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SCREENING FOR MARKERS OF RESISTANCE TO NEURAMINIDASE INHIBITORS OF INFLUENZA A VIRUS SUBTYPE N8

Influenza vaccination and treatment with antiviral drugs reduces the risk of getting sick and spreading the flu. Antivirals are needed at the initial stage of an influenza outbreak. To date, the neuraminidase inhibitors oseltamivir and zanamivir are the main available therapeutic options for combating infections caused by the influenza virus. Acquisition of resistance by avian influenza viruses to neuraminidase inhibitors due to amino acid substitutions in neuraminidase (NA) is a major public health problem. Determining the susceptibility of influenza viruses to currently available immune response inhibitors is an important part of surveillance research and risk assessment for pandemic preparedness. In the amino acid composition of the NA of the Kazakh strain A/wild goose/Kostanai/KZ/83/2021 (H5N8) of the 2021 season avian influenza virus, the G147V amino acid substitution, known to be associated with a decrease in the sensitivity of the influenza virus to the neuraminidase inhibitor zanamivir, was identified. The identified G147V mutation in the NA of the Kazakh strain A/wild goose/Kostanai/KZ/83/2021 (H5N8) of the avian influenza virus, located in the active center of the protein, can affect the biological properties of the virus. No other substitutions that cause a decrease in the sensitivity of influenza A(H5N8) viruses to the action of neuraminidase inhibitors were found.

Key words: influenza virus, subtype, neuraminidase inhibitors, amino acid substitution.

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N8 типті тұмау вирусының нейраминидаза ингибиторларына төзімділік маркерлеріне скрининг

Тұмауға қарсы вакцинация және вирусқа қарсы препараттармен емдеу ауруға шалдығу және тұмаудың таралу қаупін азайтады. Вирусқа қарсы препараттар тұмау індетінің бастапқы кезеңінде қажет. Бүгінгі таңда нейраминидаза ингибиторлары – осельтамивир және занамивир, тұмау вирусын тудырған инфекциялармен күресудің негізгі қол жетімді емдік нұсқалары болып табылады. Нейраминидазадағы (NA) аминқышқылдарының алмасуына байланысты құс тұмауы вирустарының нейраминидаза ингибиторларына төзімді болуы қоғамдық денсаулық сақтаудың негізгі проблемасы болып табылады. Тұмау вирустарының қазіргі уақытта қол жетімді иммундық жауап ингибиторларына сезімталдығын анықтау негізінде зерттеулердің және пандемияға дайын болу қаупін бағалаудың маңызды бөлігі болып табылады. 2021 жылғы құс тұмауы вирусының қазақстандық A/wild goose/Kostanai/KZ/83/2021 (H5N8) штаммының NA аминқышқылдарының құрамында G147V аминқышқылды алмасуы, тұмау вирусының нейраминидаза ингибиторы – занамивирге сезімталдығы анықталды. Ақуыздың белсенді орталығында орналасқан құс тұмауы вирусының қазақстандық A/wild goose/Kostanai/KZ/83/2021 (H5N8) штаммының NA-да анықталған G147V мутациясы вирустың биологиялық қасиеттеріне әсер етуі мүмкін. A(H5N8) тұмауы вирустарының нейраминидаза ингибиторларының әсеріне сезімталдығының төмендеуін тудыратын басқа алмастырулар табылған жоқ.

Түйін сөздер: тұмау вирусы, субтип, нейраминидаза ингибиторлары, амин қышқылының алмасуы.

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Скрининг маркеров устойчивости к ингибиторам нейраминидазы вируса гриппа подтипа N8

