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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.

Қазақстан Республикасы Ұлттық ғылым академиясы "ҚР ҰҒА Хабаршысы" ғылыми журналының Web of Science-тің жаңаланған нұсқасы Emerging Sources Citation Index-те индекстелуге қабылданғанын хабарлайды. Бұл индекстелу барысында Clarivate Analytics компаниясы журналды одан әрі the Science Citation Index Expanded, the Social Sciences Citation Index және the Arts & Humanities Citation Index-ке қабылдау мәселесін қарастыруда. Web of Science зерттеушілер, авторлар, баспашылар мен мекемелерге контент тереңдігі мен сапасын ұсынады. ҚР ҰҒА Хабаршысының Emerging Sources Citation Index-ке енуі біздің қоғамдастық үшін ең өзекті және беделді мультидисциплинарлы контентке адалдығымызды білдіреді.

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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PHYLOGENETIC ANALYSIS OF GENES OF SURFACE PROTEINS OF HIGHLY PATHOGENIC INFLUENZA A SUBTYPE H5N1 VIRUS, ISOLATED FROM GULL IN KAZAKHSTAN

Abstract. The article presents the results of phylogenetic analysis of surface protein hemagglutinin and neuraminidase genes of influenza A virus of H5N1 subtype isolated from gull in the territory of the Republic of Kazakhstan in 2015. Cluster affiliation of Kazakhstan isolate and relationship with other influenza viruses of A/H5 subtype from international database GenBank are presented. It is concluded that it is necessary to continuously monitor the avifauna of Kazakhstan in order to isolate current variants of pathogens.

Key words: influenza virus, bird, hemagglutinin, neuraminidase, H5 subtype, cluster, phylogenesis.

Birds of water and near-water complexes play a major role in the conservation of influenza A viruses in the biosphere. All known for science species of pathogens that caused pandemics and epizootics were isolated from birds, they are a reservoir and a source of new variants of pathogens [1-3].

Influenza A viruses during the evolutionary development and overcoming of interspecies barriers passed and adapted to humans and mammal animals - horses, pigs, dogs. They also cause sporadic infections in other mammals - mink, muskrat, cats, tigers, leopards [4, 5]. The influenza viruses of B genus were isolated from seals and those of C type - from pigs [6, 7]. In 2011, two antigenically and genetically different lines of D viruses were identified that infect cattle [8-11].

The global and uncontrolled spread of influenza is due to the unique variability of the pathogen, which is based on both point mutations characteristic for RNA-containing viruses and reassortment of genes. The most variable structural components of the virus particle are surface glycoproteins - hemagglutinin (HA) and neuraminidase (NA).

Classification of influenza A viruses is based on antigenic differences between HA and NA, detected in serological reactions by means of immune sera, and also in PCR using specific primers; To date, 18 subtypes of HA and 11 - NA have been identified. Combinations of these surface glycoproteins in the composition of the virion determine the subtype of the virus: H1N1, H2N2, H3N2, etc. [12]. The last identified taxonomic groups are influenza A (H17N10) and A (H18N11) viruses isolated from bats in Central America [13, 14]. Due to their lack of haemagglutinating and neuraminidase activity, some

authors propose to designate them as "NA-like" and "NA-like" (or "HL17NL10" and "HL18NL11", respectively) [15].

In the Republic of Kazakhstan in 2014-2016, eight isolates of influenza A/H5 virus were isolated from wild birds [16]. The purpose of this study was to determine the taxonomic affiliation of the Kazakhstani isolate of influenza A (H5N1) virus isolated from wild bird in Western Kazakhstan.

Materials and methods. Viruses. A/black-headed gull/Atyrau/6491/15 (H5N1) influenza virus, isolated from gull in Western Kazakhstan, was cloned and passaged by limiting dilutions on 10-11-day embryonated chicken eggs according to a conventional technique.

Extraction of RNA was performed using the QIAamp Viral RNA Mini kit (Qiagen GmbH, Hilden) in accordance with the manufacturer's recommendations.

Complementary DNA from RNA was obtained by reverse transcription using the universal primer uni-12 for influenza A viruses from the First Strand cDNA Synthesis kit (Fermentas) according to the manufacturer's instructions.

DNA was sequenced in LP "SPC of Microbiology and Virology" using terminating dideoxynucleotides on an automatic 8-capillary sequencer ABI 3500 DNA analyzer (Applied Biosystems).

