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GENETIC ANALYSIS OF NUCLEOPROTEIN OF A/H3N8 INFLUENZA VIRUS OF ASIAN AND EUROPEAN ORIGIN ISOLATED IN 2018

This paper presents the results of a genetic analysis of the nucleoprotein gene (NP) of two strains of avian influenza virus isolated in the territory of the Republic of Kazakhstan in 2018 near small lakes (Alua and Zaimishche) of the North Kazakhstan region. These strains showed the presence of 54 in the nucleotide and 4 substitutions in the amino acid sequence, thereby significantly distancing themselves from each other. In phylogenetic analysis, the strain A/northern shoveler/North-Kazakhstan/20/2018(H3N8) showed the greatest relationship with strains from Europe, the homology between strains showed 96-97%. The second strain A/garganey/North Kazakhstan/45/2018(H3N8) showed genetic similarity with strains from Asia, the NP gene identity was 99%.

Key words: influenza, virus, NP, avian IAV, H3N8, vRNP, RNA.

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2018 жылы оқшауланған азиялық және еуропалық тектес A/H3N8 тұмауы вирусының нуклеопротеинінің генетикалық талдауы

Бұл жұмыста 2018 жылы Солтүстік Қазақстан облысының шағын көлдері (Алуа және Займище) маңынан Қазақстан Республикасы аумағында бөлінген құс тұмауы вирусының екі штаммының NP геніне жүргізілген генетикалық талдау нәтижелері берілген. Бұл штаммдар бір-бірінен нуклеотидтер тізбегінде 54 орынбасудың және аминқышқылдарының тізбегінде 4 алмастырудың болуын көрсетті, осылайша бір-бірінен айтарлықтай алшақтады. Филогенетикалық талдауда A/northern shoveler/North-Kazakhstan/20/2018(H3N8) штаммы Еуропадан келген штаммдармен ең үлкен қатынасты көрсетті, штаммдар арасындағы гомология 96-97% құрады. Екінші штамм A/garganey/North Kazakhstan/45/2018(H3N8) Азия штаммдарымен генетикалық ұқсастық көрсетті, NP генінің сәйкестігі 99% құрады.

Түйін сөздер: тұмау, вирус, NP, құс тұмауының вирусы, H3N8, vRNP, РНК.

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Генетический анализ Нуклеопротеина вируса гриппа A/H3N8 азиатского и европейского происхождения, выделенного в 2018 году

В данной работе представлены результаты генетического анализа NP гена двух штаммов вируса гриппа птиц выделенных на территории Республики Казахстан в 2018 году на территории мелких озер (Алуа и Займище) Северо-Казахстанской области. Данные штаммы показали между собой наличие 54 замен в нуклеотидной последовательности и 4 замен в аминокислотной последовательности, тем самым значительно дистанцируются между собой. При филогенетическом анализе наибольшее родство со штаммами из Европы показал штамм A/northern shoveler/North-Kazakhstan/20/2018(H3N8), гомология между штаммами составила 96-97%. Второй штамм A/garganey/North Kazakhstan/45/2018(H3N8) показал генетическое сходство со штаммами из Азии, идентичность по гену NP составила 99%.

Ключевые слова: грипп, вирус, NP, вирус птичьего гриппа, H3N8, vRNP, РНК.

Introduction

Influenza A viruses belonging to the *Orthomyxoviridae* family contain eight negative-sense single-stranded RNA segments. Due to a higher error rate during replication due to the lack of a proofreading mechanism, influenza A viruses have a rich genetic diversity. Wild birds form a large gene pool of influenza A viruses in nature, based on the genetic and antigenic variability of their surface proteins HA and NA, influenza viruses are classified into different antigenic subtypes such as hemagglutinin 18 (HA) and neuraminidase 11 (NA) [1-2] .