Вакцинация против гриппа и лечение противовирусными препаратами снижает риск заболевания и распространения гриппа. Противовирусные препараты необходимы на начальном этапе вспышки гриппа. На сегодняшний день ингибиторы нейраминидазы осельтамивир, занамивир – основной доступный терапевтический вариант для борьбы с инфекциями, вызванными вирусом гриппа. Приобретение вирусами птичьего гриппа резистентности к ингибиторам нейраминидазы из-за аминокислотных замен в нейраминидазе (NA) представляет серьезную проблему для общественного здравоохранения. Определение чувствительности вирусов гриппа к доступным в настоящее время ингибиторам иммунного ответа является важной частью исследований по эпиднадзору и оценки риска для обеспечения готовности к пандемии. В аминокислотном составе NA казахстанского штамма A/wild goose/Kostanai/KZ/83/2021 (H5N8) вируса гриппа птиц сезона 2021 г. выявлена аминокислотная замена G147V, известная как ассоциированная со снижением чувствительности вируса гриппа к ингибитору нейраминидазы – занамивиру. Выявленная G147V мутация в NA казахстанского штамма A/wild goose/Kostanai/KZ/83/2021 (H5N8) вируса гриппа птиц, расположенный в активном центре белка может оказывать влияние на биологические свойства вируса. Иных замен, обусловливающих снижение чувствительности вирусов гриппа A(H5N8) к действию ингибиторов нейраминидазы, не обнаружено.

Ключевые слова: вирус гриппа, подтип, ингибиторы нейраминидазы, аминокислотная замена.

Introduction

Highly pathogenic avian influenza viruses can be carried over long distances by migratory wild birds and can sporadically infect and cause severe respiratory or systemic disease in other species, including humans [1].

Influenza is a disease caused by a virus, the constant evolution of which leads to incessant annual epidemics and epizootics. The evolution of influenza viruses is based on the accumulation of mutations that cause antigenic drift and the emergence of new virus variants, which ensures the heterogeneity of the viral population and underlies the formation of various genetic lines [2].

The study of the circulation of the influenza virus among wild birds on their flyways is the key to understanding the mechanisms of the spread of the virus. The territory of Kazakhstan plays an important geographical role in the spread of the influenza virus, facilitating its transfer by wild birds from Southeast Asia to Europe and North Africa. Two of the most important migration routes converge here: the Central Asian-Indian and West Asian-African. Birds flying along them use the territory of Kazakhstan for molting and as a stop during migration. About 540 species of birds nest and meet during migration in the republic, 32 of which are globally endangered. 57 species are included in the Red Book of Kazakhstan [3].

Influenza A virus is a member of the genus Orthomyxovirus [1]. The influenza virus is a negative-sense single-stranded RNA virus that has 8 gene segments encoding 11 proteins, contains 16 hemagglutinin (HA) serotypes, and 9 neuraminidase (NA) serotypes. Two more subtypes HA (H17 and H18) and NA (N10 and N11) were isolated from herbivorous bats [4, 5, 6, 7, 8, 9].

Neuraminidase inhibitors (oseltamivir, zanamivir), which target the NA glycoprotein of influenza A and B viruses, are widely used to prevent and treat influenza virus infection. However, amino acid substitutions in the NA gene can lead to resistance to neuraminidase inhibitors. Substitutions associated with resistance to neuraminidase inhibitors are usually specific to a certain type or subtype of NA [10].

The purpose of the study was to determine the mutation in the NA amino acid sequence and screen for markers of resistance to neuraminidase inhibitors of the N8 subtype avian influenza virus.

Materials and methods

Objects of study

The strain A/wild goose/Kostanai/KZ/83/2021 (H5N8) of the avian influenza virus, isolated from a

wild goose that lived on Lake Koybagar, Kostanay region in 2021, was used in the work.

Isolation of viral RNA

Isolation of viral RNA was carried out using the QIAamp Viral RNA Mini Kit (Qiagen GmbH, Hidden) in accordance with the manufacturer's recommendations from 140 µl of virus-containing liquid.

RT-PCR and RT-PCR-RT reactions

Reverse-transcriptase polymerase reaction (RT-PCR) and real-time RT-PCR (RT-PCR) were carried out using a One-Step RT-PCR kit (Qiagen). Primers and a probe were synthesized using an H-16 DNA/RNA synthesizer (K&A Laborgeraete, Germany). The primer and probe sequences used in this study are shown in Table 1.

Real-time RT-PCR for the primary detection of influenza A virus. Real-time RT-PCR targeting a highly conserved region of the M gene was performed using specific primers and a probe [11] in the LightCycler® 2.0 real-time PCR system (Roche Applied Science, Germany).