The alignment of the sequences of the influenza A virus genome with the complete nucleotide sequences of those from the international database was carried out using the BioEdit computer program.

Phylogenetic analysis and building of the trees was carried out with the help of BioEdit and MEGA programs versions 4-6 by the method of "joining neighbors" with bootstrap values based on 1000 replicates [17].

Results and discussion. After sequencing of DNA copies of fragments of HA and NA genes of the Kazakhstan isolate, their alignment with corresponding nucleotide sequences of viruses of this subtype from the international bank GenBank was carried out. The size of the influenza A/black-headed gull/Atyrau/6491/15 (H5N1) virus gene segments and their access numbers in the GenBank international genetic bank are shown in table 1.

Characterization of gene segments and access numbers in GenBank to the Kazakhstani isolate of the influenza virus with haemagglutinin H5

Isolate (Russian and English name)	Gene	Size (pairs of nucleotides)	GenBank №
А/черноголовый хохотун/Атырау/6491/15 (H5N1) [A/black-headed gull/Atyrau/6491/15 (H5N1)]	HA	700	GU953249
	NA	649	GU953250

The phylogenetic tree constructed on the basis of homology of nucleotide the segments of genes of Kazakhstan isolate with similar sequences of influenza subtype H5 viruses from international database GenBank database, is shown in figure 1.

As can be seen from figure 1, virus A/black-headed gull/Atyrau/6491/15 (H5N1) for the HA gene is closest to virus from India A/duck/India/11CA08/2014 (H5N1) and two strains from Nigeria and the Republic of Côte d'Ivoire - A/chicken/Nigeria/15VIR339-1/2015 (H5N1) and A/duck/Ivory_Coast/15VIR2742-2/2015 (H5N1), with which it is included in the 2.3.2.1C clade.

Results of the analysis of phylogenetic relationships of NA genes of A/black-headed gull/Atyrau/6491/15 (H5N1) influenza virus and those of A/H5 viruses circulating in various regions of the world are reflected in the form of a dendrogram in figure 2.

As follows from figure 2, Kazakhstan isolate A/blackhead gull/Atyrau/6491/15 (H5N1) has the greatest relationship with viruses from Niger and India isolated from poultry - A/chicken/Niger/15VIR2060-8/2015 (H5N1), A/duck/India/12TP04/2014 (H5N1). Together with six other viruses from Lebanon, China and Vietnam, they are related to genotypes G, V, Z by the HA gene. It is interesting to note that one of them A/tiger/Jiangsu/01/2013 (H5N1) is isolated from a predatory mammal.

Influenza A viruses of the H5 subtype occupy a special place in modern epidemiology. Previously, it was believed that avian influenza pathogens, as a rule, do not spread among humans. The events that took place at the turn of the 21st century in the countries of Southeast Asia and on the European continent have refuted this view. The "bird flu" H5N1 was released to the epidemic arena, according to WHO data, from

