

## ANNOTATION

**of the dissertation by Adilbay Karagulov on the topic "The Role of Bats in the Spread of Particularly Dangerous Diseases in Humans and Animals" submitted for the degree of Doctor of Philosophy (Ph.D) in the educational program 8D09101 – "Veterinary Medicine"**

### **Relevance of the Research Topic**

Bats, belonging to the suborder Chiroptera, are the second most numerous mammals after rodents. They inhabit all countries and continents except for the Arctic and Antarctic. There are over 1400 species of these animals worldwide, many poorly studied. Each species has its own unique characteristics and regional differences. Bats are ideal reservoirs for viruses, and their unique ability to fly aids in the spread of these viruses.

More than 27 species of bats are registered in the Republic of Kazakhstan. Several additional species are known near the borders, likely entering Kazakhstan. These 30 species make up over 70 percent of the bat fauna of the former USSR. From 1930 to now, thousands of viruses from 28 different families have been discovered in bats. Additionally, bats are carriers of bacterial infections such as leptospirosis and pseudotuberculosis. Like birds, bats carry the fungus *Histoplasma capsulatum*, which causes a lung infection (histoplasmosis) in humans and animals. The transmission of viruses from bats to humans has caused acute diseases such as Hendra and Nipah paramyxoviruses, severe acute respiratory syndrome (SARS) coronavirus, Australian bat lyssavirus, and Marburg filoviruses.

It is also worth noting that bats are likely sources of hepatitis C, hepatitis B, and epidemic mumps, which are endemic in humans. Bats are reservoirs for infections of the Lyssavirus genus. Lyssavirus infections circulate among bats worldwide, and new species of lyssaviruses are regularly identified. The Lyssavirus genus includes seven genotypes and four unclassified viruses. Of the seven genotypes, the ecology of six is associated with bats. The most well-known lyssavirus is the rabies virus. Rabies is a zoonotic infectious disease that poses a significant threat to humans and many species of wild and domestic animals.

According to the World Health Organization (WHO), rabies ranks among the top five zoonotic infectious diseases causing significant social and economic damage. Combatting infectious diseases largely depends on controlling the risks associated with bats. Risk assessment is only possible with knowing the disease status and its presence among bats. Bats are a powerful potential reservoir and source of emerging infectious diseases, epidemiologically activated by ecological transformations in the "wild animals + domestic animals + humans" system. This requires the development of a classical understanding of the bioecology of this phenomenon, which is significant for scientific and practical veterinary epidemiology (cofactors, prevention, and control measures). Its "unrecognized threat" can unexpectedly turn into an emergency at any time.

## **Purpose of the Dissertation Research**

To conduct epizootological monitoring of bats inhabiting the territory of the Republic of Kazakhstan for carriage of particularly dangerous pathogens and to determine bats' role in the epidemiology of diseases in humans and animals.

## **Research Objectives**

1. To determine the prevalence of hazardous pathogens in bat populations in Kazakhstan.
  - 1.1. Organize and conduct expeditions to various regions of the Republic of Kazakhstan to collect biological samples from bats.
  - 1.2. Determine the prevalence of hazardous pathogens in bat populations in various regions of Kazakhstan.
  - 1.3. Isolate the causative agents of hazardous pathogens and study their biological properties.
2. To determine the genetic diversity of hazardous pathogens circulating in bat populations in Kazakhstan.
3. To develop an algorithm for monitoring hazardous infections in bat populations.

## **Research Methods**

The study uses epizootological and modern research methods: locating mass gatherings of bats using bat detectors, determining the species of bats, collecting biological samples from bats in various regions, preparing samples for research, isolating nucleic acids (DNA/RNA), conducting PCR studies, determining nucleotide sequences using the Sanger method, and analyzing oligonucleotide sequences using the Basic Local Alignment Search Tool (BLAST) from the NCBI database (<https://blast.ncbi.nlm.nih.gov>). For the genetic analysis of the genes of isolated pathogens, Mega version 11 software (ThermoFisher, USA) was used. For statistical processing of the obtained data, the commercial licensed software package GraphPad (version 8.0.0, California, USA) was used.

## **Scientific Novelty of the Dissertation**

The scientific novelty of this dissertation lies in the fact that, for the first time in the Republic of Kazakhstan, comprehensive epizootological monitoring of bat populations across various regions has been conducted to assess the carriage of hazardous pathogens affecting both animals and humans. The study determined the prevalence of these pathogens in bat populations and their genetic diversity, and it also performed zoning of the territory of Kazakhstan based on the degree of epizootological hazard.

## **Theoretical and Practical Significance of the Research**

The results obtained from this work allow for developing a strategy for epidemiological surveillance of diseases spread by bats. The epizootological and epidemiological characteristics identified during the study and the specifics of these hazardous infections under Kazakhstan's conditions will form the basis for measures to optimize the infection control system. The dissertation results can have a significant positive impact on the epidemiological and epizootological situation in the Republic of Kazakhstan.

The research results enabled the identification of priority pathogens for which bats serve as reservoirs and carriers. The knowledge gained contributes to

developing a strategy for epidemiological surveillance of diseases spread by bats. Based on the research findings, an algorithm for comprehensive epizootological and epidemiological monitoring of infectious diseases, for which bats are reservoirs, has been developed. This helps reduce morbidity and prevent the social and economic damage caused by these diseases.

Based on the dissertation materials, "Methodological Recommendations for Monitoring Infectious Disease Pathogens in Bat Populations" were developed. The collected research data will enable the development of measures to reduce the risk of infection in animals and humans by hazardous diseases carried by bats. The established gene sequences of Kazakhstani isolates of alpha coronaviruses and rabies virus circulating among bats provide an essential addition to the existing viral genome database, facilitating the study of genome variability in these viruses.