Determining the subtype of the influenza virus. The determination of the subtype of hemagglutinin H5 and neuraminidase N8 of isolates was carried out by RT-PCR using subtype specific primers [12, 13].

The acquired PCR product was analyzed using agarose gel electrophoresis (1.5%) and a 100 b.p. DNA marker. (Invitrogen, Denmark).

Sequencing and phylogenetic analysis

The RT-PCR neuraminidase N8 amplificant was sequenced on an Applied Biosystems 3130 automated DNA sequencer (ABI, 3130, USA) using the Bigdye Terminator V3.1 sequencing kit (Applied Biosystems, Inc., USA) [14].

The nucleotide sequences were analyzed using the Sequencher v. 4.5 ("Gene Codes Corporation", USA). The alignment of the nucleotide sequence was carried out using the Mega 11.0 computer program. A set of nucleotide sequences from the international database GenBank (https://www.ncbi.nlm.nih.gov/) was used to build a phylogenetic tree and determine the genotype.

Table 1 – List of primers and probe for RT-PCR and RT-PCR-RT

Name	Subsequence	Product size, b.p.	Links
Influenza A virus	rRT-PCR (M+25) AGATGAGTCTTCTAACCGAGGTCG	99	[11]
	rRT-PCR (M-124) TGCAAAAACATCTTCAAGTYTCTG		
	rRT-PCR probe (M+64) FAM-TCAGGCCCCCTCAAAGCCGA-TAMRA		
Primers used for subtyping the HA genes of avian influenza viruses			
H5	H5-918F CCARTRGGKGCKATAAAYTC	249	[12]
	H5-1166R KGTCTGCWGCRTAYCCRCTY		
Primers for subtyping NA genes of avian influenza viruses			
N8	N8-93F CATRTVGTBAGYATYAYARTAAC	137	[13]
	N8-209R ACAYTRGYATTGTRCCATTG		
Sequencing primers			
N8	Ba-NA-1 TATTGGTCTCAGGGAGCAAAAGCAGGAGT	1413	[14]
	Ba-NA-1413R AGTAGAAACAAGGAGTTTTTT		
F: forward primer, R: reverse primer			

The analysis used the nucleotide sequences of influenza A virus strains available in GenBank, isolated from birds by neuraminidase: KY576087, MN165534, MG882000, MK636736, MH819861, MK208609, MK494925, MF926455, MW026090, MF073903, MW137803, ON943056, MW505409, OL442767.

Phylogenetic analysis of the sequences was carried out using the Mega 11.0 program with the following parameters: statistical method – maximum likelihood; phylogenetic test – Bootstrap method; model/method – Kimura 2-parameter model.

The calculation of genetic distances was performed using the computer program Mega 11.0 with the following parameters: analysis – Distance Estimation; variance estimation method – Bootstrap method; model/method – P distance [15].

Results and discussion

The primary nucleotide sequence of the surface NA gene of the A/wild goose/Kostanai/KZ/83/2021 (H5N8) strain of the avian influenza virus isolat-

ed from a wild goose inhabiting Lake Koybagar, Kostanay region in 2021 was determined. The nucleotide sequences of the NA gene (Figure 1) and NA amino acid sequences were determined (Figure 2).

The results of our phylogenetic analysis allow us to assume that wild birds are able to spread different variants of the influenza A virus between geographical regions as remote from each other as the territories of Kazakhstan, Europe and Central Asia. Phylogenetic analysis for the NA gene showed that the A/wild goose/Kostanai/KZ/83/2021 (H5N8) strain of the avian influenza virus belongs to the same group as the A/H5N8 viruses isolated in Kazakhstan in 2020 (ON943056) and China in 2020 (MW505409) and 2021 (OL442767). The results are presented in Figure 3.

The presented Kazakh strains A/wild goose/Kostanai/KZ/83/2021 (H5N8) and ON943056 A/chiken/Kazakhstan/23/2020 (H5N8) of the avian influenza virus are distinguished by a high level of homology in neuraminidase – 99.59%. The level of homology of Kazakhstani strains of influenza A virus with strains isolated in China in 2020-2021 amounted to 98.42 – 98.70%.

