dissertation**

The dissertation work is presented on 123 pages of computer text, contains 22 tables and 24 figures. It includes the following sections: introduction, review of scientific literature, materials and research methods, experimental part, conclusions and a list of used literature, including 222 sources, including 207 foreign authors.