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ВЕСТНИК

НАЦИОНАЛЬНОЙ АКАДЕМИИ НАУК
РЕСПУБЛИКИ КАЗАХСТАН

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NAS RK is pleased to announce that Bulletin of NAS RK scientific journal has been accepted for indexing in the Emerging Sources Citation Index, a new edition of Web of Science. Content in this index is under consideration by Clarivate Analytics to be accepted in the Science Citation Index Expanded, the Social Sciences Citation Index, and the Arts & Humanities Citation Index. The quality and depth of content Web of Science offers to researchers, authors, publishers, and institutions sets it apart from other research databases. The inclusion of Bulletin of NAS RK in the Emerging Sources Citation Index demonstrates our dedication to providing the most relevant and influential multidiscipline content to our community.

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НАН РК сообщает, что научный журнал «Вестник НАН РК» был принят для индексирования в Emerging Sources Citation Index, обновленной версии Web of Science. Содержание в этом индексировании находится в стадии рассмотрения компанией Clarivate Analytics для дальнейшего принятия журнала в the Science Citation Index Expanded, the Social Sciences Citation Index и the Arts & Humanities Citation Index. Web of Science предлагает качество и глубину контента для исследователей, авторов, издателей и учреждений. Включение Вестника НАН РК в Emerging Sources Citation Index демонстрирует нашу приверженность к наиболее актуальному и влиятельному мультидисциплинарному контенту для нашего сообщества.

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CIRCULATION OF INFLUENZA VIRUSES AMONG HUMANS AND SWINE IN THE TERRITORY OF KAZAKHSTAN DURING 2017–2018

Abstract. 251 biosamples (191 nasopharyngeal swabs and 60 blood serums) were obtained from humans at healthcare institutions located in various regions of the Republic of Kazakhstan. 369 biosamples (330 nasopharyngeal swabs and 39 blood serums) were collected from swine farms.

In the polymerase chain reaction performed on 191 samples collected from humans, the genetic material of influenza A virus was detected in 14.14% of cases, that of influenza B virus in 5.24%. When carrying out subtyping, influenza A/H1N1 virus RNA was identified in 4.71% of samples, A/H3N2 virus RNA in 4.19%. In 330 samples obtained from swine, the genetic material of influenza virus was found in 3.33% of cases, of which influenza A/H1N1 virus RNA was detected in 2.12%, and A/H3N2 virus RNA in 1.21%.

The results obtained in the polymerase chain reaction, as well as data from serological studies in the hemagglutination inhibition assay and enzyme immunoassay, indicate co-circulation of influenza A/H1N1 and A/H3N2 and B viruses among humans and influenza A/H1N1 and A/H3N2 viruses among swine in various regions of Kazakhstan during 2017-2018.

When carrying out virological study of biosamples collected from humans and swine, 10 hemagglutinating agents were isolated in chicken embryos and identified as influenza viruses with antigenic formulae A/H1N1, A/H3N2, and B.

The results of virological and serological studies underline the importance of conducting continuous monitoring of the circulation of influenza viruses among humans and swine in Kazakhstan to identify the possibility of interspecies transmission of the infectious agent.

Keywords: influenza virus, circulation, isolate, PCR diagnostics, hemagglutinin, neuraminidase, blood serum.

Introduction. A high variability of influenza A viruses due to the enormous mutation rate and rapid replication leads to the appearance of viruses with new antigenic properties, which allows it to overcome strain-specific immunity in the population and reach epidemic spread [1].

The presence of a segmented genome facilitates gene recombination between different influenza A viruses [2]. Genetic reassortment between human and avian influenza viruses in swine can play a

significant role in the emergence of pandemic strains, since swine are equally susceptible to both human and avian influenza viruses [3-6].

The viruses such as influenza A/California/04/09 (H1N1)pdm09, containing a complex combination of gene segments of swine, avian, and human influenza viruses that caused the first influenza pandemic in XXI century [7] provided an example of such reassortment. These viruses completely replaced A(H1N1) viruses circulating before and were widely distributed throughout the world along with seasonal influenza A(H3N2) and type B viruses [2, 8].

