

ANNOTATION

dissertation for the degree of Doctor of Philosophy (PhD)

In specialty 6D060700 – Biology

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Study of the biological properties of the actual equine influenza virus strains, isolated in the territory of the Republic of Kazakhstan in 2012

General characteristics of the thesis

The dissertation is devoted to the study of the biological and molecular genetic properties of equine influenza virus (EIV) strains A/H3N8 isolated on the territory of the Republic of Kazakhstan by determining their phylogenetic affiliation and antigenic activity for the subsequent development of relevant means of specific prevention.

The relevance of research

The timeless horse was traditionally an integral part of the steppe's life, a subject of his pride. Household utensils, but also entertainment. In addition, the horse made it possible to hunt and fight. The main directions of Kazakh horse breeding have always been: breeding; meat and dairy (productive); working - user; sports. In the Soviet period, 19 breeding stud farms and dozens of breeding horse farms were created in Kazakhstan, many of which have functioned so far. The Ministry of Agriculture currently recommends reconnaissance of 13 breeds and types of horses. From horsebacks - thoroughbred horseback, Arabian, Akhal-Teke; horse-drawn - Kostanay, Don, Russian trotter and Russian heavy truck; meat and dairy - Mugalzhar, Kazakh type of toad, Kushum, Adaev and cabins meat type.

The most developed horse breeding is noted in Almaty, East Kazakhstan, South Kazakhstan and Karaganda regions. The smallest in Kyzylorda, Mangistau and Atyrau regions.

Currently, the situation with dangerous infections among animals has become a problem for many countries of the world. Expansion of economic, trade and tourism ties between countries. In this regard, ensuring epizootological safety is a possible improvement in socio-economic conditions in Kazakhstan.

One of the problems of ensuring epizootological safety is the widespread infectious diseases of horses. This is due to several reasons that cause infection of vaccinated animals.

Horse influenza (grippus equorum) is an infectious, acute leaking highly contagious horse disease characterized by short-term fever, general oppression, conjunctivitis, catarrh of the upper respiratory tract, dry, jerky, deep and painful cough that causes respiratory diseases.

The incubation period lasts 1-6 days. The disease is usually acute. The degree of manifestation of signs of the disease is affected by the conditions of detention, individual resistance of animals. The first symptoms of flu in horses are often not diagnosed. the incidence is easy, with signs of rhinitis and cough, which are associated with irritation of the mucous membranes of the respiratory tract with dust of air and stern. Sick animals have an appetite, they quickly disappear, mucous sources from the nasal cavity and eyes appear, photophobia. The mucous membrane of the eyes, nasal passages is reddened, swollen, covered with a small amount of transparent mucus. The

submandibular lymph nodes are enlarged, often painful on palpation. The animals have a faster pulse, respiration. Influenza in horses often proceeds benignly and ends with clinical recovery after 2-4 days. In some cases, sudden flexibility may occur.

The causative agent of equine influenza is an RNA-containing virus belonging to the Orthomyxoviridae family of 80-120 nm in delivery. It has the necessary productive recombination mechanism, providing fast antigenic variation.

Due to the wide spread of diseases, recommendations are being made in the international communities on the main directions of the fight against equine influenza: preparation of legislation and educational work, epidemiology and epizootology, diagnostic methods, ensuring biological safety and vaccination.

The implementation of these recommendations directly depends on the study of strains of viruses circulating in the region, because their characteristic is the creation of effective diagnostic test systems, as well as an integral part of the development of technologies for the manufacture of antiviral vaccines. Therefore, studies on the biological and molecular genetic properties of equine influenza viruses isolated on the territory of the Republic of Kazakhstan are obvious and timely.

Changes in influenza viruses require constant monitoring in various regions, the identification of new cases, isolation and study of the origin of the pathogen. The timely establishment of subtypes of influenza viruses in the country and in the region will allow more effective control of morbidity data. Changes in hemagglutinin and neuraminidase, taking into account the possibility of changing antigenic properties leading to the emergence of new strains of influenza viruses, are available from time to time epidemics and pandemics. For these reasons, numerous studies are being carried out in the field of molecular, immunobiological and biochemical studies.

