

ANNOTATION

of the dissertation of Abenova Asem Zhandarbekkyzy for the degree of Doctor of Philosophy (PhD) in the educational program 8D09101 – «Veterinary Welfare of Animals», on the topic «Molecular and genetic study of rabies circulating in the territory of the Republic of Kazakhstan»

Relevance of the study. Rabies is a natural focal, highly dangerous, and fatal infectious disease caused by the rabies virus (Rabies lyssavirus), which belongs to the genus *Lyssavirus* and the family *Rhabdoviridae* [1].

Rabies is a viral disease present in more than 150 countries and territories worldwide. According to the Food and Agriculture Organization of the United Nations, it causes approximately 55,000 human deaths annually, of which 95% occur in Africa and Asia. The lack of comprehensive statistical data suggests that the actual burden of the disease may be even higher.

Rabies is a fatal viral zoonotic disease transmitted to humans primarily through bites of infected animals. The disease manifests as encephalitis and is characterized by a 100% case fatality rate after the onset of clinical symptoms. Each year, more than 55,000 people and over 1 million animals die from rabies worldwide. The direct economic losses caused by rabies exceed 4 billion euros annually.

As in many endemic countries, rabies is registered annually among animals in Kazakhstan. Vaccination programs for domestic dogs, cats, and livestock are implemented, as well as a limited vaccination program for foxes through bait distribution. However, no decrease in disease incidence among animals has been observed. Therefore, one of the aims of this study was to achieve a deeper understanding of the epidemiology of rabies in the country by investigating its geographical distribution among animals and evaluating control measures [2].

The epizootiological situation of rabies in Kazakhstan remains an important social and economic problem. In 2019, 58 outbreaks were recorded; in 2020, 124 outbreaks; and in the first eight months of 2021, 71 outbreaks were identified. As of December 31, 2020, a total of 131 outbreaks were registered, including seven that remained unresolved since 2019. Sixty-five percent of outbreaks occurred in four out of fifteen regions: Turkestan (38), East Kazakhstan (22), Pavlodar (14), and West Kazakhstan (11) regions. These regions have remained epizootiologically unfavorable for rabies for decades: from 2013 to 2020, 45% of all rabies outbreaks in the country were recorded in these areas.

The social significance of rabies necessitates continuous monitoring of the epizootiological situation and accurate surveillance. Comprehensive epizootiological data are essential for the timely implementation of preventive measures. Since the patterns of disease spread in different foci are largely associated with the properties of viral isolates, the effectiveness of veterinary measures depends on epizootiological monitoring and detailed analysis of viral isolate characteristics.

However, although epizootiological indicators characterize the rate of disease

spread, molecular genetic studies are required to accurately determine the origin and transmission routes of the infection.

In Kazakhstan, the molecular genetic and antigenic characteristics of the rabies virus have not been systematically studied at the whole-genome level. For the first time, complete genomic nucleotide sequences were determined for several rabies virus isolates obtained from animals in Kazakhstan. Previous studies were mainly focused on improving preventive measures and diagnostic test systems. These studies are of high importance, as viral genotyping methods are becoming a key element in improving rabies prevention strategies.

Analysis of nucleotide sequences allows determination of the evolutionary history of the virus, its genetic variants, and the timing of its spread. This approach enhances epizootiological control and enables a deeper understanding of disease transmission dynamics.

The study conducted in 2020 – 2022 provided comprehensive data on the geographical distribution of samples obtained from rabies-infected animals in Kazakhstan, the nucleotide sequences of viral isolates, and their genetic diversity. In addition, phylogenetic relationships with isolates from neighboring countries were identified.

Thus, the lack of data on the genetic diversity of the rabies virus in Kazakhstan determines the relevance of research in this area. The obtained genetic data enable improvement of epizootiological monitoring, clarification of transmission pathways, and scientifically grounded optimization of rabies prevention programs.

The **main objective** of the study is the molecular genetic characterization of *Rabies lyssavirus* strains isolated in Kazakhstan and the assessment of their genetic relatedness to circulating strains.

To achieve this objective, the following tasks were defined:

1. Collection of samples for cDNA synthesis of *Rabies lyssavirus* strains.
2. Determination of nucleotide sequences of fragments corresponding to the N and G genes of the rabies virus.
3. Genotypic and phylogenetic analysis of nucleotide sequences of viral isolates obtained from different regions of Kazakhstan, determination of their genetic relationships, and construction of dendrograms.
4. Creation of a database including *Rabies lyssavirus* isolates based on obtained genomic and partial genomic nucleotide sequences.