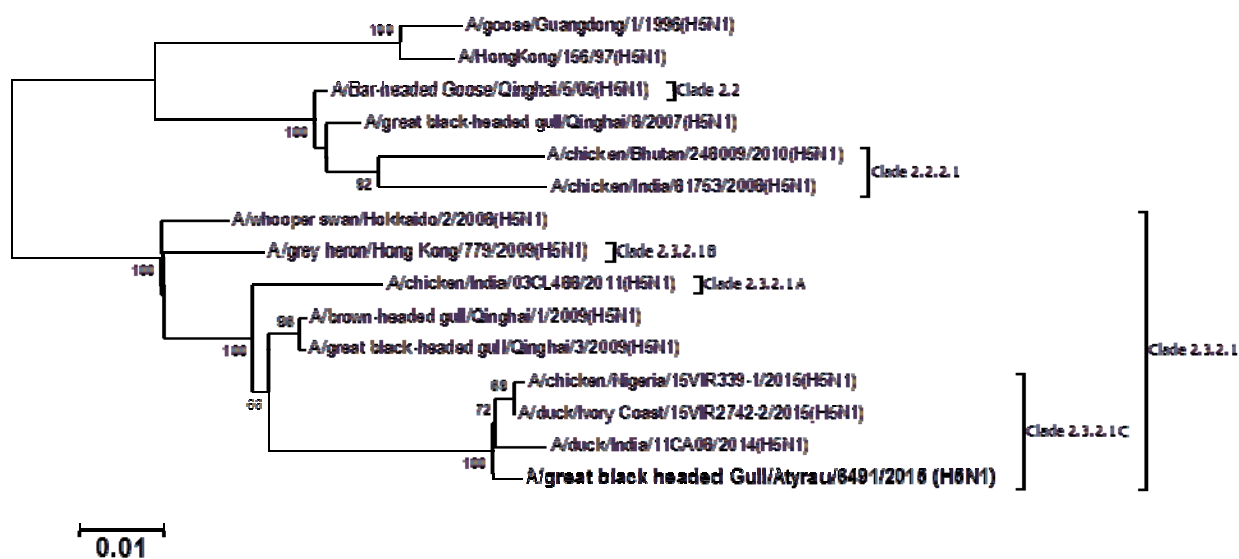


Figure 1 – The phylogenetic relationship between the HA genes of A/black-headed gull/Atyrau/6491/15 (H5N1) virus and viruses of this subtype from GenBank

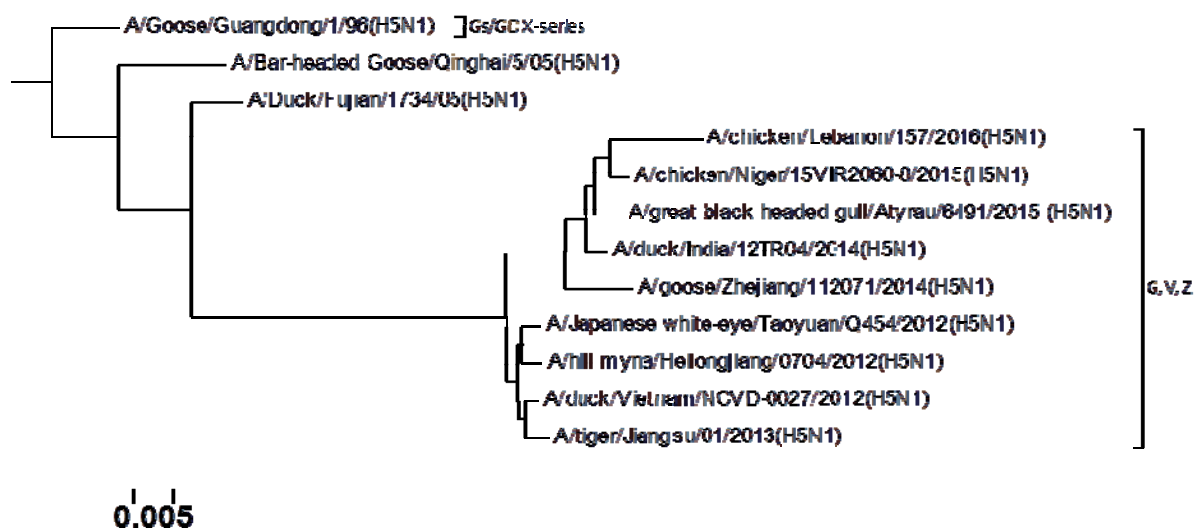


Figure 2 – Phylogenetic relationships between NA genes of A/black-headed gull/Atyrau/6491/15 (H5N1) virus and viruses of this subtype from GenBank.

May 1997 to March 2, 2018, out of 860 cases with officially confirmed diagnosis of influenza A (H5N1) in humans, 454 resulted in death [18]. In 2013, three new avian influenza A (H7N9), A (H6N1), and A (H10N8) viruses appeared that could infect humans. Virus A (H7N9) caused epidemics in 19 Chinese provinces, two other pathogens infected only a few individuals [19].

Since the first detection in humans in Hong Kong in 1997, A/goose/Guangdong/1/96-line viruses of subtype H5 (GDL) have been developed with the help of drift and shift mechanisms. At the present time, on the basis of the phylogeny of HA, GDL viruses are divided into 10 clades, designated 0-9, many of which contain additional subclades [18, 20, 21]. Their initial circulation for about 5 years, was limited to the region of Southeast Asia, later they spread in many countries of Asia, Europe, Africa and subsequently in North America [22].