>A/wild goose/Kostanai/KZ/83/2021 (H5N8)
GATTATTGGTCTCAGGGAGCAAAAGCAGGAGTTTAAAATGAATCCAAATCAGAAAATATCGACCATTGGCTCCAT
CTCATTGGGGCTAGTTGTATTCAATGTTCTACTGCATGCCTTGAGCATCATATTAATGGTGTTAGCCATGGGGAAA
AGTGAAAACAATGGAATCTGCAATGGAACTATAGTAAGGGGATATAATGAAACAGTTAGGATAGAGAAAGTGAC
CCAGTGGTACAACACTAGTGTAGTCGAATATGTACCGCATTGGAACGAGGGCGCTTATATAAACAACACCGAACC
AATATGTGATGTCAAGGGCTTTGCACCCTTTTCCAAGGACAACGGGATAAGACTTGGCTCCAGAGGACATATTTTT
GTCATAAGGGAGCCTTTCGTCTCTTGTTCACCTGTAGAGTGCAGAACTTTCTTTCTCACTCAGGGAGCTCTACTCAA
TGACAAACACTCAAATGAAACAGTGAAGGATAGGAGCCCATTCAGAACTCTCATGAGTGTCGAAGTGGGTCAATC
ACCCAATGTGTATCAAGCAAGGTTTGAAGCTGTAGCATGGTCAGCAACAGCCTGTCATGATGGCAAGAAATGGAT
GACGATTGGTGTGACAGGGCCAGATTCGAAAGCAATAGCAGTAGTCCATTACGGAGGAGTGCCTACTGATATTGT
TAACTCCTGGGCAGGAGACATATTACGGACTCAGGAGTCATCTTGTACTTGCATTCAAGGTAATTGTTATTGGGTA
ATGACTGACGGTCCATCCAATAGACAGGCGCAGTATAGAATATACAAAGCAAATCAAGGCAAAATAATTGACCAA
GCGGATGTCAGCTTTAGTGGAGGGCATATTGAGGAATGCTCTTGTTATCCAAATGATGGTAAAGTGGAATGCGTG
TGTAGAGACAACTGGATGGGAACTAACAGGCCTGTGCTAGTTATCTCGCCTGACCTCTCTTACAGGGTTGGGTATT
TATGTGCGGGATTGCCCAGTGACACTCCAAGAGGGGAAGATGCCCAATTTGTCGGTTCATGCACTAGTCCCATGG
GAAATCAGGGGTATGGAGTAAAAGGTTTCGGGTTTCGACAGGGAACTGATGTGTGGATGGGGCGGACAATTAGT
CGAACCTCCAGGTCAGGGTTTGAAATAATAAGGATAAAGAATGGTTGGACGCAGACAAGCAAAGAACAGATTAG
AAGGCAGGTGGTTGTTGATAATTTGAATTGGTCGGGATACAGTGGGTCTTTCACTTTACCAGTAGAATTGTCTGGG
AGGGAATGTTTAGTCCCCTGTTTTTGGGTCGAAATGGTCAGAGGCAGGCCAGAGGAAAGAACAATCTGGACCTCT
AGTAGCTCCATTGTAATGTGTGGAGTTAATCATGAAATTGCCGATTGGTCATGGCACGATGGAGCTATTCTTCCCT
TTGACATCGATGGGATGTAATTTACGGAAAAAACTCCTTGTTTCTACTAATACGAGACCAT

Figure 1 – Nucleotide sequence of neuraminidase strain A/wild goose/Kostanai/KZ/83/2021 (H5N8)