Research materials and methods. The study was conducted in 2020 - 2022 within the framework of the research project «Molecular biological analysis of rabies virus circulating in the Republic of Kazakhstan» (AP08053353), 2023 – 2025 AP19679670 «Improvement of preventive measures against infectious diseases of animals (on the rabies example), based on the use of information and communication technologies». A total of 117 brain samples from animals with clinical suspicion of rabies were used as research material. The samples were collected in various regions of Kazakhstan during 2020 – 2022 and included foxes, wolves, dogs, cats, cattle, small ruminants, and horses.

Epizootiological assessment, cartographic, and statistical analyses were

performed at the Department of Veterinary Sanitation of S. Seifullin Kazakh Agrotechnical Research University. Viral RNA extraction was carried out at the National Reference Center for Veterinary Medicine of the Ministry of Agriculture of the Republic of Kazakhstan (Astana, Laboratory of Infectious Disease Diagnostics). Work with reverse-transcribed cDNA samples and molecular genetic analyses was conducted at the Applied Genetics Laboratory of the National Center for Biotechnology LLP.

The study employed a complex of molecular biological, next-generation sequencing (NGS), bioinformatic, phylogenetic, and epizootiological methods.

Viral RNA extraction was performed according to the manufacturer's protocol using commercial test kits produced by «Fractal Bio» LLP. Complementary DNA (cDNA) was synthesized from the extracted RNA by reverse transcription. Initial detection of the *rabies virus* was carried out using real-time PCR on a Rotor-Gene amplifier.

Primers for full amplicon coverage of the rabies virus genome were designed in silico using *MAFFT* and *Primer3* software based on sequences from the NCBI Virus database. Primer performance was validated experimentally.

DNA libraries were prepared from amplicons using the Illumina DNA Prep protocol, and whole-genome sequencing was performed on the Illumina MiSeq platform. Sanger sequencing was used to confirm selected fragments.

Quality control of sequencing data was performed using FastQC by assessing read quality. Read preprocessing and filtering were performed using *Seqtk* and *Sickle*. Genome assembly was carried out de novo using *SPAdes*.

Alignment of sequencing reads to a reference genome was performed using BWA, while nucleotide variants were identified and consensus sequences generated using *FreeBayes*, *BCFtools*, and *SAMtools*.

Genotypic classification of rabies virus isolates was performed using the RABV-GLUE platform. Phylogenetic analysis was conducted using Neighbor-Joining and Bayesian approaches in *MEGA X* and *BEAST* software. Visualization and annotation of phylogenetic trees were performed using the *iTOL* web platform.

Standard statistical methods were applied to calculate epizootiological indicators and to perform cartographic analysis. During the processing of molecular genetic data, the reliability of phylogenetic trees was assessed using 1,000 bootstrap replicates. All statistical computations were carried out with the use of Microsoft Excel software, together with the internal algorithms of specialized bioinformatics packages, including *MEGA X* and *BEAST*.

Object of the study. Pathological materials obtained from animals with clinical suspicion of rabies in different regions of Kazakhstan, together with the Rabies lyssavirus strains isolated from them.

Subject of the study. Molecular genetic features of rabies virus isolates, including their genotypic and phylogenetic characteristics, as well as the patterns of their distribution across the territory of Kazakhstan.

Scientific novelty. During this study, the genetic structure of rabies virus isolates circulating in Kazakhstan in 2020–2022 was characterized for the first time using full-genome nucleotide sequences. The obtained data showed that the virus

belongs to the cosmopolitan major clade and the *CAI* minor clade, as well as that individual isolates form genetically distinct lineages.

Within the framework of the study, a laboratory protocol enabling amplification and sequencing of the full coding regions of the *N*, *P*, *M* and *G* genes was validated for the first time. The proposed protocol has applied significance for conducting molecular genetic monitoring of the rabies virus.

As a result of the study, 31 complete and partial genomic nucleotide sequences of the rabies virus were identified for the first time and registered in the international *GenBank* database. These sequences were systematized within the PRJNA832888 BioProject and published under the accession numbers OP477379 – OP477391 and ON366706 – ON366710. As a result, a structured national-level dataset containing molecular genetic characteristics of *Rabies lyssavirus* strains circulating in Kazakhstan was formed for the first time.

