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PHYLOGENETIC RELATIONSHIPS OF HEMAGGLUTININ GENE OF KAZAKHSTAN INFLUENZA A VIRUS ISOLATES (H13N6)

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Summary

The results of phylogenetic analysis of the hemagglutinin gene of influenza A virus strains A/Great Black-headed Gull/Atyrau/773/04 and A/Great black-headed Gull/Atyrau/2966/08 (H13N6), isolated in different years from the same colony of gulls are presented. It was shown that the both Kazakhstan isolates belong to the H13N6 viruses of North American-Eurasian lineage ascending to the prototype strain A/gull/Maryland/704/77.

Currently in the literature there are plenty of reports on the isolation of influenza A viruses from the gulls [1, 2]. Ecological peculiarities of gull species associated with high population density on breeding colonies, possible contact with mammals, as well as with other families of birds through the flyways determine their role and significance as a reservoir of influenza virus in animals in nature. Influenza A viruses of subtype H13 were isolated for the first time in 1976-77 from the Delaware and the Franklin gulls in the United States [3]. In that period of time, virus of the same subtype was isolated from Laridae species in the Caspian Sea [4].

In the Northern Caspian between 1976-1999 during virological survey over 3,000 individuals of 37 species of birds belonging to the families Laridae, Charadriidae, Anatidae, Ardeidae, Phalacrocoracidae were isolated 288 strains of influenza A virus. Among the isolates dominated strains with the antigenic formula H13N6, whose hemagglutinin (HA) was characterized with high degree of antigenic variability. According to S. Yamnikova et al. [5] since 1976 annually increased the number of isolates that were not recognized by monoclonal antibodies to the prototype strain of this subtype A/gull/Maryland/704/77.

As a result of virological examination 2072 samples collected from wild birds in the Kazakhstan part of the Caspian Sea in 2002-2008., we have isolated 58 isolates of influenza A virus from representatives of orders: Podicipediformes, Anseriformes, Gruiformes, Charadriiformes, Passeriformes [6].

Influenza A virus isolates were characterized by different combinations of the HA and neuraminidase (NA) N4N6, H5N1, H11N2, H13N6, N16N3 with subtype N13N6 as dominating. The majority number of viruses with H13 subtypes were solated from chicks in the colonies of Great black-headed gulls (Larus ichtyaetus) on the islands of the Northern Caspian Sea after month post-hatching period in late June - early July. Influenza A viruses of this subtype caused sporadic death of young gulls at the age of juvenile flight plumage in 2002, 2004 and 2008.

The aim of this study was phylogenetic characterization of HA gene of influenza virus subtype H13N6 strains isolated in different years from the same one breeding colony of great black-headed gulls.

Materials and Methods

Collection of materials from wild waterfowl and wetland ecological complexes was performed in 2002-2008. on the coastal area and islands in the northern and eastern parts of the Caspian Sea, in the delta. Urals.

Biological samples as cloacal and tracheal swabs were obtained from birds during the breeding season, the spring and autumn migrations. Samples collected with sterile cotton swabs then placed into viral transport storage 199 medium containing the antibiotic complex (2000 units penicillin/ml, streptomycin 2 mg/ml, gentamicin 50 ug/ml 50 units of nystatin/ml) and bovine serum albumin at a final concentration of 0.5%. Samples in liquid nitrogen or at -80° C stored until virology analysis.

Virus isolation was performed by inoculating each sample of the material in the allantoic cavity of three 10-11-day-old chick embryos, followed by incubation at +35 ° C for 48 hours. Allantoic fluid was harvested and tested for presence of virus in the micro HA assay with 0.5% suspension of chicken red blood cells. The hemagglutinating agents identified by diagnostic set Directigen Flu A (Becton Dickinson, USA) and verified on RT-PCR with primer pairs to the M gene of influenza virus A.

Antigenic formula of influenza A virus isolates was determined in hemagglutination inhibition (HI) and neuraminidase activity (NI) assays using a set of diagnostic polyclonal and monospecific sera for 16 subtypes of HA and 9 subtypes of NA, according to the WHO recommendations [7].

Isolation of viral RNA was performed using a set of QIAamp Viral RNA Mini Kit (Qiagen GmbH, Hidden), according to manufacturer's recommendations from 140 ul of virus-containing allantoic fluid [8].

Reverse transcription was performed with reverse transcriptase AMV (Promega, Madison) with primers Uni 12 (agcaaaagcagg).

Amplification of cDNA was performed using the primers recommended for sequencing segments of the genome of influenza A viruses [9] and set the Expand High Fidelity PCR System (Roche Diagnostics, Manheim, Germany) according to the manufacturers protocol.