Due to the fact that reassortment leads to the emergence of viruses with new biological and antigenic properties capable of wide epidemic spread, the infection surveillance and timely pathogen diagnosis, both in humans and in swine, are extremely important areas for combating influenza [9, 10].

The purpose of this study was to examine the characteristics of the circulation of influenza viruses among humans and swine in the territory of Kazakhstan during the 2017-2018 epidemic seasons.

Materials and methods. Nasopharyngeal swabs from humans and swine were collected in vials containing 2 mL of Medium 199 with 0.5% bovine serum albumin and antibiotic complex (50000 U/mL of penicillin, 50 μg/mL of streptomycin, 3000 μg/mL of gentamicin, 5000 U/mL of nystatin). The samples were kept for 4 days at 4 °C and stored in liquid nitrogen (-196 °C).

RIBO-prep and REVERTA-L reagent kits were used to isolate RNA from the samples under study and perform reverse transcription reaction to obtain c-DNA. Primary screening was carried out in real-time polymerase chain reaction (RT-PCR) with hybridization-fluorescence detection using the reagent kits for detection of influenza virus RNA AmpliSens Influenza virus A/B-FL and AmpliSense Influenza virus A-type-FL (FSBI Rospotrebnadzor Central Research Institute for Epidemiology, Russia) on the Rotor-Gene Q6plex (QIAGEN, Germany).

Hemagglutinating agents (HAA) were isolated in 9-10 day old developing chicken embryos. 0.75% suspension of cock and human erythrocytes of 0 (1) blood group was used to indicate virus in the hemagglutination assay (HA) [11].

Identification of viruses was performed in the hemagglutination inhibition (HAI) and neuraminidase activity inhibition (NAI) assays with the kits of polyclonal diagnostic serums, according to WHO recommendations [12].

Serological studies of blood serums were carried out in the HAI assay and enzyme-linked immunosorbent assay (ELISA). The HAI assay was performed using the influenza A/HINI and A/H3N2 virus antigen detection kits (Enterprise for the Production of Diagnostic Preparations LLC, St. Petersburg, Russia). ELISA was performed using a test system for influenza A viruses (subtypes H1N1 and H3N2) (FSBI Rospotrebnadzor Central Research Institute for Epidemiology, Russia).

Results. Biomaterials were obtained from humans and swine in the Kostanay, Karaganda, Almaty, and Aktobe oblasts in 2017-2018. A total of 620 biosamples were collected (521 nasopharyngeal swabs and 99 blood serums).

To study the circulation of influenza viruses among the population, 251 biosamples (191 nasopharyngeal swabs and 60 serums) were obtained from patients with primary diagnoses of ARVI, influenza, bronchitis, and pneumonia at healthcare institutions. 330 nasopharyngeal swabs and 39 serums were obtained from 4-6-month-old swine at the livestock farms.

The characteristics of collected material and results of RT-PCR screening are presented in table 1.

As presented in Table 1, during primary screening of 191 nasopharyngeal swabs collected from humans, the genetic material of influenza virus was found in 27 samples (14.14% of the total number of samples examined). Influenza A virus RNA was detected in 17 biosamples (8.9%), and influenza B virus RNA in 10 biosamples (5.24%). Subtyping of influenza A positive samples revealed influenza A/H1N1 virus RNA in 9 samples (4.71%) and A/H3N2 virus RNA in 8 samples (4.19%).

Primary screening of 330 nasopharyngeal swabs collected from swine, resulted in detection of influenza virus RNA in 11 samples (3.33% of the total number of samples), A/H1N1 influenza virus RNA was found in 7 samples (2.12%), A/H3N2 virus RNA in 4 samples (1.21%).

Primary screening of nasopharyngeal swabs in RT-PCR therefore showed that influenza A/H1N1, A/H3N2, and B viruses circulate among humans and A/H1N1, A/H3N2 viruses among swine in the territory of Republic of Kazakhstan.

