UDC 575.
https://doi.org/10.2298/GENSR1902511G
Original scientific paper

GENETIC ANALYSIS OF HEMAGGLUTININ PROTEINS OF H3 AND H1 SUBTYPES IN KAZAKHSTAN

Dilyara GRITSENKO [1,2,3*], Aleksandr POZHARSKY[4], Nina DERYABINA[1], Aliya KASSENOVA[5], Nurbol GALIAKPAROV[1]

[1]Laboratory of molecular biology, Institute of plant biology and biotechnology, Almaty, Kazakhstan
[2]Laboratory of molecular genetics and biochemistry, Institute of plant protection and quarantine, Almaty, Kazakhstan
[3]Department of molecular biology and genetics, Al-Farabi Kazakh National University, Almaty, Kazakhstan
[4]Asfendiyarov Kazakh National Medical University, Kazakhstan
[5]Nazarbayev University, Astana, Kazakhstan

Gritsenko D., A. Pozharsky, N.Deryabina, A. Kassenova, N. Galiakparov (2019): *Genetic analysis of hemagglutinin proteins of H3 and H1 subtypes in Kazakhstan*.- Genetika, Vol 51, No.2, 511-524.

The influenza is one of the most dangerous and widespread infectious diseases on the planet. A natural reservoir of the influenza A virus is wild waterfowl, which subsequently contribute to the spread of viral infection among domestic animals. Currently, different influenza A subtypes were isolated from various animal species and their genetic diversity were investigated with the subsequent possibility to predict the places of outbreaks and the transfer between species. An investigation of genetic diversity of influenza A virus is also important for a timely response by developing suitable vaccines to the emergence of new strains. In the work, we investigated two subtypes of hemagglutinin (H3, H1) from wild waterfowl in the Republic of Kazakhstan (RK). This work was aimed to determine the homology between these subtypes and currently known isolates from the NCBI database. H3N8 isolates from Kazakhstan were located in a

Corresponding author: Gritsenko Dilyara:phone +77073865386, e.mail:d.kopytina@gmail.com

monophyletic group together with isolates from Mongolia and Altai according to phylogenetic analysis of the hemagglutinin sequences . The hemagglutinin homology of H3N8 isolates from Kazakhstan and A/eq/Richmond/1/2007 vaccine strain was 86.07% and with A/eq/Ohio/2003 vaccine strain was 86, 24%. In the case of H1N1 isolates from Kazakhstan, the highest hemagglutinin homology was with isolates from Europe. The homology with the A/California/07/2009 (H1N1) vaccine strain was 81.27%. Important amino acids of cleavage and receptor binding sites were not variable in both H3 and H1 subtypes. The investigation of antigenic sites showed presence of variations in all five sites for H3 subtype and in 4 sites for H1.

Keywords: Influenza virus, subtype, genetic diversity, hemagglutinin, vaccine.

INTRODUCTION

The influenza is one of the most dangerous and a widespread infectious disease on the planet because of its frequent emergence of new strains (variants). At present time, about 100 million cases of influenza with a fatal outcome in 250000-500000 cases are registered annually (GOLDSMITH, 2006). The pandemic of 1918 caused the death of 20-50 million people around the world (SRIWILAIJAROEN and SUZUKI, 2012). The pandemic of 1918 was caused by the H1N1 strain of avian origin. 2009 was also marked by the appearance of the H1N1 strain of swine origin. Wild waterfowls are a natural reservoir of the influenza A virus, which subsequently contribute to the spread of viral infection among domestic animals (FOUCHIER *et al*., 2005; WEBSTER *et al*., 1992). The influenza virus can infect domesticated birds, pigs, horses, dogs, humans and so on. According to the results of phylogenetic analysis, it has been shown that mammalian influenza viruses originate from avian influenza viruses (WEBSTER *et al*., 1992). At the moment, the influenza A virus was isolated from 150 species of wild birds belonging to 26 different families (ALEXANDER, 2007). The emergence of new strains, in addition to point mutations in genes, may be due to genetic reassortment of different subtypes, which can lead to the emergence of pandemic strains. The Asian H2N2 influenza that caused a severe pandemic in 1958 and the Hong Kong H3N2 (1968-1969) influenza resulted from reassortment of human and avian influenza subtypes. Due to the high mutability of the influenza virus genome, it is necessary to respond in a timely manner and produce appropriate vaccines for each season, depending on the circulation of a strain. The hemagglutinin and neuraminidase genes are frequently used for the development of vaccines. At present, 18 subtypes of hemagglutinin (HA) and 11 subtypes of neuraminidase (NA) are known (TONG *et al*., 2012). 16 of 18 subtypes of hemagglutinin and 9 of 11 subtypes of neuraminidase were isolated from birds, 2 HA subtypes are found in bats (H17N10 and H18N11) (WEBSTER *et al*. 1992; TONG *et al*., 2012; OLSEN *et al*., 2006). H1, H2, H3 of HA and N1, N2 of NA are dangerous (epidemic) subtypes for human. Antibodies to these proteins lead to an effective immune response to viral infection. The influenza virus connects the host cells by hemagglutinin, which forms a bond with the sialic acid residues on the cell surface. Neuraminidase is another key surface element of the envelope of the virus. Neuraminidase is an exosialidase which cleaves α-ketosidic linkage between the sialic (N-acetylneuraminic) acid and an adjacent sugar residue (VARGHESE and COLMAN, 1991). The functions of neuraminidase are not yet fully understood, but one thing is clear and experimentally confirmed: this antigen facilitates the release of newly synthesized virions from the infected and destroyed cell if viral hemagglutinin has formed a bond with sialic acid on the membrane of the same cell.