### **Theoretical Significance**

The theoretical significance lies in assessing the prevalence and diversity of hazardous pathogens in bat populations across various regions of the Republic of Kazakhstan. As a result of this work, 11 RdRp gene sequences (460 bp) of alpha coronaviruses from bats in 5 areas of Kazakhstan and the nucleoprotein gene sequence of the rabies virus from Western Kazakhstan were deposited in the NCBI GenBank database.

### **Main Provisions of the Dissertation**

- Prevalence of hazardous pathogens in bat populations across various regions of Kazakhstan.
- Genetic diversity of hazardous pathogens circulating in bat populations in Kazakhstan.
- GIS maps show hazardous pathogens carriage by specific bat species across different regions.
- Zoning of the country's regions based on the degree of hazard.
- An algorithm for comprehensive epizootological monitoring of hazardous infections in bat populations.
- The developed methodological recommendations for monitoring infectious disease pathogens in bat populations.

### **Testing and Publication of the Work**

The research results were published at international scientific and practical conferences, including:

- The International Scientific and Practical Conference "Modern Challenges for Biotechnology, Veterinary Medicine, and Medicine during the COVID-19 Pandemic" is dedicated to the 30th anniversary of Kazakhstan's independence (Gvardeisky, 2021).
- The International Scientific and Practical Conference of Students, Master's, and Doctoral Candidates "The Path to Science – 2022" (Oral, 2022).
- The International Scientific Conference "Astana Biotech 2024" (Astana, 2024).

The main results of the dissertation were annually presented at meetings of the Higher School "Veterinary and Biological Safety" of the Faculty of Veterinary Medicine and Biotechnology, scientific and technical councils of the Zhangir Khan West Kazakhstan Agrarian Technical University, and regional seminars and

meetings of veterinary specialists with the participation of specialists from the regional territorial inspection of the Veterinary Control and Supervision Committee of the Ministry of Agriculture of the Republic of Kazakhstan.

The dissertation results were included in the Research Report on Project No. AP 08053258 (2020, 2021, 2022).

The main content is reflected in 7 scientific works, including:

- 1 article in a journal indexed in the Web of Science or Scopus database with a non-zero impact factor.

- 3 articles in national scientific journals included in the Committee for Control in the Sphere of Education and Science of the Ministry of Education and Science of the Republic of Kazakhstan.

- 3 articles in the proceedings of international conferences.

**Main results and conclusions.** For the first time in the Republic of Kazakhstan, comprehensive epizootological monitoring of bat populations living in various regions of Kazakhstan for the carriage of particularly dangerous animal and human pathogens was carried out.

As a result of the conducted research, priority pathogens have been identified by the reservoir and/or vectors, which are bats in Kazakhstan. During the dissertation, the author collected 1,149 biological samples from four species of bats (*Vespertilio murinus*, *Nyctalus noctula*, *Myotis blythii*, *Eptesicus serotinus*) in 9 regions of Kazakhstan. As a result of the research, it was found that the prevalence of coronavirus in the Almaty region was 100%, Atyrau region 18.18%, Aktobe region 13.92%, West Kazakhstan region 5.16%, Turkistan region 1.39%, and Kyzylorda region 0.66%. The infection rate of *Vespertilio murinus* bats was 0.85%, *Eptesicus serotinus* 9.94%, and *Myotis blythii* 27.27%. The phylogeny of the RdRp gene of 12 samples allowed them to be classified as alphacoronaviruses, and divided into two groups. The main new group (n=11) was closely related to bat coronaviruses from Ghana, Zimbabwe and Kenya. The second group (n=1) was close to viruses previously isolated in the south of Kazakhstan.

Infection of bats with lissaviruses in the territory of the Republic of Kazakhstan has been established for the first time. Rabies virus RNA was detected in 27 (7.74%) samples from 349 bats studied. The prevalence of lissaviruses among bats in the North Kazakhstan region was 12.77%, Atyrau region 17.02%, Aktobe region 1.64%, West Kazakhstan region 11.11%, Turkestan region 5.08%, Zhambyl region 10.0%. The infection rate of *Vespertilio murinus* bats was 7.28%, *Eptesicus serotinus* 8.74%. Phylogenetic analysis of the sequence N of the OP585396 KZ(West)/bat/111/2021 gene showed its close relationship with isolates from the Cosmopolitan rabies virus group (Central Asia).

Morbillivirus RNA in 23 (5.85%) biological samples from bats. Morbillivirus prevalence among bats in West Kazakhstan region was 20.7%, Kyzylorda region 7.5%, Turkistan region 6.8%. The infection rate of *Vespertilio murinus* bats was 4.0%, *Eptesicus serotinus* 7.1%, *Nyctalus noctula* 14.3%.

As a result of the work, 11 sequences of the RdRp gene (460 bp) of alphacoronaviruses from bats from 5 regions of Kazakhstan, as well as the

sequence of the rabies virus nucleoprotein gene from western Kazakhstan, were deposited into the NCBI GenBank database.

Based on the materials of the dissertation work, an algorithm for complex epizootological monitoring of particularly dangerous infections in bat populations has been developed, and "Recommendations for catching and sampling bats for molecular biological studies" have been compiled. The collected research data will allow us to develop measures to reduce the risk of infection of animals and humans with particularly dangerous diseases carried by bats.

#### **Volume and Structure of the Dissertation**

The dissertation is 109 pages long and consists of abbreviations and acronyms, an introduction, a literature review, materials and methods, results, conclusions, 154 references, 28 figures, and 17 tables.