Table 1 – Characteristics of biosamples and RT-PCR screening of nasopharyngeal swabs collected from humans and swine in different regions of Kazakhstan during 2017-2018

Sampling point	Nasopharyngeal swab	Serum	Number of PCR-positive samples for				
			influenza viruses	influenza type A virus	virus sybtypes		influenza type B virus
					A/H1N1	A/H3N2	
Human biosamples							
Kostanay oblast	52	14	2	0	0	0	2
Karaganda oblast	10	10	4	4	2	2	0
Almaty oblast	116	30	18	11	6	5	7
Aktobe oblast	13	6	3	2	1	1	1
Total	191	60	27	17	9	8	10
Swine biosamples							
Kostanay oblast	85	39	3	3	1	2	*
Karaganda oblast	10	0	4	4	2	2	*
Almaty oblast	137	0	2	2	2	0	*
Aktobe oblast	98	0	2	2	2	0	*
Total	330	39	11	11	7	4	*
*A study was not conducted.							

During primary infection of 9-10-day-old chicken embryos with the samples collected from humans in different regions of Kazakhstan, eight HAAs were isolated: three from patients living in Karaganda and five from Almaty, hemagglutination titers were of 1:4 - 1:16. Two HAAs with hemagglutination titers of 1:2 - 1:4 were isolated from the swine samples collected in the Kostanay oblast.

The results of determining hemagglutinin subtypes of new influenza virus in the HAI assay are presented in table 2.

As seen from table 2, hemagglutinating activity of four isolates from humans (04/18, 05/18, 14/18, and 15/18) was inhibited by immune serums against A/H1N1 and A/H1N1pdm viruses (from 1/4 to homologous titer), that of three viruses (06/18, 07/18, and 08/18) by serum against influenza type B virus (from 1/2 to homologous titer). Hemagglutinating activity of Karaganda/16/18 isolate was inhibited to 1/2 of homologous titer by serum against A/H3N2 virus.

Table 2 – Identification of hemagglutinin subtypes in influenza virus isolates obtained from humans and swine during 2017-2018 in the HAI assay

Isolate	Antihemagglutinin titer with diagnostic serums against reference strains			
	A/H1N1	A/H1N1pdm	A/H3N2	type B
Almaty/04/18	40	80	< 20	< 20
Almaty /05/18	80	< 20	< 20	< 20
Almaty /06/18	< 20	< 20	< 20	160
Almaty /07/18	< 20	< 20	< 20	80
Almaty /08/18	< 20	< 20	< 20	80
Karaganda/14/18	40	160	< 20	< 20
Karaganda/15/18	40	80	< 20	< 20
Karaganda/16/18	< 20	< 20	80	< 20
swine/Kostanay/471/18	40	20	< 20	< 20
swine/Kostanay/522/18	80	20	< 20	< 20
Homologous titer of serums against reference viruses	160	160	160	160
Note. The reciprocals of antibody titers are presented here and in table 3.				

Hemagglutination of two swine isolates from the Kostanay oblast (471/18 and 522/18) was inhibited from 1/2 to 1/8 of homologous titers by immune serum against a reference virus with antigenic formula A/H1N1.

Identification of the neuraminidase subtype of influenza A virus isolates in the NAI assay is presented in table 3.

Table 3 – Identification of neuraminidase subtype of Kazakhstan influenza A virus isolates of 2017-2018 in neuraminidase activity inhibition assay

Isolate	Anti-neuraminidase antibody titer with immune serums against viruses	
	H1N1	H3N2
Almaty/04/18	100	< 20
Almaty/05/18	100	< 20
Karaganda/14/18	100	< 20
Karaganda /15/18	100	< 20
Karaganda /16/18	< 20	100
swine/Kostanay/471/18	100	< 20
swine/Kostanay/522/18	100	< 20

As seen from table 3, enzymatic activity of isolates with hemagglutinin A/H1 was inhibited by polyclonal diagnostic serum against A/H1N1 virus, with hemagglutinin A/H3 by serum against A/H3N2 virus.

Identification carried out in the HAI and NAI assays showed that human isolates belong to influenza viruses with the antigenic formula A/H1N1 (Almaty/04/18, Almaty/05/18, Karaganda /14/18, Karaganda/15/18), A/H3N2 (Karaganda/16/18) and to influenza type B viruses (Almaty/06/18, Almaty/07/18 and Almaty/08/18). Two HAAs isolated from swine (swine/Kostanay/471/18, swine/Kostanay/522/18) were classified as A/H1N1 influenza viruses.