In the development of vaccines, one of the main problems is the range of strains and subtypes against which the vaccine works, usually the vaccine is active against a subtype of HA or NA for which it was directly developed or to closely related subtypes. In this work, we investigated two subtypes of hemagglutinin (H1, H3) from wild waterfowl in the Republic of Kazakhstan (RK). The aim of the work was to determine the homology with the currently known isolates of the NCBI database. These genes were cloned into viral vector based on Grapevine virus A genome and considered as candidates for vaccine development.

MATERIALS AND METHODS

Materials

Total RNA was isolated from fecal samples of wild waterfowl dwelling on the territory of the Republic of Kazakhstan. Isolation of RNA was performed by The QIAamp Viral RNA Mini Kit. Two isolates of H1N1 subtype and two isolates of the H3N8 subtype were obtained from the black-headed gull (Atyrau- 2017) and from red-crested pochard (Korgalzhyn- 2017), respectively.

An investigation of genetic diversity of avian influenza viruses in Kazakhstan is particularly important, because the country lays on the paths the migration of waterfowl in Siberian-Black Sea-Eastern African and the Central-Asian-Indian directions. So, there is a high probability of spreading new strains of influenza viruses.

Reverse transcription

Reverse transcription was performed with the reverse primer (5'-ATATCGTCTCGTATTAGTAGAAACAAGGGTGTTTT-3 ') for the two subtypes of HA. Reverse transcription was carried out in 2 steps. Step 1- incubation with primers: 13 µl of the reaction mixture (1 µl (150 ng) of total RNA, 1 µl (10 mM) of the specific reverse primer for each gene, 11 µl of water) was incubated for 10 min at 65° C. Further, the reaction mixture was cooled in ice for 5 minutes. Step 2- reverse transcription: 4 µl of 5 × RT buffer (250 mM Tris-HCl (pH 8.3 at 25° C), 250 mM KCl, 20 mM MgCl2, 50 mM DTT), 2 µl dNTP (10 mM dNTP mixture) and 1 µL (200U) reverse transcriptase (RevertAid H Minus Reverse Transcriptase) were added to the 13 µl reaction mixture obtained in Step 1. The final reaction mixture was incubated at 42° C for 1.5 hours. Subsequently, cDNA was used for PCR with specific forward and reverse primers.

Cloning and sequencing of HA genes

To amplify HA, the reaction mixture contained 4 µL DNA from reverse transcription (cDNA), primer 1F (5'-TATTCGTCTCAGGGAGCAAAAGCAGGGG-3 ') and 1R (5'-ATATCGTCTCGTATTAGTAGAAACAAGGGTGTTTT-3') at a concentration of 0, 25 mM, PCR buffer, dNTP at a concentration of 0.2 mM, deionized sterile water, 1.25 units of Pfu-DNA polymerase. The amplification mixture was 25 µL. PCR program for amplification of HA: Stage 1: 1 cycle – 2 min at 94° C; Stage 2 (35 cycles): step 1 – 15 seconds at 94° C; step 2 – 15 seconds at 55° C; step 3- 4 min at 72° C. Stage 3: 1 cycle – 10 min at 72° C.

After the amplification, the PCR products were analyzed by electrophoresis in a 1% agarose gel. PCR products of HA were excised from the agarose gel and purified using the GeneJET Gel Extraction Kit (Thermo Scientific) kit. Further, the HA genes of H3 and H1 subtypes were cloned into the T-pGEM vector by T / A method (GRITSENKO *et al*. 2016). Also,

the HA genes were subcloned into a viral vector based on the genome of the grapevine virus A (GRITSENKO *et al.* 2017; GRITSENKO and GALIAKPAROV, 2017; GRITSENKO *et al*., 2019). Viral vector was developed by using full genome and "deconstructed" genome strategies. HA was under control of subgenomic promoter of capsid protein gene. Data on the expression of hemagglutinin in the viral vector is not given in this work.

HA sequencing was performed in the T-pGEM vector with the T7 forward and M13 reverse primers. Sequencing was carried out using the BigDye Terminator v3.1 Cycle Sequencing Kit, POP-4® polymer on the ABI PRISM® 310 Genetic Analyzer (Applied Biosystems).

Genetic analysis of the HA genes

The analysis of the quality of sequencing of HA genes was carried out using the DNAMAN program. The creation of phylogenetic trees based on Neighbor-joining method was carried out using the MEGA7 program. The robustness of the branch support was determined using 1000 bootstrap replicates. Multiple sequence alignment analysis was performed by using MultAlin (CORPET, 1988). Investigation of antigenic sites was performed by WebLogo 3 webserver (CROOKS *et al*., 2004).

RESULTS

Phylogenetic analysis of HA (H3, H1)

The hemagglutinin amino acid sequences of the two isolates (H3N8 subtype) were differed only in one amino acid at 344 position. Isoleucine at 344 position for H3N8-1 KZ and Threonine at 344 position for H3N8-2 KZ, Fig. 1.

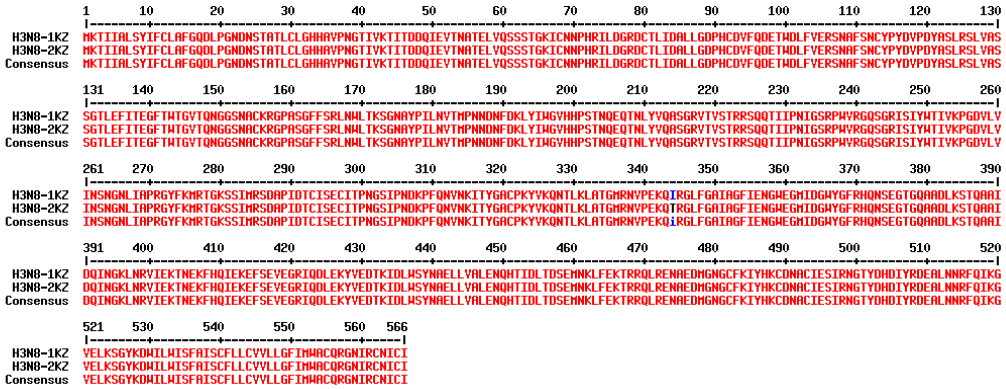

Fig. 1 Comparison of hemagglutinin amino acid sequences of 2 isolates (H3N8 subtype) from Kazakhstan