Objective: to study the biological and molecular genetic properties of equine influenza virus strains isolated on the territory of the Republic of Kazakhstan. To achieve this goal, the following **tasks** was formulated:

1. To carry out epizootological monitoring of equine influenza in the territory of the Republic of Kazakhstan with the subsequent isolation and identification of EIV isolates.
2. To determine the optimal parameters for the cultivation of EIV strains in cell culture and developing chicken embryos.
3. Investigate the morphometric characteristics of EIV.
4. Develop full-size segments and conduct genome-wide sequencing of EIV strains.
5. Conduct phylogenetic analysis of surface genes (HA, NA), to determine the phylogenetic relationship with other strains.
6. To develop a system for diagnosing and subtyping new isolated strains of EIV using a biological microchip and a real-time polymerase chain reaction method.

Objects of study

The objects of research were the strains *A/equine/Baizak/09/2012*, *A/equine/Kostanai/09/2012*, *A/equine/Matybulak/10/2012* and *A/equine/LKZ/09/2012* EIV isolated in 2012 territory of Kazakhstan, and their nucleotide sequences deposited in the GenBank database.

Subject of study

Determination of phylogenetic affiliation and antigenic activity of EIV strains A/H3N8 isolated on the territory of the Republic of Kazakhstan for the subsequent development of topical means of specific prevention.

Research methods

Classical virological and molecular genetic methods such as RGA, RTGA, PCR, sequencing and phylogenetic analysis using the programs BioEdit, Mega7.0 and the analytical database NCBI.

Scientific novelty of research

The scientific novelty and originality of the research lies in the first established phylogenetic affiliation of new epizootic strains of EIV: *A/equine/Baizak/09/2012*, *A/equine/Kostanai/09/2012*, *A/equine/Matybulak/10/2012* and *A/equine/LKZ/09/2012* to American clyde, sub Florida 2 subclade, with H3N8 antigenic formula.

The main biological properties that allow the cultivation of a virus with high antigenic activity, which is the main criterion in the development of specific prophylaxis, are determined.

The nucleotide sequence of the complete genome of EIV strains *A/equine/Baizak/09/2012*, *A/equine/Kostanai/09/2012* and the genes (HA, NA, M, NP, NS) of A/H3 strains was first identified and published in the international GenBank database *A/equine/Matybulak/10/2012* and *A/equine/LKZ/09/2012*.

A comparative and genetic analysis were carried out, which established the possibility of using the studied strains in the development of modern diagnostic test systems and vaccines.

The theoretical significance of the work

The theoretical significance of the work lies in the determination and publication in the international GenBank database of the nucleotide sequence of the complete genome of EIV strains *A/equine/Baizak/09/2012*, *A/equine/Kostanai/09/2012* and genes (HA, NA, M, NP, NS) strains *A / equine / Matybulak / 10/2012*, *A / equine / LKZ / 09/2012*, which is freely available to other scientists and can be used in comparative and phylogenetic analyzes.

The practical value of the study is the isolation of EIV strains *A/equine/Baizak/09/2012*, *A/equine/Kostanai/09/2012*, *A/equine/Matybulak/10/2012* and *A/equine/LKZ/09/2012*, related to clyde American, subclay Florila 2, which allows us to establish the phylogenetic variability of isolates relative to previous epizootics and recommend them when developing topical vaccines.

Virus-containing materials with high infectious and hemagglutinating activity have been obtained, which will provide intense immunity if used as vaccine candidates.

The manufactured experimental series of oligonucleotide microarrays can be used in the diagnosis of influenza A/H3N8 virus. Methods have been developed for the early diagnosis and subtyping of EIV strains by RT-PCR for the influenza A/H3N8 subtype.

The main provisions to be defended:

EIV isolates from various regions of the Republic of Kazakhstan belong to the equine influenza A/H3N8 subtype.

The selection of cultivation parameters made it possible to obtain virus-containing materials with an infectious activity of 7.8–8.3 lg EID₅₀ / cm³, and a hemagglutinating

titer of 1: 1024–2048 GAE, which corresponds to the requirements of vaccine strains, for the development of specific prophylactic agents.

According to the phylogenetic analysis of the nucleotide sequence of the HA hemagglutinin and NA neuraminidase genes, strains *A/equine/Baizak/09/2012*, *A/equine/Kostanai/09/2012*, *A/equine/Matybulak/10/2012* and *A/equine/LKZ/09/2012* belong to the Clyde American, subclay Florila 2.

The developed diagnostic system, including PCR and the oligonucleotide microchip, allows the identification and subtyping of influenza A/H3N8 viruses.

