

## ANNOTATION

to the doctoral thesis for Doctor of Philosophy (PhD) degree on the specialty  
«6D060700-Biology»

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«Molecular and biological characteristics of swine influenza viruses circulating in  
Kazakhstan»

**General characterization of the dissertation research.** The research work is devoted to identification and isolation of swine influenza virus strains circulating in the territory of the Republic of Kazakhstan, study of their molecular and biological properties and determination of phylogenetic affiliation to reveal the regularities of their distribution and evolution.

**Research relevance.** Swine influenza is a highly contagious viral infection caused by influenza A virus (IAV) of the *Orthomyxoviridae* family. Influenza virus is divided into 18 subtypes based on surface proteins for hemagglutinin and 11 subtypes for neuraminidase. IV infects humans as well as birds and various mammalian species, including swine. Three major subtypes of IAV circulate in the swine population: H1N1, H3N2, and H1N2. The epithelium of the pig's respiratory tract has cellular receptors for sialic acid residues with NAcNeu $\alpha$ 2-3Gal terminal structures susceptible to avian and NAcNeu $\alpha$ 2-6Gal receptors susceptible to human IAV strains, which favors simultaneous replication and reassortment of different subtypes IAVs in pigs. The main routes of transmission of swine influenza viruses (SIVs) are airborne and direct contact: through the excreta of infected animals. In the field, IV is transmitted through water in reservoirs contaminated with poultry feces. Pigs can serve as a natural reservoir and vessel for mixing and emergence of new potentially dangerous strains, as confirmed by the 2009 pandemic caused by a reassortant swine H1N1 virus that contained neuraminidase and M protein genes of Eurasian swine virus and six genes of North American triple reassortant virus.

Systematic molecular biological screening of biological samples and isolation of SIV strains from nasopharyngeal flushes collected from pigs in different Kazakhstan regions significantly expand knowledge in the field of identifying patterns of distribution in the ongoing evolution of SIV, and may also contribute to relevant vaccine strains selection and minimize the role of pigs as a source of emergence of new, potentially dangerous variants of IAV.

**Research objects:** swine influenza viruses, nasopharyngeal washings, blood sera.

**Research aim.** Investigation of the molecular and biological characteristics and identification of the influenza virus's variability circulating in the population of pigs in the Republic of Kazakhstan.

**Research objectives:**

1. Molecular biological screening and isolation of IV strains from nasopharyngeal washings collected from pigs in different Kazakhstan regions.

2. Phylogenetic analysis of variable genes (hemagglutinin, neuraminidase, NS and M) of SIV strains.

3. Comparative study of biological properties and antigenic relationships of SIV strains.

4. Preparation of lyophilically dried preparations of SIV strains and rabbit immune sera.

**Research Methods.** The research work was performed using modern certified virological and molecular biological methods. Molecular genetic studies (NGS sequencing on the Illumina MiSeq platform and sequencing using Sanger technology) were conducted using appropriate methodologies and material and technical facilities of virus biochemistry and molecular virology laboratory of the «Research and production center of Microbiology and Virology» LLP.

### **Scientific novelty**

During the epidemic periods from 2018 to 2021, in livestock farms in Kazakhstan different regions, a monitoring study was conducted for the first time and the co-circulation of two subtypes of IAV was identified: H1N1 and H3N2, with H1N1 IAV predominating.

Six strains were isolated from nasopharyngeal flushes collected from pigs in pig farms in the northern and Almaty regions of Kazakhstan for the first time.

The nucleotide sequence of the complete genome of SIV A/pig/Karaganda/3/2020 (MZ396822.1 - MZ396828.1) and A/pig/Karaganda/4/2020 (MZ363969.1 - MZ363976.1) was determined and published for the first time in the GenBank database.

The phylogenetic affiliation of SIV strains A/pig/Karaganda/03/2020, A/pig/Karaganda/04/2020 to the European branch and HCV isolates A/pig/Petropavlovsk/01/2018, A/pig/Petropavlovsk/02/2018, A/pig/Petropavlovsk/03/2018, A/pig/Pavlodar/43/2019 to the Asian branch was established for the first time.

For the first time, the S31N mutation responsible for the formation of resistance to Remantadin was detected in the structure of the M2 protein gene of Karaganda SIV (03/20; 04/20) and Almaty strain (45/19).

A comparative study of biological properties of North Kazakhstan and Almaty strains of SIV 2018-2019 with SIV isolates circulating in pig farms of Kostanay and Aktobe regions in 2012-2014 and reference strains of SIV was carried out. Heterogeneity of 2018-2019 SIV populations by a number of biological properties was shown.