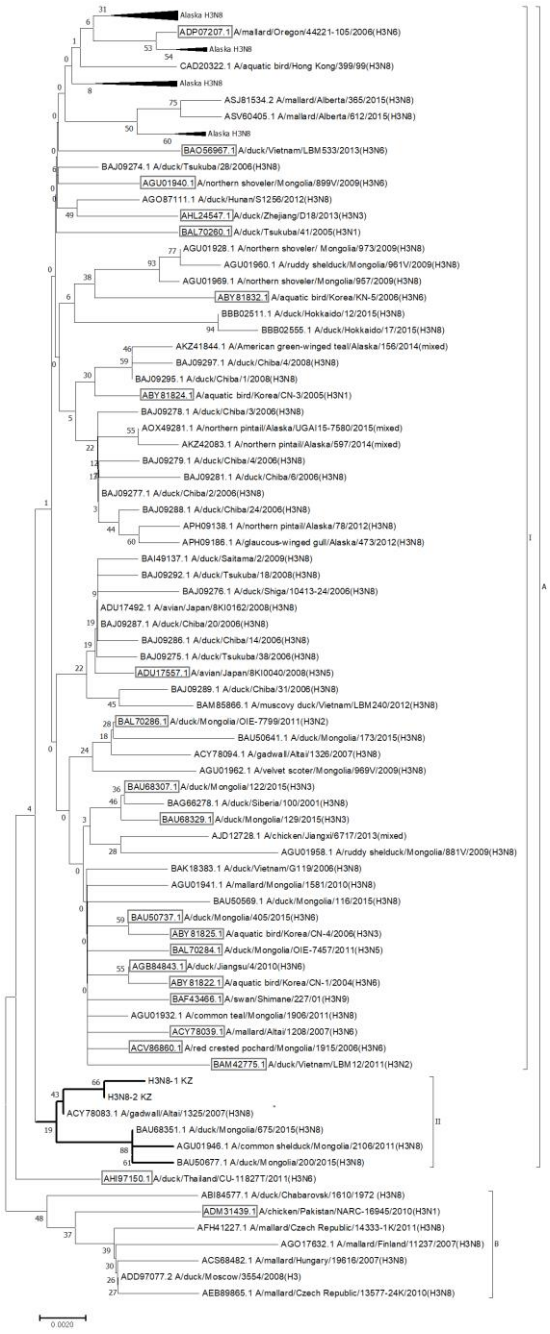

Fig. 2 Phylogenetic tree for H3 subtype. Subtypes other than H3H8 are marked with a rectangle

The main interest in the investigation of sequences of hemagglutinin from RK was the identification of the relationship of local isolates with isolates from around the world, in order to determine the closest variants. The investigation of local isolates in relation with the world's isolates can help in prediction of the origin of seasonal strains and its spreading with the migration of birds. H3 subtype isolates of the Republic of Kazakhstan were compared with isolates of the NCBI database. 84 isolates with coverage of 100% and homology of at least 99% from different countries of the world were selected for the analysis. All isolates are known to be isolated from birds. In addition to the subtype H3N8, there were isolates of H3N5, H3N6, H3N3, H3N1, H3N2, H3N9 subtypes among the 84 isolates. The number of isolates other than the H3N8 subtype is 25.5% of the total selection with the predominance of the H3N6 subtype. The other subtypes do not form separate clusters but are located in monophyletic groups together with isolates of the H3N8 subtype. Based on the results of phylogenetic analysis, a division into two large A and B clusters can be observed. Cluster A represents isolates mainly of Asia and Alaska. 33% of cluster A consist of H3N8 isolates of Alaska and 27% of H3N8 isolates from Mongolia. Cluster B includes isolates of predominantly Eastern Europe, except for the isolate of H3N1 subtype from Pakistan. The isolates of H3N8 Kz were distributed within cluster A. As it can be seen from the dendrogram, Fig. 2, H3N8 Kz isolates were placed together with the isolate of H3N8 from Altai and 3 isolates of H3N8 from Mongolia in subcluster A II. Amino acid sequences of the hemagglutinin of H1 subtype isolated from RK were compared with the isolates of NCBI database, none of the isolates in the database had 100-99% homology with the RK isolates investigated under 100% coverage. The H1N1-1 KZ isolate differ from the H1N1-2 KZ isolate with two amino acids at 20 and 468 positions, Fig. 3.

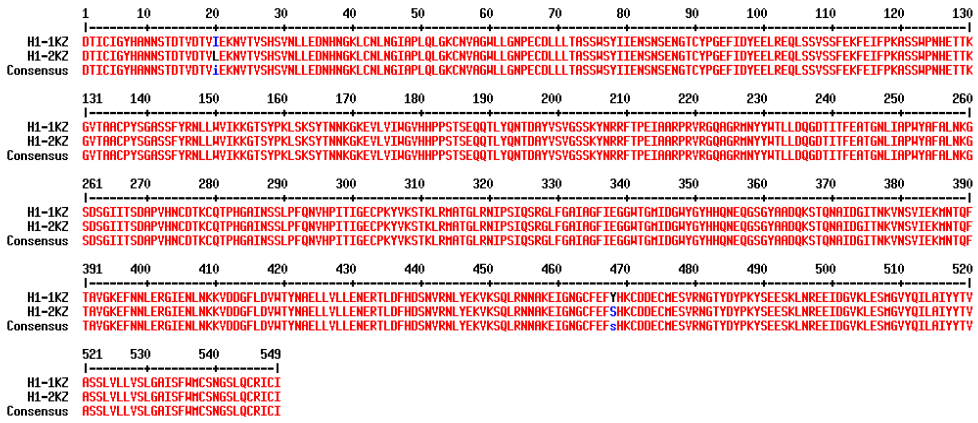

Fig. 3 Comparison of hemagglutinin amino acid sequences of 2 isolates (H1N1 subtype) from Kazakhstan. First amino acid is aspartic acid (D)