For sequencing cDNA the method of Sanger dideoxysequencing was used [10]. DNA sequencing was performed on an automated 96-capillary sequencer Genetic Analyser 3730 xl, Applied Biosystems according to the instruction. Alignment of sequenced genes of influenza A viruses with complete nucleotide sequences of viruses belonging to American and Eurasian lines was carried out by a computer program BioEdit. Phylogenetic trees were constructed with the program MEGA version 4 by the "neighbor joining" with Bootstrap values based on 1,000 repetitions. The system described by S.S. Yamnikova et al. was used to indicate the clusters [ ].

Results and Discussion

Performed sequencing of the coding region of the gene HA isolates of influenza A virus subtypes with H13, allocated in 2004 and 2008 from wild waterfowl in the North Caspian Sea.

The nucleotide sequences of the studied genes were compared with those of strains of an international data bank GenBank(Figure 1) [NCBI Influenza Virus Research Database; http://www.ncbi.nlm.nih.gov/genomes/FLU/Database/nph-

Percent Identity

1	2	3	4	5	6	7	8	9	10	11	12	13
14	15	16	17	18								
1	98,9	98,7	97,7	95,9	93,7	92,8	92,8	78,3	77,9	77,8	77,6	77,5
77,4	77,4	77,4	77,4	76,8	1	A/gb-headed_gull/Atyrau/2966/08 (H13N6)						
21,1		99,0	97,9	96,3	94,0	93,1	93,0	78,3	77,9	77,7	77,6	77,5
77,7	77,2	77,4	77,4	76,7	2	A/Mongolian_gull/Mongolia/405/07 (H13N6)						
31,3	1,0		98,0	96,2	93,9	93,0	92,8	78,0	77,6	77,4	77,2	77,2
77,1	76,9	77,1	77,1	76,4	3	A/herring gull/Norway/10_2336/06 (H13N6)						
42,4	2,2	2,1		97,2	94,7	93,9	93,5	78,7	78,2	78,1	77,8	77,9
77,9	77,5	77,8	77,8	76,9	4	A/gb-headed gull/Atyrau/773/04(H13N6)						
54,2	3,9	4,0	2,9		96,4	95,4	95,1	78,2	77,8	77,8	77,3	77,6
77,4	77,2	77,4	77,4	76,4	5	A/duck/Siberia/272/98 (H13N6)						

6	6,7	6,4	6,6	5,6	3,8		98,5	98,2	78,6	78,3	78,0	77,8	77,5
	77,7	77,7	78,1	78,1	77,0	6	A/gull/Astrakhan/226/84 (H13N6)						
7	7,8	7,4	7,5	6,5	4,8	1,5		98,9	78,9	78,6	78,2	77,9	77,8
	77,7	78,1	78,5	78,5	77,4	7	A/Larus ichthyaetus/Astrakhan/10/88 (H13N6)						
8	7,8	7,6	7,8	6,9	5,2	1,9	1,1		78,8	78,4	77,7	77,6	77,4
	77,6	77,7	78,1	78,1	77,1	8	A/gull/Astrakhan/998/90 (H13N6)						
9	26,5	26,5	27,0	25,9	26,7	26,1	25,6	25,8		97,7	96,6	93,3	93,7
	91,6	93,4	97,1	97,1	92,0	9	A/gull/Astrakhan/1314/79 (H13N2)						
10		27,2	27,2	27,7	26,7	27,2	26,4	25,9	26,3	2,3		98,5	94,3
	95,1	92,4	94,6	99,2	99,2	92,8	10	A/gb-headed gull/Astrakhan/591/82(H13N2)					
11		27,3	27,4	27,9	26,7	27,3	26,9	26,6	27,4	3,6	1,6		94,7
	95,4	92,9	95,4	97,7	97,7	93,2	11	A/gull/Astrakhan/176/86 (H13N2)					
12		27,6	27,6	28,1	27,3	28,0	27,3	27,0	27,5	7,2	6,1	5,6	
	97,7	94,8	95,6	93,7	93,7	95,4	12	A/b-headed/gull/Netherlands/1/00 (H13N8)					
13		27,7	27,7	28,2	27,1	27,5	27,6	27,1	27,7	6,8	5,2	4,8	2,3
	95,1	96,5	94,4	94,4	95,9	13	A/gull/Astrakhan/1818/98 (H13N6)						
14		27,8	27,4	28,3	27,1	27,9	27,4	27,4	27,5	9,1	8,2	7,6	5,5
	5,2		94,1	91,8	91,8	94,7	14	A/b-headed gull/Mongolia/1766/06 (H13N6					
15		27,9	28,1	28,6	27,7	28,0	27,3	26,6	27,3	7,1	5,7	4,9	4,6
	3,6	6,3		94,0	94,0	94,7	15	A/black-headed gull/Sweden/1/99 (H13N6)					
16		27,9	27,9	28,4	27,4	27,9	26,7	26,2	26,8	3,0	0,8	2,4	6,7
	5,9	8,9	6,4		100,0	92,2	16	A/Larus ichthyaetus/Astrakhan/75/83 (H13N2)					
17		27,9	27,9	28,4	27,4	27,9	26,7	26,2	26,8	3,0	0,8	2,4	6,7
	5,9	8,9	6,4	0,0		92,2	17	A/gb-headed gull/Gurjev/76/83 (H13N2)					
18		28,8	29,0	29,5	28,6	29,4	28,4	27,7	28,3	8,8	7,7	7,3	4,9
	4,3	5,6	5,6	8,5	8,5		18	A/Larus minutus/Astrakhan/3357/02 (H13N6)					