The main research results and conclusions:

Comprehensive studies on the morphometric, antigenic and molecular genetic characteristics of strains *A/equine/Baizak/09/2012*, *A/equine/Kostanai/09/2012*, *A/equine/Matybulak/10/2012* and *A/equine/LKZ/09/2012*, the influenza virus of horses isolated on the territory of Kazakhstan, allowed their phylogenetic analysis. It was found that the strains belong to the American line of the Florida-2 family of equine influenza viruses. The determination of the nucleotide sequence of the complete genome of strains was used in the development of oligonucleotide microarrays for the diagnosis and subtyping of influenza A / H3N8 virus.

The results obtained allow us to draw the following conclusions:

1. When conducting epizootological monitoring, 1563 samples of blood serum and 857 samples of nasopharyngeal swabs delivered from various regions of the Republic of Kazakhstan were analyzed. Four strains were isolated: *A/equine/Baizak/09/2012*, *A/equine/Kostanai/09/2012*, *A/equine/Matybulak/10/2012* and *A/equine/LKZ/09/2012* of the equine influenza virus.

2. The optimal conditions for the cultivation of equine influenza virus in developing chicken embryos and in MDCK cell culture were selected (temperature - 34 ° C, incubation time - 72 hours and an infection dose of 1000 EID₅₀ / 0.2 cm³, 0.1 TCD₅₀) in compliance with these parameters A viral suspension with a high hemagglutinating activity of at least 1: 256 and an infectious titer of the order of 7.25 lg was obtained.

3. The strains *A/equine/Baizak/09/2012*, *A/equine/Kostanai/09/2012* and *A/equine/Matybulak/10/2012* (H3N8) differ in both morphometric (shape and size of virions) and physical characteristics (sedimentation constant and floating density). It has been established that the size of the rounded virions is 80-200 nm, the length of some filiform forms of viral particles reaches 750 nm or more, and the surface of the viral particles is covered with spines 6-8 nm high.

4. Using selected specific primers, full-sized segments of the genome of the studied strains were developed. The nucleotide sequence of the complete strain genome obtained by sequencing is published in the GenBank international database. Access numbers for strain *A/equine/LKZ/09/2012*: MH173319, MH173320, MH173321, KP202378, KP202382, MH173322, KP202374, KP202386, for strain *A/equine/Kostanai/09/2012*: MH173056, MH173058, MH173058, MH173058, MH173058, K KP202384, MH173059, KP202376 and KP202388.

5. As a result of phylogenetic analysis, it was found that strains *A/equine/Baizak/09/2012*, *A/equine/Kostanai/09/2012*, *A/equine/Matybulak/10/2012* and *A/equine/LKZ/09/2012* (H3N8) belong to the American line of the Florida-2 family of equine influenza viruses. It has been established that the differences in amino acids

at positions 387 and 394 can serve to determine equine influenza viruses isolated in 2012 as a separate phylogenetic branch.

6. Selected and synthesized specific oligonucleotide probes and primers for influenza A / H3N8. Diagnosis and subtyping of the studied strains of equine influenza viruses using a biological microchip and RT-PCR were performed. An optimal design of the location of the probes on the platform has been developed. An experimental series of microchips was manufactured.

All the tasks set in the thesis are completed.

Personal contribution of the author

The author independently carried out an analysis of literary data on the topic of research, experimental work, statistical processing, and analysis of research results and the manuscript of the dissertation.

Relationship with the plan of basic scientific work

The dissertation was carried out as part of the program “O.0534 Influenza Equines: Epizootological Monitoring, Development of Specific Prevention and Diagnostic Tools for 2010-2012”.

Work approbation

Materials of the dissertation were reported and discussed at the following conferences:

- at the 7th International Scientific Conference "European Science and Technology", Germany, Munich (1), 2014;
- Symposium on Eurasian Biodiversity, 23-27 May 2016, Antalya, TURKIYE;
- at the International Scientific Conference “Scientific Forum: Medicine, Biology and Chemistry” Moscow, Russia, 2018

Publications

Based on the dissertation materials, 9 works were published, including 2 articles in peer-reviewed foreign scientific journals indexed in the Web of Science or Scopus databases with a non-zero impact factor; 3 articles in republican magazines from the list of the Committee for control in the field of education and science of the Republic of Kazakhstan; 1 article in a journal included in the list of RSCI; 3 abstracts in materials of international conferences and 4 patents of the Republic of Kazakhstan.

Volume and structure of the dissertation

The dissertation presented on 115 pages of computer text and consists of the following sections: notation and abbreviations, introduction, literature review, research materials and methods, results and discussion, conclusion, list of references from 180 items. The work includes 29 tables, 35 figures and 4 applications.