19 isolates with 100% coverage and homology of at least 98% were selected from NCBI database. The results of phylogenetic analysis showed that isolates of H1N1-KZ were distributed to separate cluster from isolates of the database, Fig. 4. Surprisingly, most isolates with a homology of 98% were originated from the Netherlands (52%). In prospect of the geographical location of Kazakhstan in relation to the Netherlands and Italy, a question arises

about the high homology with isolates from these countries, rather than the nearby countries of Eastern Europe or Asia. The probable reason for this homology can be the migration of birds.

Nevertheless, it can be noted that 3 isolates from Mongolia (neighbor country) are also present. But these isolates also have homology higher with isolates from the Netherlands and Italy than with isolates from RK. In addition to isolates of the H1N1 subtype, high homology of H1 has an isolate from the Netherlands of H1N4 subtype and isolate from Hainan of H1N2 subtype.

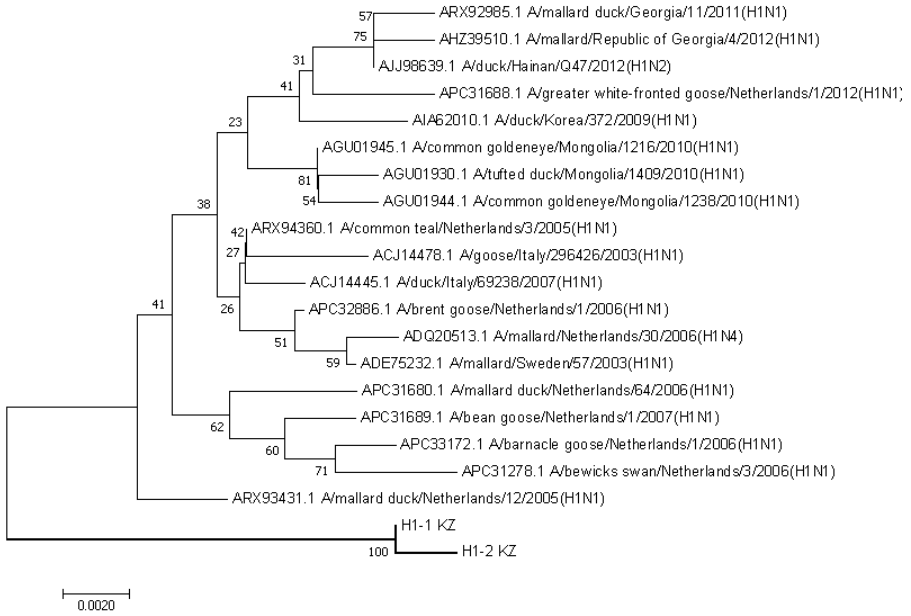

Fig. 4 Phylogenetic tree for H1 subtype

Genetic variation of important hemagglutinin sites
H3 subtype

The results of multiple alignment of 84 sequences of the HA (H3 subtype) from the NCBI database and two isolates from Kazakhstan showed no variation in amino acid residues of the important cleavage and the receptor binding sites, Table 1. The receptor binding site determines the specificity of host, which can be infected by virus, and the mutations in this site play a crucial role in the transmission of the virus from one species to another. The selection of amino acids positions of important hemagglutinin sites was carried out in accordance with previous investigations (BAILEY *et al*. 2016; WILSON and COX 1990). The first amino acid is methionine, Fig. 1. As previously noted in a number of investigations, the main amino acid in the receptor binding site is amino acid at 226 position (CONNOR, 1994). Leucine at 226 position determines the binding of hemagglutinin to α2-6-linked receptors (human-like). The presence of glutamine determines the binding of hemagglutinin to α2-3-linked receptors (avian-like).

Table 1 Genetic variation in the cleavage and receptor binding sites of H3

Isolate	Important amino residues of cleavage site				Important amino residues for receptor binding site				
	326	327	328	329	183	190	225	226	227
2 isolates of H3N8 Kz	K	Q	N	T	T	F	S	Q	Q
84 isolates from NCBI database	K	Q	N	T	T	F	S	Q	Q

The investigation of antibody-recognizing (antigenic) sites showed presence of variations in all five sites, Fig. 5. 31 of 84 isolates from NCBI database have variations in antigenic sites. Of the 5 antigenic sites, the greatest number of variations is noted in site A, the smallest in site C. The level of varying amino acid positions of A site is 36%, the varying amino acids are G140S, T142S, T144A, N153S. 12.5% of varying positions are noted in B site: A160T, N189Y. 3.2% of the varying positions are detected for C site: Y273F.