The wild bird reservoir plays an important role in the emergence, evolution, maintenance and spread of zoonotic influenza viruses. For example, the highly pathogenic avian influenza A (H5N1) virus, which has been repeatedly transmitted to humans since it was first reported in 1996, has indeed spread worldwide through bird migration [2]. The novel H7N9 avian influenza virus has been causing serious infections in humans in China since March 2013 and is a genetic reassortment of wild bird influenza virus and poultry avian influenza virus [3]. More recently, H5N10 influenza viruses, including H5N6 and H5N8 viruses, have been circulating in poultry and wild birds and are causing economic damage to livestock production. It should be noted that the H5N6 virus has crossed the species barrier and caused multiple infections among people in China and posed a threat to human health. As of January 15, 2021, 27 laboratory-confirmed cases of human infection with influenza A (H5N6) virus have been reported to WHO from China [4,5]. Once these zoonotic influenza viruses acquire the ability to transmit effectively from person to person, a pandemic will arise that will endanger the lives of people around the world. Three subtypes (H1N1, H2N2 and H3N2) of influenza viruses, with the exception of the 1918-1919 H1N1 influenza virus, some of whose genes originated from a wild waterfowl reservoir, have caused influenza pandemics in humans [6]. Waterfowl are the reservoir of influenza A viruses and are responsible for the evolution and spread of the virus over long distances. Occasionally, spread to poultry or domesticated mammals can lead to infection in humans and sustained transmission within a new mammalian host, as evidenced by the equine influenza (H3N8) (EIV) virus . H3N8 influenza viruses have been detected in a range of wild bird species, including *Anseriformes* (primarily from migratory ducks) and *Charadriiformes* (primarily from waders) of humans, and can trigger a population epidemic of the virus. In addition, sporadic cases of interspecies transmission of the H3N8 influenza virus have been reported in various species such as pigs, dogs, horses, seals and donkeys [12–16]. Although infected birds remain healthy or show only mild disease, H3N8 viruses can cause severe respiratory illness in mammalian hosts and even death. Of particular note, H3N8 avian influenza viruses have established stable strains in dogs and horses. Although sporadic cases of H3N8 avian IAV have not been reported in humans, a previous study showed that the H3N8 seal virus can be transmit-

ted by airborne infection in ferrets, which is widely used to assess the pandemic potential of influenza viruses in humans [17]. The above studies show that H3N8 AIVs are a potential threat to human and animal health.

The study of the molecular genetic properties of all 8 genes is necessary to understand the structure and reproduction of the virus. The nucleoprotein (NP) gene of the avian influenza virus plays a central role in viral replication. As a structural protein with no intrinsic enzymatic activity, it is the most abundant viral protein in infected cells. NP is a critical component of the viral ribonucleoprotein (vRNP) complex, and recognized functions of NP include, among others, organization of RNA packaging, nuclear transport, and transcription and replication of viral RNA. NP is a multifunctional protein and is indispensable for virus replication [18].

Materials and methods

Objects of study

Since 2018, materials (cloaca swabs) have been collected from various species of wild birds. The territory for collecting material was small lakes (Alua and Zaimishche) of the North Kazakhstan region. In total, samples of cloacal swabs from 90 wild birds of the families *Anatidae*, *Accipitridae*, *Rallidae*, *Podicipedidae* were studied. Cloac samples were collected aseptically and stored and transported in liquid nitrogen. Samples were stored at -40°C until the study.

Isolation of viral RNA

Viral RNA isolation was performed using the QIAamp Viral RNA Mini Kit according to the manufacturer's recommendations from 140 µl of virus-containing liquid.

Synthesis of CDNA

Reverse transcription for cDNA synthesis was carried out with reversease – 200 U/µl M-MLV enzyme synthesizing cDNA on an RNA template using the Uni12(AGCAAAAGCAGG) primer.

Setting up a polymerase chain reaction

The PCR mixture was collected using a commercial Platinum SuperFi DNA Polymerase kit (Invitrogen) according to the manufacturer's recommendations using the primers listed in Table 1. The temperature-time regimen of amplification was carried out according to the program: 1) 50°C, 30 min.; 94 °C – 2 min.; 2) 35 cycles 94 °C – 30 sec.; 50 °C – 30 sec.; 68 °C – 1 min., post-amplification 68 °C – 7 min. Amplification was carried out on a Mastercycler X50s cycler, Eppendorf (USA).

Table 1 – Primers used

Primer name	Sequence 5' – 3'	Product size, bp
Bm-NP-1	AGC AAA AGC AGG GTA	1565
Bm-NP-1565R	AGT AGA AAC AAG GGT ATT TTT	

Sequencing

Samples for sequencing were prepared using the BigDye Terminator v3.1 Cycle Sequencing kit from Applied Biosystems. Sequencing was performed on a 3130xl Genetic Analyzer automated 16-capillary sequencer (Applied Biosystems/Hitachi). Nucleotide sequences were analyzed and assembled using the Sequencer v 4.5 program [26].