Practical significance. This study scientifically substantiates the effective use of molecular genetic methods applied in the diagnosis of rabies. Characterization of the genetic structure of the rabies virus makes it possible to differentiate circulating regional strains and to assess their epizootic significance.

The results of molecular genetic analysis make it possible to identify the features of virus circulation in populations of domestic and wild animals and to clarify the directions of infection spread.

The obtained data can be directly applied in planning anti-rabies preventive measures in the Republic of Kazakhstan, including the scientific justification of vaccination organization, improvement of rapid response algorithms in epizootic foci, and identification of risk areas.

Main provisions for defense:

1 During the study, a comprehensive description of the genetic diversity and evolutionary traits of the rabies virus circulating in Kazakhstan was carried out for the first time. This forms a basis for improving molecular epidemiological monitoring of regional strains.

2 A relationship between the phylogenetic structure of the rabies virus and its geographic distribution was established, and the main foci of circulation and pathways of spread were identified.

3 The nucleotide sequences obtained in the course of the research were deposited in international databases (*GenBank* and others), creating a reference resource accessible to the global scientific community.

4 It was substantiated that the identified genetic variants of the rabies virus can be used to improve the sensitivity and accuracy of diagnostic systems.

5 The results of the study make it possible to provide a scientific basis for evaluating the effectiveness of rabies vaccines in Kazakhstan and, if required, for updating them.

6 Taking into account the characteristics of rabies virus circulation, scientific approaches to epizootic surveillance and prediction of risk areas were proposed.

Publications and approval. The main scientific results of the dissertation were published in 11 publications. Among them, 2 articles were published in journals indexed in the *Web of Science* and *Scopus* databases [3, 4] (Appendix A).

3 articles were accepted by journals recommended by the *Ministry of Science and Higher Education of the Republic of Kazakhstan* [5, 6] (Appendix Θ), and 1 methodological guideline (Appendix Б) was published. 2 act on the implementation of the research results into the educational process were introduced (Appendix B).

The main results of the dissertation work were annually presented at the Scientific Council of S. Seifullin Kazakh Agrotechnical Research University in 2020 – 2023 and discussed in reports. In addition, part of the research materials was presented at 3 international scientific conferences (Appendix Γ) and at 2 forums:

- International scientific and practical conference «Seifullin Readings – 18: Youth and Science – a look into the future» - 2022 [7].

- International scientific and practical conference «Seifullin Readings - 18(2): Science of the XXI century – the era of transformation» 2022 [8].

- 12th international scientific and practical conference «Siberian village: 200 years of development of the Omsk region – from the reforms of M.M. Speransky to the agro-industrial center of Siberia», held at the Federal State Budgetary Educational Institution of Higher Education Omsk State Agrarian University on September 21–23, 2022 [9] (Appendix F).

- The 1st China Kazakhstan Veterinary Medicine Science and Technology Postgraduate Academic Forum Co-organized by Harbin Veterinary Research Institute, Seifullin Kazakh Agro Technical University, China-Kazakhstan Belt and Road Joint Laboratory on Agricultural Sciences, Belt and Road Joint Center for Animal Infectious Diseases Prevention and Control. 28 May 2022 (Appendix F).

- International scientific and practical conference dedicated to the 120th anniversary of the foundation of the Kazakh Research Veterinary Institute, 2025 [10].

The author's personal contribution. The majority of the work carried out within the framework of the dissertation topic was performed with the direct participation of the author. The collection and analysis of literature data, the formulation of the research direction, the definition of goals and objectives, the conduct of experimental studies, the processing and interpretation of the obtained results, the preparation of scientific articles, and the drafting of the dissertation text constitute the author's personal contribution. The formatting of the manuscript and the introduction of the obtained results into scientific circulation were also the author's responsibility.

Structure and volume of the dissertation work. The work consists of a total of 100 pages. It includes normative references, definitions, designations, and abbreviations, an introduction, a literature review, materials and methods, research results and their discussion, a conclusion, and a list of 118 references used.

The main text of the dissertation contains 31 figures and 16 tables, while 29 figures are presented in the appendices. All illustrative materials allow for a full disclosure of the analyses carried out in the text and provide a visual presentation of the obtained results.