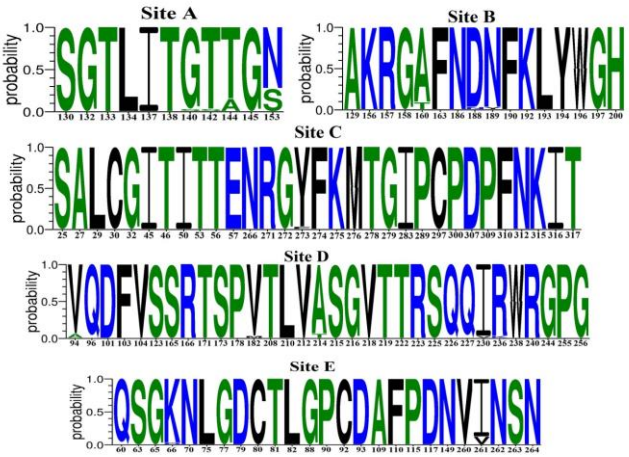

Fig. 5 Variation in antigenic site of H3 subtype. Hydrophilic amino acids are marked in blue color; neutral in green color; hydrophobic in black color

D site includes 18.75% of varying positions: V94A, T171X, V182M, A214T, I230V, R236K. E site showed variations in 16% of amino acid positions: K66R, D79X, S117N, I261V. The indicated variations in 5 antigenic sites were not detected in the isolates of H3N8 KZ, as well as in isolates of Altai and Mongolia, constituting a monophyletic group with isolates from Kazakhstan. Isolates of database with variations in antigenic sites and H3N8 Kz isolates were used to create a phylogenetic tree, Fig.6.

As can be seen from the results of the phylogenetic analysis, figure 6, the isolates from the RK were distributes into a separate cluster of A group as in the case of phylogenetic analysis for 84 isolates. Red color indicates isolates, in which two variable amino acids are noted in antigenic

sites. Blue marked an isolate, which has 3 variant amino acids in antigenic sites. The remaining isolates have only one variant amino acid in antigenic sites. The only ABY81832.1 A / aquatic bird / Korea / KN-5/2006 (H3N6) isolate containes 2 varying amino acids in one antigenic site, site E. In the case of other isolates having 2 and 3 varying amino acids, variants were distributed in different antigenic sites.

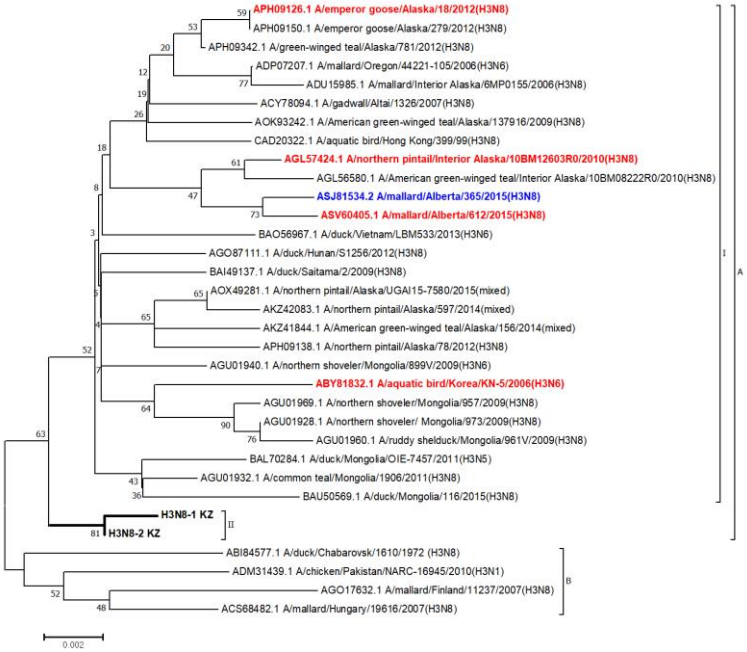

Fig. 6 Genetic diversity depending on variations in antigenic sites of H3 subtype

H1 subtype

We also did not find any variations in the important amino acid residues of the cleavage and the receptor binding sites when comparing H1 isolates from Kazakhstan and the 19 isolates of the database, Table 2.

Table 2 Genetic variations in the cleavage and receptor binding sites of H1

Isolate	Important residues of cleavage site						Important residues for receptor binding							
	322	323	324	325	326	327	91	133	150	180	187	192	222	223
2 isolates of H1N1 Kz	P	S	I	Q	S	R	Y	T	W	H	E	Y	G	Q
19 isolates from NCBI database	P	S	I	Q	S	R	Y	T	W	H	E	Y	G	Q

As was shown earlier in a number of investigations, the most important positions in the receptor binding site for the H1 subtype are 187 and 222. A receptor binding site containing E187 and G222 is able to bind to α2-3-linked receptors of bird, while D187 and G222 allow hemagglutinin to bind to α2-3-linked and to α2-6-linked receptors of pigs. The H1 subtype containing D187 and D222 in the receptor binding site efficiently interacts with human α2-6-linked receptors (STEVENS *et al.* 2006; TUMPEY *et al*. 2007; MATROSOVICH *et al.* 1997). The selection of important sites of the hemagglutinin amino acid sequence was carried out in accordance with previous investigations (SRIWILAIJAROEN and SUZUKI 2012; LIN *et al*. 2009). First amino acid selected was aspartic acid, Fig.3. The amino acid positions of the important sites correspond to the selected framework, where the first amino acid is aspartic acid.

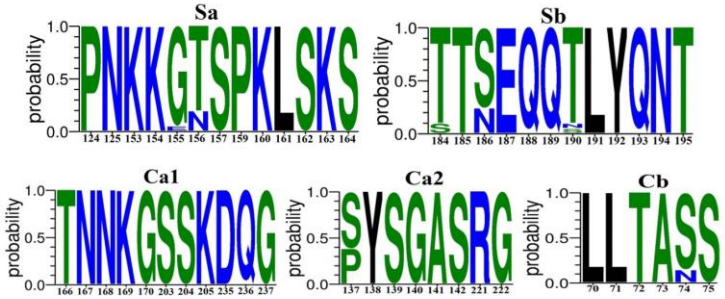

Fig.7 Variation in antigenic site of H1 subtype. Hydrophilic amino acids are marked in blue color; neutral in green color; hydrophobic in black color

The investigation of antigenic sites of hemagglutinin of 2 isolates from the RK and 19 isolates from the database showed that the most variable site is Sb – 25% of the variable positions. Moreover, 190 position has 3 different amino acids, Fig.7. The varying positions of the Sb site are 184, 186, 190. Isolates from Kazakhstan at 184 position have S, at 186 position-S and at 190 position-T. Previously, it was also shown that the Sb site is the most variable when comparing 5 antigenic sites (Raymond et al. 1986). The site Ca1 was marked as conservative, not a single variant was found. We noted 15.4% of the varying positions (155, 156) in the Sa site, for Kz isolates 155 position is G and 156 position is T. Ca2 site has 12.5% of varying positions (137), 137 position of Kz isolates is P.