Comparative and phylogenetic analysis

Comparative analysis by nucleotide sequences was carried out in the software modules of the NCBI website (http://www.ncbi.nlm.nih.gov/genbank/). The search for homologous nucleotide sequences of influenza A virus genes was carried out using the BLAST program in the GeneBank database. Phylogenetic analysis of the sequences was performed using the Mega 10 program. The phylogenetic tree was built using the Neighbor-joining method and the Kimura 2-parameter model with the inclusion of substitutions d: Transitions + Transversions, as well as the inclusion of codons 1st + 2nd + 3rd + Non-Coding and repetition bootsraps 500.

Results and discussion

As a result of the research, genetic studies of the degree of genetic relationship of Kazakhstani strains of European avian influenza virus A/northern shoveler/North-Kazakhstan/20/2018(H3N8) and Asian origin A/garganey/North Kazakhstan/45/2018(H3N8) with reference viruses were carried out. from the international gene database (GenBank). The results of sequencing of the accumulated PCR products of the NP gene, the results of which determined their nucleotide sequences. The nucleotide sequences of the NP gene are shown in Figure 1.

When conducting a comparative analysis of the nucleotide sequences of the NP gene of two Kazakh influenza virus strains A/northern shoveler/North-Kazakhstan/20/2018(H3N8) and A/garganey/North Kazakhstan/45/2018(H3N8) isolated in 2018 in the northern regions Kazakhstan, it was found that the isolates differ in the presence of 54 nucleotide substitutions and 4 amino acid substitutions. The results of the comparative analysis are presented in Figure-2.3.

>ON682725 A/northern shoveler/North-Kazakhstan/20/2018(H3N8)
AGCAGGGTAGATAATCACTCACTGAGTGACATCAAAATCATGGCGTCTCAAGGCACCAAACGATCTTATGAACA-
GATGGAAACTGGTGGAGAACGCCAGAATGCCACTGAAATCAGAGCATCTGTTGGAAGGATGGTTGGTGGAATTGGG-
AGGTTCTACATACAGATGTGCACTGAACTCAAACTCAGCGACTATGAAGGGAGGCTGATCCAGAACAGCATA-
ACGATAGAGAGAATGGTTCTCTCTGCATTTGATGAAAGGAGGAACAAATACCTGGAAGAACATCCCAGTGC-
GGGGAAGGACCCGAAGAAAACTGGAGGTCCAATTTATCGAAGGAGAGATGGGAAATGGATGAGAGAACTGATCCT-
GTATGACAAAGAGGAGATCAGAAGGATCTGGCGTCAAGCGAACAATGGAGAGGACGCAACTGCTGGTCTCACT-
CACCTGATGATCTGGCATTCCAATCTAAATGATGCCACATACCAGAGGACAAGAGCTCTCGTGCGTACTGGGATG-
GACCCCAGAATGTGCTCTCTGATGCAAGGATCAACTCTCCCGAGGAGATCTGGAGCTGCTGGTGCAGCAGTA-
AAGGGAGTCGGAACGATGGTGATGGAACTAATTCGGATGATAAAACGAGGGATTAATGACCGGAATTTCTGGAGA-
GGCGAAAACGGACGGAGAACAAGGATTGCATATGAGAGAATGTGCAACATCCTCAAAGGGAAATTCCAAA-
CAGCAGCACAAAGAGCAATGATGGATCAGGTGCGTGAAAGCAGGAATCCTGGCAATGCTGAAATTGAAGATCT-
TATCTTTCTGGCACGGTCTGCACTCATCCTGAGAGGATCAGTGGCCCATAAGTCCTGCTTGCCTGCTTGTGTATAC-
GGACTCGCTGTGGCCAGTGGATACGACTTTGAGAGAGAAGGGTACTCTCTAGTTGGAATAGATCCTTTCCGTCT-
GCTTCAAAACAGCCAGGTCTTCAGTCTCATTAGACCAAATGAGAACCCAGCACACAAGAGTCAATTGGTTTGGATG-
GCATGCCATACTGCAGCATTTGAAGACCTGAGAGTCTCAAGTTTCATCAGAGGGACAAGAGTGGTCCCAAGAGGA-
CAACTATCCACCAGAGGAGTTCAAATTGCTTCAAACGAGAACATGGAAACAATGGACTCCAGCACTCTTGAATT-
GAGGAGCAGATATTGGGCTATAAGAACCAGGAGTGGAGGAAACACCAACCAACAGAGAGCATCTGCAGGACA-
GATCAGTGTACAGCCCACTTTCTCGGTACAGAGAAATCTTCCCTTCGAAAGAGCGACCATTATGGCGGCGTTCA-
CAGGAAATACTGAAGGCAGAACATCTGACATGAGGACTGAAATCATAAGAATGATGGAAAGTGCCAGACCAGAA-
GATGTGTCCTTCCAGGGGCGGGGAGTCTTCGAGCTCTCGGACGAAAAGGCAACGAACCCGATCGTGCCTTCCTTT-
GACATGAGTAATGAAGGATCTTATTTCTTCGGAGACAATGCAAAGGAGTATGACAATTAAAGAAAAATACCCTT-
GTTTCTACT