> A/wild goose/Kostanai/KZ/83/2021 (H5N8)
MNPNQKISTIGSISLGLVVFNVLLHALSIILMVLAMGKSENNGICNGTIVRGYNETVRIEKVTQWYNTSVVEYVPHWNE
GAYINNTEPICDVKGFAPFSKDNGIRLGSRGHIFVIREPFVSCSPVECRTFFLTQGALLNDKHSNETVKDRSPFRTLMSVE
VGQSPNVYQARFEAVAWSATACHDGKKWMTIGVTGPDSKAIAVVHYGGVPTDIVNSWAGDILRTQESSCTCIQGNC
YWVMTDGPSNRQAQYRIYKANQGKIIDQADVSFSGGHIEECSCYPNDGKVECVCRDNWMGTNRPVLVISPDLSYRVG
YLCAGLPSDTPRGEDAQFVGSCTSPMGNQGYGVKGFGFRQGTDVWMGRTISRTSRSGFEIIRIKNGWTQTSKEQIRR
QVVVDNLNWSGYSGSFTLPVELSGRECLVPCFWVEMVRGRPEERTIWTSSSSIVMCGVNHEIADWSWHDGAILPFDI
DGM

Figure 2 – Amino acid sequence of neuraminidase strain A/wild goose/Kostanai/KZ/83/2021 (H5N8)

The phylogenetic tree was built using the MEGA software version 11.0 (www.megasoftware.net/) using the maximum likelihood method. The A/wild goose/Kostanai/KZ/83/2021 (H5N8) strain of avian influenza virus isolated in Kazakhstan in 2021 is circled.

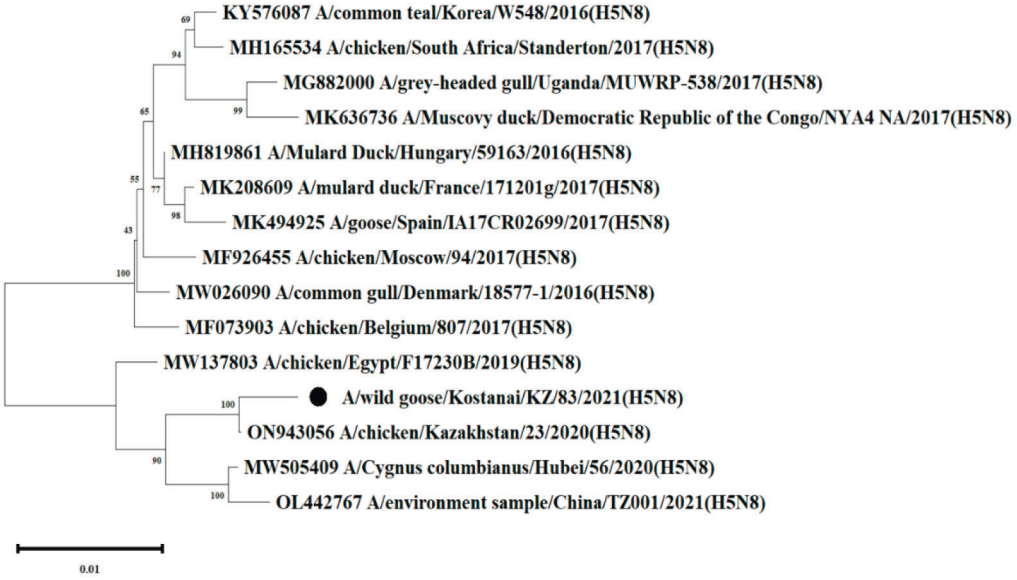

Figure 3 – Phylogenetic tree built according to the amino acid sequences of neuraminidase of influenza A/H5N8 viruses

An assessment of evolutionary divergence showed that the A/wild goose/Kostanai/KZ/83/2021 (H5N8) strain of the avian influenza virus distances itself from the strains of the A/H5N8 avian influenza virus presented in the Genbank. Pairwise values of genetic differentiation based on neuraminidase of influenza viruses A/H5N8 showed that the strain of avian influenza virus A/wild goose/Kostanai/KZ/83/2021 (H5N8) is significantly distant from strains of the European line (D> 0.01669 – 0.04001). The strain of avian influenza virus A/wild goose/Kostanai/KZ/83/2021 (H5N8) in terms of the neuraminidase sequence was the closest to influenza viruses A/H5N8 (ON943056) A/chicken/Kazakhstan/23/2020 (H5N8) (D>0.00430) and (MW505409) A/Cygnus-columbianus/Hubei/56/2020(H5N8) (D>0.01447) (Figure 4).