Cb site has 16.6% of varying positions, 74 position. 74 position of Kz isolates is S. The variation in amino acids in these antigenic sites leads to a change in the antigenic properties of hemagglutinin. Due to the variation in amino acids in these positions, the influenza virus can be masked from the host's immune system and cause infection. The high level of natural mutability of the genome of the virus is due to poor proofreading activity of RNA polymerase (Liu et al. 2009). In view of this, it is necessary to develop new vaccines every season, which effectively prevent the spread of infection

DISCUSSION

Transmission of influenza A virus between species is a serious problem and causes a constant fear associated with the emergence of a new reassortant that can cause a pandemic. The appearance of seasonal subtypes occurs due to the evolution of the virus through the point mutations of hemagglutinin or neuraminidase. Every season, different influenza A subtypes are

being isolated from various animal species and their genetic diversity are being investigated, and this can help to predict the places of outbreaks and the interspecific transmission. The investigation of genetic diversity of influenza A virus is also important in order to respond in a timely manner to the emergence of new strains by developing suitable vaccines. Currently, intensive influenza investigation involves subtypes capable to infect human and it is not logical, since these subtypes originate from the subtypes of birds and animals. It is necessary to connect methods of bioinformatics analysis to predict the possible transfer of new isolates between species. For instance, when mutations occur in receptor binding sites of hemagglutinin, it is necessary to be able to predict what kind of receptors the mutant hemagglutinin can bind with. Currently available genetic data on the transmission of the virus between the species are only being obtained *a posteriori* fact of such transfer. . Also, when identifying mutations in antigenic sites, it is necessary to be able to predict the spectrum of the action of the vaccine. Therefore bioinformatics analysis can help reduce the cost of vaccines development.

At the moment it is known that the H3N8 subtype is capable to infect birds, horses, seals and dogs. The spread among dogs has been obtained through the transition from horses (BRYANT *et al*., 2011). Also, the H3 subtype compared with other subtypes is isolated from wild birds in most cases (MUNSTER *et al*., 2007). H3N8 isolates from Republic of Kazakhstan are located in a monophyletic group together with isolates from Mongolia and Altai according to phylogenetic analysis of the hemagglutinin amino acid sequences. We did not notice a variation in the receptor binding and cleavage sites. We investigated variations in antigenic sites, the greatest number of varying positions were noticed in A site and the smallest number of variations in C site. H3N8 isolates circulating among horses cause a strong infection and initially evolved into 2 lineages of the American and European. Further, the American lineage evolved into two sublineage, the sublineage of South America and the sublineage of Florida. Currently, there are two large clades of the Florida sublineage (clade 1 and clade 2) (BRYANT *et al*., 2011). The isolates of clade 1 and clade 2 cause outbreaks of infection around the world and at the moment OIE allocates 3 isolates recommended for use in the development of vaccines. A/eq/South Africa/04/2003 isolate or A/eq/Ohio/2003 isolate, which represent clade 1. A/eq/Richmond/1/2007 isolate belongs to clade 2. In 1999, the H3N8 subtype was isolated from dogs in the USA, which originates from H3N8 horses. The homology of canine influenza hemagglutinin (A/canine/Florida/43/2004 (H3N8)) and equine influenza hemagglutinin (A/eq/South Africa/04/2003 or A/eq/Ohio/2003) is 95.59%. In addition, there was a transfer of H3N2 avian influenza to dogs in 2005-2006 in Asia. H3N8 equine influenza was obtained from H3N8 avian influenza. We analyzed the hemagglutinin amino acid sequences of H3N8 Kz isolates and 2 strains of equine influenza recommended for the vaccines development. The hemagglutinin homology of isolates from Kazakhstan and A/eq/Richmond/1/2007 straine is 86.07% and with A/eq/Ohio/2003 strain is 86, 24%.

The virus of H1N1 subtype has certainly been studied well due to the three pandemics caused by it (KOÇER *et al*., 2012). The pandemic of 1918 was caused by a virus, which is presumably originated from avian influenza, 8 genes of H1N1 (1918) were cloned and sequenced (TUMPEY *et al*., 2005). The second pandemic of 1977 was caused by H1N1 in Russia (GREGG *et al*., 1978). The 2009 pandemic was caused by the H1N1 subtype resulting from the reassortment of viruses of different origins. Viruses possessed PB2 and PA genes of North American avian virus origin, a PB1 gene of human H3N2 virus origin, HA (H1), NP, and NS genes of classical swine virus origin, and NA (N1) and M genes of Eurasian avian-like swine

virus origin (NEUMANN and KAWAOKA, 2009). At the moment, hemagglutinin of H1 subtype is isolated from different hosts in various countries of the world and genetically characterized. Hemagglutinins of the H1N1 isolates of Republic of Kazakhstan were compared with 2009 pandemic strains. The homology of the RK isolates with the A/California/07/2009 (H1N1) vaccine strain is 81.27%. Results of phylogenetic analysis showed that H1N1 isolates of RK were distributed into separate cluster from 19 isolates of the database. Most of the selection included isolates from the Netherlands, not from nearby Eastern European countries, which is probably related to bird migration. Molecular genetic studies of genes such as hemagglutinin and neuraminidase of influenza A virus are promising and will be relevant for a long time. Since these genes encode important proteins – antigens, which are necessary for the development of vaccines. A investigation of the genetic diversity of these proteins throughout the world will allow a more accurate assessment of the evolution of the influenza A virus.