The results of serological testing of 60 serums in ELISA and HAI assay collected from humans in different regions of Kazakhstan are presented in figure 1.

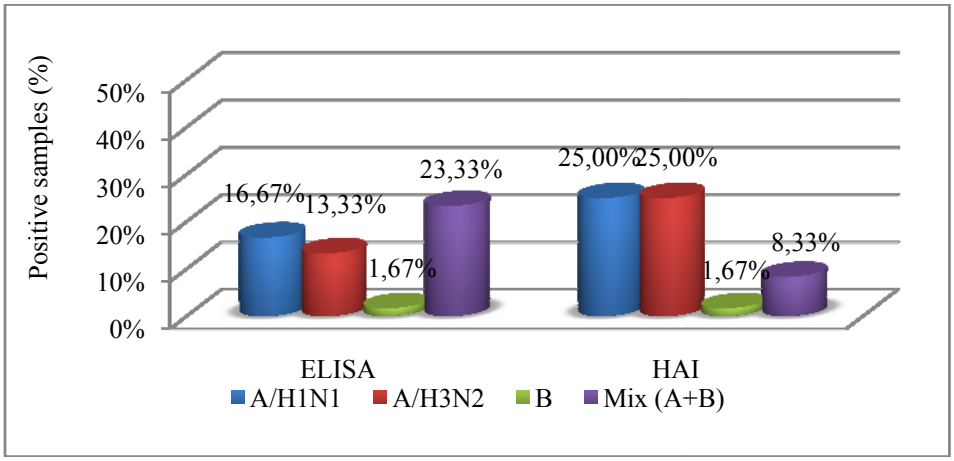

Figure 1 – Detection of antibodies against influenza viruses in human serums in ELISA and HAI assay

As seen in figure 1, in ELISA antibodies against A/H1N1 influenza virus were detected in 16.67% of cases (10 samples), against A/H3N2 virus in 13.33% (8 samples), against influenza B virus in 1.67% (1 serum). In 23.33% of cases (14 samples), antibodies simultaneously against two influenza viruses (A+B) were found in serums.

In the HAI assay antihemagglutinins against influenza A/H1N1 virus, as well as A/H3N2 virus, were detected in 25.00% of the total number of samples (15 samples each), and in 1.67% (1 sample) against influenza B type virus. The antibody titers were of 1:40 - 1:160. Seropositive serums simultaneously

against influenza A and B viruses were detected in 8.33% of cases (5 samples) with antibody titers of 1:80 - 1:160.

The results of serological testing for influenza of 39 serums collected from swine in the Kostanay oblast are presented in figure 2.