Past influenza pandemics caused by infection with avian influenza viruses, as well as the growing number of cases of human infection with avian influenza virus, have shown the importance of information on resistance to avian NA inhibitors that can cross the species barrier [16].

Amino acid substitutions in influenza NA are associated with resistance or reduced susceptibility to neuraminidase inhibitors. Neuraminidase inhibitors are small chemical compounds that bind to the active site of the viral NA enzyme.

Neuraminidase inhibitors reduce influenza virus replication by preventing the release of virions from host cells and their spread to new target cells.

Currently, there are only two worldwide licensed neuraminidase inhibitors: oseltamivir and zanamivir with licensed peramivir are used in Japan, South Korea, the EU and the US, while laninamivir is licensed and used only in Japan [17].

The development of drugs from the group of neuraminidase inhibitors is a big achievement for modern science made in recent years, since modern knowledge of the tertiary structure of NA as an antigen and enzyme was used in the synthesis [18]. Antineuraminidase drugs imitate the structure of natural substrates of the NA catalytic site, which drives the virus to interact with them more [19]. The first official reports on drugs with antineuraminidase activity were made in 1993 (zanamivir) and 1997 (oseltamivir) [20, 21]. With the emergence of resistant viruses in the population of epidemic A(H1N1) strains, as well as among highly pathogenic strains of the A(H5N1) avian influenza virus, WHO experts supplemented the recommendations in the Pandemic Preparation Plans by including zanamivir in the list [22].

Alignment of the amino acid sequence of neuraminidase of influenza viruses A/H5N8 showed ten amino acid substitutions (at positions 8, 36, 46, 52, 66, 73, 106, 145, 477 and 449) in the N8 subtype of strain A/wild goose/Kostanai/KZ/ 83/2021 (H5N8). According to five amino acid substitutions (at positions 46, 106, 145, 477 and 449) in N8, the studied strain A/wild goose/Kostanai/KZ/83/2021 (H5N8) differs from the Kazakh strain (ON943056)

A/chicken/Kazakhstan/23 /2020 (H5N8), isolated in 2020. The results are presented in Figure 5.

From the literature data, it is known that new substitutions (G/N147V/I, A246V and I427L) and previously described substitutions E119A/D/G/V, Q136K) specific for subtypes N4, N5, N6 and N8 of avian influenza virus have been identified in NA proteins. . The new G/N147 substitution is located in loop 150 (residues 147–152), which forms part of the active site of the NA enzyme [23].

Structural analysis showed that residue 147 plays a significant role in the conformation of the 150-loop. Most avian influenza viruses contain G at residue 147 of the NA protein, while avian influenza viruses of the N5 subtype and human subtype N2 viruses mostly have N at this location [23]. A G→N change at residue 147 conferring resistance to a neuraminidase inhibitor have not been previously reported [24].

The novel G147V in the N5 virus and N147I in the N8 subtype virus are causing reduced inhibitory action of zanamivir [25].

NA of the avian influenza virus subtype N8 has amino acid substitutions associated with a decrease in sensitivity to the neuraminidase inhibitor: N8 V116D (oseltamivir and zanamivir); N8 Q136K (zanamivir); N8 G147V (zanamivir); N8 S367C (oseltamivir); N8 N216D+I379V (oseltamivir) [25].