The sensitivity of North Kazakhstan and Almaty strains of SIV 2018-2019 to modern anti-influenza drugs included in the state register of drugs and medical devices, and in the clinical protocol of diagnosis and treatment of influenza of Ministry of Health of the RK was studied for the first time.

Patent No. 34782 dated 09.04.2021 was obtained for the strain A/pig/Petropavlovsk/03/18 (H1N1) intended for the production of diagnostic drugs.

### **Theoretical and practical significance**

A vast arsenal of knowledge has been accumulated on the specifics of the influenza circulation and the role of pigs in the evolution and transmission of this pathogen. The study of molecular and biological characteristics of IV isolates isolated from pigs is a fundamental contribution to influenza ecology. In addition to theoretical significance, the thesis work is also of practical value related to the possibility of using the strain A/pig/Petropavlovsk/03/18 (H1N1) for the production of modern diagnostic drugs.

The original strain of SIV A/pig/Petropavlovsk/03/18 (H1N1) is deposited (registration number M-3-19/D from 27.03.2019) in the national collection of microorganisms of "Research Institute of Biological Safety Problems" MSHE RK. The original strain of SIV A/pig/Petropavlovsk/03/18 (H1N1), intended for the production of diagnostic preparations, was granted a patent (No. 34782 dated 09.04.2021).

Lyophilic dried preparations of Kazakhstani strains of SIV circulating in pig populations in 2018-2019 and rabbit immune sera are recommended for use in virology laboratories of public health and veterinary services of the republic to assess the intensity of collective immunity to influenza and decipher emerging outbreaks of acute respiratory viral infections and identification of newly isolated isolates of IV.

The nucleotide sequences of all SIV genes A/pig/Karaganda/03/2020 and A/pig/Karaganda/04/2020 under numbers MZ396822.1 - MZ396828.1 and MZ363969.1 - MZ363976.1 are freely available in the international GenBank database, which makes it possible to use them for local and global comparative phylogenetic analysis with currently circulating SIV strains.

### **Provisions, submitted for the defence:**

1. During 2018 – 2021, in pig farms of different Kazakhstan regions, 2144 biological samples were collected from pigs. During screening of samples in RT-PCR and hemagglutination inhibition (HI) reaction, the co-circulation of H1N1 and H3N2 HAV was established. Five strains of influenza virus A/H1N1 and one A/H3N2 on MDCK cultural cells and chicken embryo cells were isolated from samples obtained from the Northern and Southern regions of Kazakhstan.

2. Phylogenetic analysis of variant genes (NA, NA and NS) of IV strains circulating in swine populations in RK in 2018 - 2020 revealed that Karaganda VHS (03/20; 04/20) belonged to the European branch and North Kazakhstan strains (43/19; 03/18; 02/18; 01/18) belonged to the Asian branch.

3. According to the main biological properties, the isolates represent mainly a homogeneous group, showing heterogeneity in some properties. Close antigenic affinity of North Kazakhstan strains (44/19; 43/19; 03/2018; 02/2018 and 01/2018) with reference H1N1 HAVs (A/New Jersey/8/76; A/swine/USA/1976/31 and A/swine/Iowa/15/30), Almaty strain (45/19) - with A/Wisconsin/67/05(H3N2) virus was established.

4. Lyophilically dried SIV 2018 - 2019 antigens and rabbit immune sera retained activity after six to nine months of storage, which allows us to recommend these preparations for use as diagnostic preparations.

### **Research results and conclusions:**

1. As a result of molecular biological screening in RT-PCR of 2055 swine nasopharyngeal flushes and serologic analysis in HI reaction of 89 blood sera of pigs collected in livestock farms of Almaty, Karaganda, Kostanay, North-Kazakhstan and Pavlodar regions of Kazakhstan during epidemic periods from 2018 to 2021, co-circulation of two subtypes of SIV was identified: H1N1 and H3N2, with predominance of H1N1 HAV.

2. Six strains were isolated from 116 PCR-positive samples for MDCK cell culture and Chicken embryo cells: A/Pig/Almaty/45/19(H3N2), A/Pig/Pavlodar/44/19(H1N1), A/Pig/Pavlodar/43/19(H1N1), A/Pig/Petropavlovsk/03/18(H1N1), A/Pig/Petropavlovsk/02/18(H1N1), A/Pig/Petropavlovsk/01/18(H1N1).