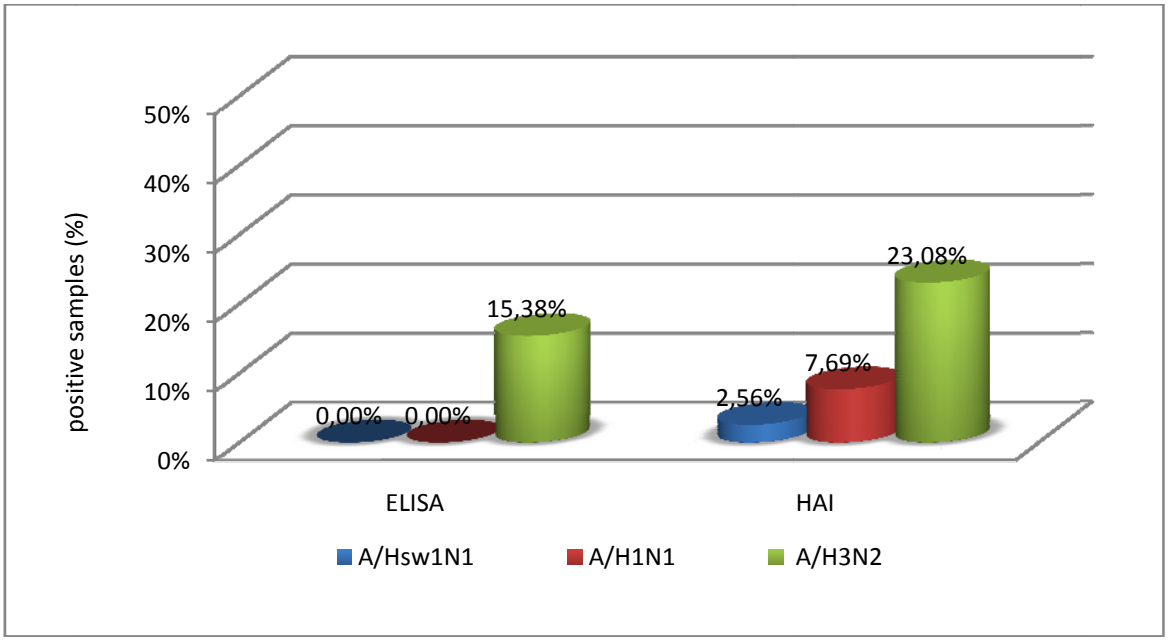

Figure 2 – Detection of antibodies against influenza viruses in swine serums in ELISA and HAI assay

As seen in figure 2, in ELISA antibodies only against influenza A/H3N2 virus were detected in swine serums in 15.38% (6 samples).

In the HAI assay antihemagglutinins against A/Hsw1N1 virus were detected in 2.56% (1 sample) of the total number of examined serums, against A/H1N1 in 7.69% (3 samples), and against A/H3N2 in 23.08% (9 samples). The antibody titers were of 1:20 - 1:40.

The results of primary screening of nasopharyngeal swabs in RT-PCR and serological studies of blood serums therefore indicate co-circulation of influenza A/H1N1 and A/H3N2 and B viruses among humans, influenza A/H1N1 and A/H3N2 viruses among swine in different regions of Kazakhstan during 2017–2018.

Discussion. Literature data suggests that interspecies transmission of human and swine influenza A (H1N1) viruses is an important factor in studying the evolution, ecology, and epidemiology of pathogens. There are theoretical substantiations of the possibility of interspecific transmission of influenza A virus between birds and marine animals, birds and swine, seals and humans, swine and humans [13, 14]. The appearance of reassortants with new qualities and the mechanisms that cause the adaptation of animal and avian influenza viruses to the human body are still largely unknown.

Today, the most acute problem is influenza caused by the new virus A/H1N1 (the so-called swine influenza). This virus is a typical emergent infection (from English “emergency” which means a sudden, unpredictable case), with the possibility of transmitting a known pathogen to a new host. Swine influenza can acquire pandemic proportions [15].

The unpredictable variability of influenza A viruses does not allow any prognosis regarding “swine” influenza virus, since analysis of its pathogenic properties has shown that the evolution of this virus can follow the path of recovering some signs of pathogenicity, and this, in turn, can lead to transition of this virus to the category of highly pathogenic [9].

In this regard, the most important areas in the fight against influenza are the obtainment of new data on the evolution of influenza pathogens that contribute to the effective infection surveillance, as well as timely diagnosis of the pathogen and disease prevention.

Conclusions. Primary screening of 521 nasopharyngeal swabs in RT-PCR and serological studies of 99 blood serums in the haemagglutination inhibition assay and enzyme immunoassay indicate co-circulation of influenza A/H1N1 and A/H3N2 and B viruses among humans, influenza A/H1N1 and A/H3N2 viruses among swine in different regions of Kazakhstan during 2017-2018.

The results of virological and serological studies highlight the need for continuous monitoring of the circulation of influenza viruses among humans and swine in Kazakhstan to identify interspecies transmission of the infectious agent.

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2017–2018 Ж. ҚАЗАҚСТАН АУМАҒЫНДАҒЫ АДАМ ЖӘНЕ ШОШҚА АРАСЫНДА ТҰМАУ ВИРУСЫНЫҢ АЙНАЛЫМЫ

Аннотация. 2017-2018 ж. індет аралығында Қазақстан Республикасының әр түрлі аймақтарындағы емдік мекемелерінен 251 биосынама (191 мұрын-жұтқыншақ сынамасы және 60 қан сарысуы) алынды. Шошқа шаруашылықтарынан 369 материал (330 танау-жұтқыншақ және 39 қан сарысуы) жиналды.