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. A/wild goose/Kostanai/KZ/83/2021(H5N8)		0,00190	0,00335	0,00354	0,00369	0,00492	0,00518	0,00495	0,00496	0,00559	0,00522	0,00467	0,00498	0,00512	0,00555
2. ON943056 A/chicken/Kazakhstan/23/2020(H5N8)	0,00430		0,00268	0,00300	0,00311	0,00442	0,00478	0,00449	0,00454	0,00518	0,00480	0,00444	0,00451	0,00468	0,00517
3. MW505409 A/Cygnus columbianus/Hubei/56/2020(H5N8)	0,01447	0,01009		0,00170	0,00265	0,00427	0,00462	0,00432	0,00444	0,00499	0,00457	0,00432	0,00442	0,00459	0,00496
4. OL442767 A/environment sample/China/TZD01/2021(H5N8)	0,01667	0,01227	0,00358		0,00308	0,00451	0,00483	0,00456	0,00466	0,00519	0,00480	0,00456	0,00469	0,00482	0,00513
5. MW137803 A/chicken/Egypt/F17230B/2019(H5N8)	0,01669	0,01302	0,01008	0,01226		0,00402	0,00433	0,00407	0,00415	0,00478	0,00431	0,00399	0,00415	0,00433	0,00478
6. MW026090 A/common gull/Denmark/18577-1/2016(H5N8)	0,03156	0,02781	0,02774	0,02997	0,02177		0,00240	0,00209	0,00214	0,00295	0,00234	0,00170	0,00209	0,00252	0,00320
7. KY576087 A/common teal/Korea/W548/2016(H5N8)	0,03534	0,03156	0,03148	0,03373	0,02548	0,00790		0,00253	0,00263	0,00252	0,00156	0,00194	0,00235	0,00264	0,00266
8. MF073903 A/chicken/Belgium/807/2017(H5N8)	0,03234	0,02857	0,02850	0,03074	0,02253	0,00574	0,00862		0,00230	0,00313	0,00269	0,00195	0,00235	0,00274	0,00329
9. MF926455 A/chicken/Moscow/94/2017(H5N8)	0,03305	0,02929	0,03000	0,03224	0,02401	0,00646	0,00863	0,00718		0,00319	0,00277	0,00192	0,00235	0,00266	0,00336
10. MG882000 A/grey-headed gull/Uganda/MUWRP-538/2017(H5N8)	0,03998	0,03617	0,03607	0,03834	0,03001	0,01155	0,00864	0,01301	0,01301		0,00252	0,00270	0,00303	0,00326	0,00207
11. MH165534 A/chicken/South Africa/Standerton/2017(H5N8)	0,03609	0,03231	0,03222	0,03448	0,02622	0,00718	0,00358	0,00935	0,00935	0,00864		0,00209	0,00246	0,00281	0,00277
12. MH819861 A/Mulard Duck/Hungary/59163/2016(H5N8)	0,03159	0,02783	0,02776	0,03000	0,02179	0,00430	0,00502	0,00502	0,00502	0,00937	0,00574		0,00126	0,00183	0,00285
13. MK208609 A/mulard duck/France/171201g/2017(H5N8)	0,03309	0,02858	0,02851	0,03075	0,02401	0,00646	0,00718	0,00718	0,00718	0,01155	0,00790	0,00215		0,00165	0,00313
14. MK494925 A/goose/Spain/IA17CR02699/2017(H5N8)	0,03538	0,03084	0,03076	0,03301	0,02625	0,00863	0,00935	0,00936	0,00936	0,01375	0,01008	0,00430	0,00358		0,00336
15. MK636736 A/Muscovy duck/Democratic Republic of the Congo/NVA4 NA/2017(H5N8)	0,04001	0,03619	0,03610	0,03837	0,03004	0,01374	0,01010	0,01448	0,01448	0,00574	0,01083	0,01083	0,01302	0,01522	

Figure 4 – Pairwise values of genetic differentiation based on neuraminidase of influenza A/H5N8 viruses