3. As a result of phylogenetic analysis of the three most variable SIW genes: HA, NA and NS protein, Karaganda SIVs (03/20; 04/20) were assigned to the European branch and North Kazakhstan strains (43/19; 03/18; 02/18; 01/18) to the Asian branch.

4. S31N mutation responsible for the formation of resistance to Remnantandin was found in the structure of M2 protein gene of Karaganda SIV (03/20; 04/20) and Almaty strain (45/19).

5. A recombination was detected in the nucleotide sequence of the M gene in strain A/pig/Pavlodar/43/19. The probable insertion is located at position 331-684. The main parental sequence of this region is strain A/mallard/Illinois/10OS3243/2010.

6. The nucleotide sequence of the complete genome of SIV A/pig/Karaganda/3/2020 (MZ396822.1 - MZ396828.1) and A/pig/Karaganda/4/2020 (MZ363969.1 - MZ363976.1) was published in GenBank.

7. Kazakhstan SIV strains of 2018 - 2019 showed similarity among themselves and with reference strains in terms of adsorbing and eluting abilities and inhibitor sensitivity, differing in hemagglutinating and infectious activity; degree of sensitivity to antiviral drugs (Tamiflu, Remantadine) and resistance of hemagglutinin to temperature factor.

8. Antigenic analysis determined close affinity of strain A/Almaty/45/19 to A/Wisconsin/67/05 (H3N2) virus and North Kazakhstan strains (44/19; 43/19; 03/2018; 02/2018; 01/2018) to reference IV viruses: A/New Jersey/8/76; A/swine/USA/1976/31 and A/swine/Iowa/15/30.

9. Lyophilically dried preparations of SIW 2018 - 2019 and rabbit immune sera were found to retain activity over nine months of storage, which allows us to recommend them for use as standard diagnostic preparations in deciphering emerging outbreaks of acute respiratory viral infections.

**Relation to the main scientific works's plan.** The dissertation work was carried out within the framework of two projects: grant funding of the Ministry Science and Higher Education of the Republic of Kazakhstan AP05130989 "Molecular and genetic variability of swine influenza viruses in Kazakhstan" (2018 - 2020) and international ISTC K-2231 "Molecular characterization of swine influenza viruses circulating in different regions in Kazakhstan and comparison with contemporary human influenza viruses" (2018 - 2021) in LLP "Research Center for Microbiology and Virology". Scientific supervisor of the projects - Candidate of Biological Sciences N.G. Klivleeva.

**Work approbation.** The main results of the dissertation work were published and reported at three international scientific conferences: "Influenza 2018: Centenary of the 1918 Pandemic" (London, 2018); Congress "10th Edition of Options for the Control of Influenza" (Singapore, 2019. ); 22nd Annual Meeting of the European Society for Clinical Virology (Copenhagen, 2019), in the abstracts of international scientific-practical conferences in the near abroad: "Viral infections and society: problematic issues of diagnosis, treatment and prevention" (Yekaterinburg, 2018); "Molecular bases of the European Society for Clinical Virology" (Yekaterinburg, 2018); "Molecular bases of the European Society for Clinical Virology" (Yekaterinburg, 2018). ); "Molecular bases of epidemiology, diagnostics, prevention and treatment of topical infections" (St. Petersburg, 2018) and the Republic of Kazakhstan: "Modern challenges for biotechnology, veterinary medicine and veterinary medicine" (Gvardeysky, 2020. ), at the annual scientific seminars of the Department of "Molecular Biology and Genetics" of the Faculty of "Biology and Biotechnology" of Al-Farabi KazNU and partially included in the Reports on research work under the projects AR05130989, ISTC K-2231 and OR11465435-OT-22.

**Publications.** According to the requirements of the Regulations on the Dissertation Council of Al-Farabi KazNU the materials of the dissertation are reflected in 17 publications, including 2 articles in journals included in Q3 of Web of Science, 3 articles in journals included in the list of the Committee for Quality Assurance in Education of the Ministry of Science and Higher Education of the Republic of Kazakhstan, 3 abstracts in the abstracts of international conferences, 2 abstracts in the abstracts of international conferences in the near abroad and 1 abstract in the abstracts of an international conference in the Republic of Kazakhstan. And also 1 patent for invention.

**The content and structure of the dissertation.** The dissertation is set out on 127 pages and consists of following sections: list of abbreviations, introduction, literature review, materials and methods, results, conclusions, 250 references, 200 of them in English. The thesis contains 30 tables, 32 figures and two appendices.