ACKNOWLEDGEMENTS

Scientific grant from Ministry of science and education of Republic of Kazakhstan. Number of grant is AP05132367.

Received, July 21th, 2018
Accepted May 18th, 2019

REFERENCES

ALEXANDER, D. (2007): Summary of avian influenza activity in Europe, Asia, Africa, and Australasia, 2002-2006. Avian Dis., *51*:161-6.

BAILEY, E., L., LONG, N., ZHAO, J., HALL, J., BAROCH, J., NOLTING, L., SENTER, F., CUNNINGHAM, G., PHARR, L., HANSON, R., SLEMONS, T., DELIBERTO, X., WAN (2016): Antigenic Characterization of H3 Subtypes of Avian Influenza A Viruses from North America. Avian Dis., *60*:346-53.

BRYANT, N., A., RASH, A., WOODWARD, E., MEDCALF, M., HELWEGEN, F., WOHLFENDER, F., CRUZ, C., HERRMANN, K., BORCHERS, A., TIWARI, T., CHAMBERS, J., NEWTON, J., MUMFORD, D., ELTON (2011): Isolation and characterisation of equine influenza viruses (H3N8) from Europe and North America from 2008 to 2009. Vet Microbiol., *147*:19-27.

CONNOR, R., Y., KAWAOKA, R., WEBSTER, J., PAULSON (1994): Receptor specificity in human, avian, and equine H2 and H3 influenza virus isolates. Virology, *205*(1):17-23.

CORPET, F. (1988): Multiple sequence alignment with hierarchical clustering. Nucleic Acids Res., *16*(22):10881-90.

CROOKS, G., G., HON, J., CHANDONIA, S., BRENNER (2004): WebLogo: a sequence logo generator. Genome Res., *14*(6):1188-90.

FOUCHIER, R., V., MUNSTER, A., WALLENSTEN, T., BESTEBROER, S., HERFST, D., SMITH, G., RIMMELZWAAN, B., OLSEN, A., OSTERHAUS (2005): Characterization of a novel influenza A virus hemagglutinin subtype (H16) obtained from black-headed gulls. J. Virol., *79*(5):2814-22.

GOLDSMITH, C. (2007): Influenza: The Next Pandemic?. In: Goldsmith C (ed) It's just the flu, Twenty-First Century Books, Minneapolis, pp. 7–20.

GREGG, M., A., HINMAN, R., CRAVEN (1978): The Russian flu. Its history and implications for this year's influenza season. JAMA, *240*: 2260–2263.

GRITSENKO, D., N., DERYABINA, N., GALIAKPAROV (2017): Modification of genome of grapevine virus A. Eurasian J. App. Biotech., *4*:17-25.

GRITSENKO, D., N., GALIAKPAROV (2017): Insertion of 2A 'self-cleaving' peptides between ORF4 and ORF5 of grapevine virus A genome for vector development. Asian J. Microbiology, Biotech. Environ. Sci., *19*:1034-1040.

GRITSENKO, D., R., KENZHEBEKOVA, N., DERYABINA, N., GALIAKPAROV (2016): Sozdanie t-vektora dlya pryamogo klonirovaniya ptsr-produktov. Eurasian J. App. Biotech., *4*:43-50.

GRITSENKO, D., R., KENZHEBEKOVA, N., DERYABINA, N., GALIAKPAROV (2019): Development of a "deconstructed" vector based on the genome of grapevine virus A. Plant Biotech. Rep., *13*:169-177.

KOÇER, Z., S., KRAUSS, D., STALLKNECHT, J., REHG, R., WEBSTER (2012): The potential of avian H1N1 influenza A viruses to replicate and cause disease in mammalian models. PLoS One, *7*(7):e41609.

LIN, T., G., WANG, A., LI, Q., ZHANG, C., WU, R., ZHANG, Q., CAI, W., SONG, K., YUEN (2009): The hemagglutinin structure of an avian H1N1 influenza A virus. Virology, *392*(1):73-81.

LIU, N., G., WANG, K., LEE, Y., GUAN, H., CHEN, Z., CAI (2009): Mutations in influenza virus replication and transcription: detection of amino acid substitutions in hemagglutinin of an avian influenza virus (H1N1). FASEB J., *23*: 3377–3382.

MATROSOVICH, M., A., GAMBARYAN, S., TENEBERG, V., PISKAREV, S., YAMNIKOVA, D., LVOV, J., ROBERTSON, K., KARLSSON (1997): Avian influenza A viruses differ from human viruses by recognition of sialyloligosaccharides and gangliosides and by a higher conservation of the HA receptor-binding site. Virology, *233*: 224–234.

MUNSTER, V., C., BAAS, P., LEXMOND, J., WALDENSTRÖM, A., WALLENSTEN, T., FRANSSON, G., RIMMELZWAAN, W., BEYER, M., SCHUTTEN, B., OLSEN, A., OSTERHAUS, R., FOUCHIER (2007): Spatial, temporal, and species variation in prevalence of influenza A viruses in wild migratory birds. PLoS Pathog., *3*(5):e61.

NEUMANN, G., T., NODA, Y., KAWAOKA (2009): Emergence and pandemic potential of swine-origin H1N1 influenza virus. Nature, *459*: 931–939.