Figure 1 - The similarities and differences of the Kazakhstan A/H13 influenza virus wild bird isolates HA genes with strains from GenBank

As can be seen from Figure 1, the Kazakhstan isolate A/Great Black-headed Gull/Atyrau/2966/08 showed the highest similarity (98.9%) with viruses A/Mongolian gull/Mongolia/405/07 and A/herring gull/Norway/10_2336/06 (98.7%). Kinship of the Kazakhstan isolates 2966/08 and 743/04 between each other was 97.2%.

Phylograms in Figure 2 shows that, within subtype H13 the formation of four lineages is traced - two genetically distant from each other by the American lineages, one Eurasian, and having a significant divergence from their lineage consisting of viruses of different hemisphere. Within the last lineage except Eurasian viruses there were strains isolated in North America: A/American white pelican/Minnesota/AI-07-1819/2007 (line H13A), A/gull/Maryland/704/1977 (H13D), as well as in South America (A/kelp gull/Argentina/LDC4/2006).

Figure 2 - Phylogenetic relationships between genes on the Kazakhstan A/H13 isolates of influenza viruses and viruses of this subtype from GenBank.

It is obvious that the formation of the Eurasian lines is not associated with the geographic or time division, as both are presented predominantly by isolates from the North Caspian period 1980-90-ies. Kazakh isolates of A/Great Black-headed Gull/Atyrau/2966/08 and A/Great Black-headed Gull/Atyrau/773/04 entered H13D group consisting of the North Caspian, Siberian and Norwegian isolates genetically related to the reference strain.

For the American lineage a temporary division is characteristic, as H13B lineage formed by viruses phylogenetically related to the Eurasian lineage H13A isolated in 2004, 2006 and 2008. American lineage H13C is an isolated and consists entirely of viruses 1980s along with going back to him strain A /ruddy turnstone/New Jersey/1407/2001.

Thus, closely related influenza viruses - A/Great Black-headed Gull /2966/08 (H13N6) and A /Great Black-headed Gull/Atyrau/773/04 (H13N6), isolated in Kazakhstan, together with strains of Mongolia (2007), Norway (2006), Siberia (1998) and Astrakhan isolates from late 1980s by HA gene formed American - Eurasian group of viruses that are phylogenetically ascending to the reference strain A/gull/Maryland/704/1977. In the HA structure of Kazakhstan isolates of influenza virus subtype H13 eight potential glycosylation sites at positions 29 (NSSE), 54 (NHTG), 181 (NNTT), 182 (NTTG), 304 (NRTF), 487 (NDSC), 496 (NGTY), 556 (NGSC), are contained which allows them to overcome the effect of specific neutralizing antibodies and contributes to their further circulation in wild bird populations.

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ТҰМАУ А(H13N6) ВИРУСЫНЫҢ ҚАЗАҚСТАНДЫҚ БӨЛІНДІЛЕРІНІҢ ГЕМАГГЛЮТИНИН ГЕНІНІҢ ФИЛОГЕНЕТИКАЛЫҚ ӨЗАРА-БАЙЛАНЫСТАРЫ

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Түйін

Мақалада шағала тәрізді құстардың бір колониясынан әр жылдары оқшаулап алған тұмау А/қарабас өгіз шағала/Атырау/773/04 (H13N6) және А/қарабас өгіз шағала/Атырау/2966/08 (H13N6) вирустары гемагглютинин генін филогенетикалық талдау нәтижелері келтірілген. Қазақстандық H13N6 бөлінділердің эталодық A/gull/Maryland/704/77 штамынан тарайтын американдық-еуразиялық вирустар желісіне жататындығы көрсетілген.