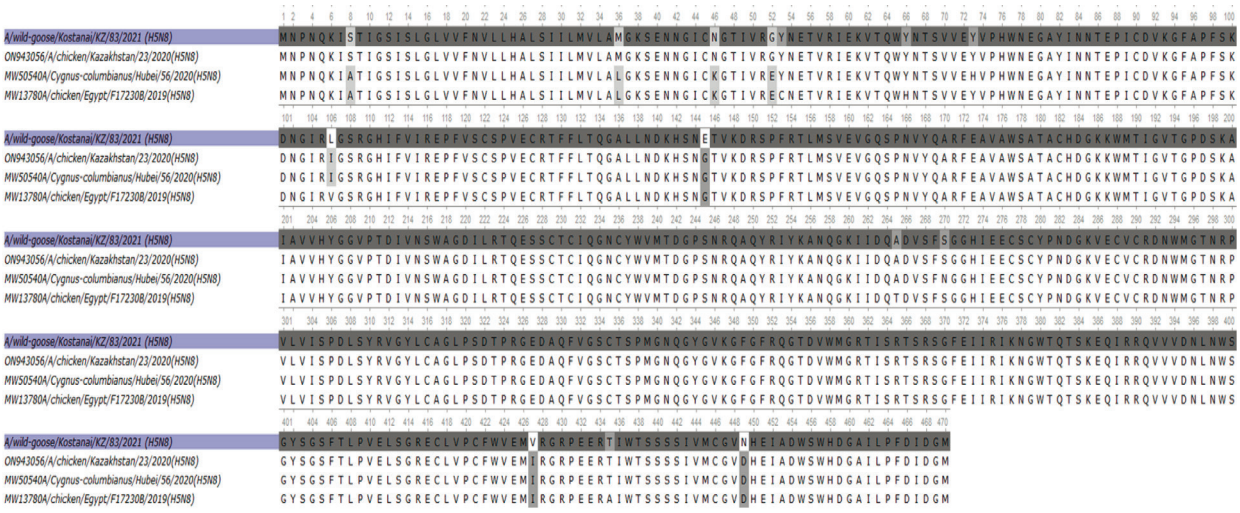

Figure 5 – Alignment of the amino acid sequence of neuraminidase of influenza A / H5N8 viruses

We have identified only one mutation N8 G147V (zanamivir) in the NA protein of the Kazakh strain A/wild goose/Kostanai/KZ/83/2021 (H5N8) of the avian influenza virus isolated during the 2021 season. It is known that the amino acid residue at position 147 is located in the NA active site and, therefore, a mutation at this position can potentially affect the biological properties of the virus. The G147V mutation has previously been described as being associated with an increase in the ability of NA to bind to receptors. The G147V substitution reduces the sensitivity of the virus to zanamivir.

No other mutations (V116D, Q136K, E119A/D/G/V, A246V, R292K, N294S, S367C, I427L) associated with decreased sensitivity to neuraminidase inhibitors were identified.

Conclusion

Genetic studies of the H5N1 and H7N7 avian influenza viruses isolated in epizootics indicate the presence of mutations that lead not only to an increase in pathogenicity for chickens, but also to the acquired ability to overcome the interspecies barrier with direct infection of humans, causing severe clinical manifestations with frequent lethal outcomes. A pandemic can occur as a result of mutations in the genes of viruses or of reassortment of the avian influenza virus genome with genes of human strains in the event of simultaneous infection of a person with an avian influenza virus and an epidemic strain of influenza A viruses (H1N1 or H3N2). Vaccine and chemoprophylaxis remain as the main measures to combat influenza. However, early production of a vaccine is not possible because the antigenic structure of a future pandemic influenza virus cannot be predicted. Out of the 4 anti-influenza chemotherapy drugs available – amantadine, rimantadine, oseltamivir and zanamivir, the latter 2 are preferred because they are more effective than amantadine and rimantadine. In addition, the resistance of influenza viruses to these drugs develops significantly less frequently. Chemotherapy has an immediate effect and does not affect the immunogenicity and protective efficacy of inactivated influenza vaccines [26]. To date, no human-to-human transmission of the avian influenza virus has been reported; all cases of infection were caused by birds.

The NA of the A/wild goose/Kostanai/KZ/83/2021 (H5N8) strain of avian influenza virus isolated during the 2021 season differs in genetic variability from the Kazakh strain (ON943056) A/chicken/Kazakhstan/23/2020 (H5N8) , isolated in 2020 based on five amino acid substitutions (at positions 46, 106, 145, 477, and 449).

In the active center of the NA strain A/wild goose/Kostanai/KZ/83/2021 (H5N8) of the avian influenza virus isolated in 2021 the amino acid substitution G147V, known to be associated with a decreased sensitivity of the influenza virus to the neuraminidase inhibitor zanamivir, was identified. No other mutations that cause a decrease in the sensitivity of the influenza A(H5N8) virus to the action of neuraminidase inhibitors were found.

Conflict of interests

All authors are familiar with the content of the article and have no conflict of interest.

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