The evolution of GDL viruses is marked by the spread of a number of clades in different geographic regions in which their evolution took place. An example is Egypt and Indonesia, where the virus lines specific to these countries developed. In 1997-2001, in China, a rapid emergence of the genotypic

diversity of these infectious agents in process of reassortment with other viruses from the bird reservoir was noted. Thus, in 2001, during the survey of domestic birds in the southeast of the PRC, up to six genotypes of viruses were revealed [23]. Their differentiation is based on a partial sequences of genomes, in particular, polymerase genes. In 2002, a new genotype Z appeared, spreading in 2004 to Cambodia, Japan, the Lao People's Democratic Republic, the Republic of Korea, Thailand and Vietnam, with the last two countries reporting human cases [24, 25]. Almost all of these genotypes arose as a result of two reassortations; the first generated viruses of genotypes B, Z and W, the second - viruses of the X genotype. All modern genotypes are the result of further reassortment of earlier viruses (for example, genotypes G and V originated from genotype Z). Two main cases of genotype replacement were observed in China - from genotype B to Z in 2002 and from genotype Z to V in 2005. Both replacements are associated with an increase of H5N1 activity in this region. It was found that viruses of clade 2.3.4 (Fujian-like), formerly belonging to genotype Z [26], are now defined as genotype V.

An important event in the evolution of GDL viruses in 2005 was their first advance from Southeast Asia towards the African continent. It began with the mass death of wild birds on Lake Kukunor (Qinghai Province) in China, caused by a virus different from genotype Z, and later classified as clade 2.2 [27, 28], then this pathogen was discovered in Russia, Kazakhstan, some European countries, further on the African continent in Egypt, in a number of West African countries, and also isolated in India and Bangladesh [29, 30].

The next significant change in the epidemiology of GDL occurred also in South-East Asia and consisted in the appearance of two separate lines of the virus. These lines 2.3.2 and 2.3.4, co-circulated for some time, eventually the line 2.3.2 became dominant and subsequently divided into groups during the genetic drift. Like the viruses of group 2.2, several years before, viruses 2.3.2 (2.3.2.1a) spread to the west, in mid-2009 they were found in Russia and then in Europe [31]. They penetrated into India and Bangladesh and completely replaced representatives of line 2.2, which have been endemic in these countries since 2005 [32]. In 2015, clade 2.3.2.1c viruses were isolated in Russia, eventually, they occurred, bypassing Egypt, in western Africa.

The latest event in the evolution of GDL H5 was the unexpected and wide-scale spread of clade 2.3.4.4 viruses. Most of them do not belong to the subtype H5N1 and contains other NA, including N2, N6, N8 [33]. Reports about their isolation came from countries in Asia, Africa, and Europe, at the end of 2014 they infected for the first time bird populations in the American continent. The modern epizootic situation is dynamic, variable and characterized by the spread of representatives of the group 2.3.4.4 in Egypt and in the regions of West Africa, where previously the viruses of other clades were endemic. Soon after the occurrence of H5N8 viruses of 2.3.4.4b clade in Hungary and Poland, they were found among wild birds in Germany, where from November 2016 to September 2017 caused more than 1500 cases of infection of wild birds and 107 outbreaks among poultry [34].

In general, the results obtained indicate that important, highly pathogenic influenza viruses are circulating in the wild ornithofauna of Kazakhstan. Inhabiting a huge number of migratory birds on the territory of the republic and their species diversity explains the need for constant virological monitoring in order to isolate current variants of pathogens.

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ҚАЗАҚСТАНДА ШАҒАЛАДАН БӨЛІП АЛЫНҒАН ТИПТАРМАҒЫ H5N1 ЖОҒАРЫПАТОГЕНДІ ТҰМАУ А ВИРУСЫНЫҢ БЕТКЕЙЛІК АҚУЫЗДАРЫ ГЕНДЕРІН ФИЛОГЕНЕТИКАЛЫҚ ТАЛДАУ

Аннотация. Мақалада 2015 жылы Қазақстан Республикасы аумағында шағаладан бөліп алынған тұмау А вирусының H5N1 типтармағының беткейлік – гемагглютинин және нейраминидаза ақуыздары гендерін филогенетикалық талдау нәтижелері келтірілген. Қазақстандық бөліндінің кластерлік тегі және GenBank халықаралық деректер қорындағы тұмау вирусының басқада А/Н5 типтармақтарының байланысы анықталды. Қоздырушылардың ағымдағы нұсқаларын бөліп алу мақсатында Қазақстан орнитофаунасына үздіксіз мониторинг жүргізу қажет екендігіне қорытынды жасалады.

Түйін сөздер: тұмау вирусы, құс, гемагглютинин, нейраминидаза, H5 типтармағы, кластер, филогенез.

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