Полимеразды тізбекті реакциясында адамдардан жиналған 191 сынамада А вирусының генетикалық материалы 14,14 % анықталды, В тұмау вирусы 5,24 % құрады. Субтиптеу кезінде A/H1N1 тұмау вирусының РНҚ 4,71% сынамада анықталды, A/H3N2 – 4,19%. Шошқалардан жиналған 330 сынамадан тұмау вирусының генетикалық материалы 3,33% анықталды, солардың ішінде A/H1N1 тұмау вирусының РНҚ - 2,12%, құраса, A/H3N2 тұмау вирусы – 1,21%.

Полимеразды тізбекті реакциясында алынған нәтиже, сонымен қоса иммуноферментті талдау және гемагглютинин тежеу реакциясында серологиялық зерттеу нәтижелері, 2017-2018 жылдары Қазақстан Республикасының әр түрлі аймақтарындағы адамдар арасында A/H1N1, A/H3N2 және В тұмау вирустарының айналымын көрсетеді, ал шошқалар арасында A/H1N1 және A/H3N2 тұмау вирустарын көрсетеді.

Адам және шошқалардан жиналған биосынамаларды тауық эмбриондарында вирусологиялық зерттеу нәтижесінде антигендік формуласы A/H1N1, A/H3N2 және В болып табылатын 10 гемагглютининдеуші агент бөлініп алынды.

Вирусологиялық және серологиялық зерттеу нәтижелері, Қазақстан аумағындағы адам және шошқа арасында инфекция қоздырғышның түр аралық берілу мүмкіндігін айқындау үшін, тұмау вирусының айналымына үнемі мониторинг жасаудың маңыздылығын көрсетеді.

Түйін сөздер: тұмау вирусы, айналым, изолят, ПТР-диагностикасы, гемагглютинин, нейраминидаза, қан сарысуы.

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ЦИРКУЛЯЦИЯ ВИРУСОВ ГРИППА СРЕДИ ЛЮДЕЙ И СВИНЕЙ НА ТЕРРИТОРИИ КАЗАХСТАНА В 2017–2018 ГГ.

Аннотация. В эпидемические сезоны 2017–2018 гг. в различных регионах Республики Казахстан от людей в лечебных учреждениях получена 251 биопроба (191 носоглоточный смыв и 60 сывороток крови). В свиноводческих хозяйствах собрано 369 материалов (330 носоглоточных смывов и 39 сывороток крови).

В полимеразной цепной реакции в 191 образце, собранном от людей, генетический материал вируса гриппа А был обнаружен в 14,14% случаев, вируса гриппа В – в 5,24%. При субтипировании РНК вируса гриппа A/H1N1 идентифицирована в 4,71% проб, A/H3N2 – в 4,19%. В 330 образцах, полученных от свиней, генетический материал вируса гриппа был обнаружен в 3,33% случаев, из них РНК вируса гриппа A/H1N1 выявлена в 2,12%, вируса гриппа A/H3N2 – в 1,21%.

Результаты полученные в полимеразной цепной реакции, также как и данные серологических исследований в реакции торможения гемагглютинации и иммуноферментном анализе, указывают на социркуляцию среди людей в 2017–2018 гг. в различных регионах Казахстана вирусов гриппа A/H1N1 и A/H3N2 и В, среди свиней – вирусов гриппа A/H1N1 и A/H3N2.

При вирусологическом исследовании биопроб, полученных от людей и свиней, на куриных эмбрионах выделено 10 гемагглютинирующих агентов, идентифицированных как вирусы гриппа с антигенными формулами A/H1N1, A/H3N2 и В.

Результаты вирусологических и серологических исследований свидетельствуют о важности проведения постоянного мониторинга циркуляции вирусов гриппа среди людей и свиней на территории Казахстана для выявления возможности межвидовой передачи возбудителя инфекции.

Ключевые слова: вирус гриппа, циркуляция, изолят, ПЦР-диагностика, гемагглютинин, нейраминидаза, сыворотка крови.

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