OLSEN, B., V., MUNSTER, A.,WALLENSTEN, J., WALDENSTRÖM, A., OSTERHAUS, R., FOUCHIER (2006): Global patterns of influenza a virus in wild birds. Science, *312*(5772):384-8.

RAYMOND, F., A., CATON, N., COX, A., KENDAL, G., BROWNLEE (1986): The antigenicity and evolution of influenza H1 hemagglutinin, from 1950–1957 and 1977–1983: two pathways from one gene. Virology, *148*: 275–287.

SRIWILAIJAROEN, N., Y., SUZUKI (2012): Molecular basis of the structure and function of H1 hemagglutinin of influenza virus. Proc. Jpn. Acad. Ser. B Phys. Biol. Sci., *88*(6):226-49.

STEVENS, J., O., BLIXT, L., GLASER, J., TAUBENBERGER, P., PALESE, J., PAULSON, I., WILSON (2006): Glycan microarray analysis of the hemagglutinins from modern and pandemic influenza viruses reveals different receptor specificities. J. Mol. Biol., *355*: 1143–1155.

TUMPEY, T., C., BASLER, P., AGUILAR, H., ZENG, A., SOLÓRZANO, D., SWAYNE, N., COX, J., KATZ, J., TAUBENBERGER, P., PALESE, A., GARCÍA-SASTRE (2005): Characterization of the reconstructed 1918 Spanish influenza pandemic virus. Science, *310*(5745):77-80.

TUMPEY, T., T., MAINES, N., VAN HOEVEN, L., GLASER, A., SOLORZANO, C., PAPPAS, N., COX, D., SWAYNE, P., PALESE, J., KATZ, A., GARCIA-SASTRE (2007): A two-amino acid change in the hemagglutinin of the 1918 influenza virus abolishes transmission. Science, *315*: 655–659.

TONG, S., Y., LI, P., RIVAILLER, C., CONRARDY, D., CASTILLO, L., CHEN, S., RECUENCO, J., ELLISON, C., DAVIS, I., YORK, A., TURMELLE, D., MORAN, S., ROGERS, M., SHI, Y., TAO, M., WEIL, K., TANG, L., ROWE, S., SAMMONS, X., XU, M., FRACE, K., LINDBLADE, N., COX, L., ANDERSON, C., RUPPRECHT, R., DONIS (2012): A distinct lineage of influenza A virus from bats. Proc. Natl. Acad. Sci. USA, *109*:4269–4274.

VARGHESE, J., P., COLMAN (1991): Three-dimensional structure of the neuraminidase of influenza virus A/Tokyo/3/67 at 2.2 A resolution. J. Mol. Biol., *221*(2):473-86.

WEBSTER, R., W., BEAN, O., GORMAN, T., CHAMBERS, Y., KAWAOKA (1992): Evolution and ecology of influenza A viruses. Microbiol. Rev., *56*(1):152-79.

WILSON, I., N., COX (1990): Structural basis of immune recognition of influenza virus hemagglutinin. Ann. Rev. Immunol., *8*:737-71.

GENETIČKA ANALIZA HEMAGGLUTININSKIH PROTEINA H3 I H1 SUBTIPA U KAZAHSTANU

Dilyara GRITSENKO [1,2,3*], Aleksandr POZHARSKY[4], Nina DERYABINA[1,3], Aliya KASSENOVA[5], Nurbol GALIAKPAROV[1]

[1]Laboratorija za molekularnu biologiju, Institut za biologiju i biotehnologiju biljaka, Almati, Kazahstan
[2]Laboratorija za molekularnu genetiku i biohemiju, Institut za zaštitu bilja i karantin, Almati, Kazahstan
[3]Departman za molekularnu biologiju i genetiku, Al-Farabi Kazakh Nacionalni Univerzitet, Almati, Kazahstan
[4]Asfendiyarov Kazakh Nacionalni Medicinski Univerzitet, Kazahstan
Nazarbayev Univerzitet, Astana, Kazahstan

Izvod

Grip je jedna od najopasnijih i najrasprostranjenijih zaraznih bolesti na planeti. Prirodni rezervoar virusa influence A su divlje vodene ptice, koje kasnije doprinose širenju virusne infekcije kod domaćih životinja. Trenutno su iz različitih životinjskih vrsta izolovani različiti podtipovi influence A i ispitivana je njihova genetička raznolikost sa mogućnošću predviđanja mesta pojave i prenosa između vrsta. Istraživanje genetičke raznolikosti virusa influence A takođe je važno za blagovremeni odgovor razvijanjem odgovarajućih vakcina do pojave novih sojeva. U radu smo istražili dva podtipa hemaglutinina (H3, H1) iz divljih vodenih ptica u Republici Kazahstan (RK). Ovaj rad je imao za cilj da odredi homologiju između ovih podtipova i trenutno poznatih izolata iz NCBI baze podataka. Izolati H3N8 iz Kazahstana bili su locirani u monofitelnoj grupi zajedno sa izolatima iz Mongolije i Altaja prema filogenetskoj analizi sekvenci hemaglutinina. Homologost hemaglutinina izolata H3N8 iz Kazahstana i A / ek / Richmond / 1/2007 soja vakcine iznosila je 86,07%, a A / ek / Ohio / 2003 vakcinski soj bio je 86,24%. U slučaju izolata H1N1 iz Kazahstana, najviša homologija hemaglutinina bila je sa izolatima iz Evrope. Homologija sa A / California / 07/2009 (H1N1) sojem vakcine bila je 81,27%. Kod važnih amino kiselina mesta cepanja i vezivanja receptora nisu bile promenljive u oba podtipa H3 i H1. Istraživanje antigenih lokacija pokazalo je prisustvo varijacija u svih pet lokacija za podtip H3 i na 4 lokacije za H1.

Primljeno 21.VII.2018.
Odobreno 18. V. 2019.