>MT126635 (A/garganey/North Kazakhstan/45/2018(H3N8)
AGCAGGGTAGATAATCACTCACTGAGTGACATCAACATCATGGCGTCTCAAGGCACCAAACGATCTTATGAA-
CAGATGGAAACTGGTGGAGAGCGCCAGAATGCCACTGAGATCAGAGCATCTGTTGGAAGAATGGTTGGTG-
GAATTGGGAGGTTCTACATACAGATGTGCACTGAGCTCAAACTCAGCGACTATGAAGGAAGGCTGATCCAGAA-
CAGCATAACAATAGAGAGAATGGTTCTATCTGCATTTGATGAAAGGAGGAACAAATATCTGGAAGAACATCCCAGT-
GCGGGGAAGGACCCGAAGAAAACTGGAGGTCCAATTTATCGGAGGAGAGATGGGAAATGGGTGAGAGAACT-
GATCCTGTACGACAAAGAGGAGATCAGGAGGATCTGGCGTCAAGCGAACAATGGAGAAGACGCAACTGCTG-
GTCTCACTCACCTGATGATCTGGCATTCCAATCTAAATGATGCCACATACCAGAGGACAAGAGCTCTCGTGC-
GTACTGGGATGGACCCCAGGATGTGCTCTCTTATGCAAGGATCAACTCTCCCAAGGAGATCTGGAGCTGCTG-
GTGCAGCAGTAAAGGGAGTCGGGACAATGGTGATGGAACTAATTCGGATGATAAAGCGAGGAATTAAT-
GATCGGAACTTCTGGAGAGGTGAGAATGGACGAAGGACAAGGATTGCATATGAGAGAATGTGCAACATCCT-
CAAAGGGAAATTCCAAACAGCAGCACAAAGAGCAATGATGGACCAGGTGCGTGAAAGCAGGAATCCTGGCAAT-
GCTGAAATTGAAGATCTCATCTTTCTGGCACGGTCTGCACTCATCCTGAGAGGGTCAGTGGCCCATAAGTCCTGCTT-
GCCTGCTTGTGTGTACGGACTCGCTGTGGCCAGTGGATACGACTTTGAGAGAGAAGGGTACTCTCTAGTTGGAATA-
GATCCTTTCCGTCTGCTTCAAAACAGCCAGGTCTTCAGTCTCATTAGACCAAATGAGAATCCAGCACACAAGAGT-
CAATTGGTGTGGATGGCATGTCATTCTGCAGCATTCGAGGATCTGAGAGTCTCAAGTTTCATCAGAGGGACAAGAG-
TAGTTCCAAGAGGACAACTATCCACCAGAGGAGTTCAAATTGCTTCAAATGAGAATATGGAAACAATGGACTC-
CAGCACTCTTGAACTGAGAAGCAGATATTGGGCTATAAGAACCAGGAGTGGAGGAAACACCAACCAACAAAGAG-
CATCTGCAGGACAAATCAGTGTACAGCCCACTTTCTCGGTACAGAGAAATCTTCCCTTTGAAAGAGCGACCAT-
TATGGCGGCGTTCACAGGGAATACTGAGGGCAGAACATCCGACATGAGGACTGAAATCATAAGAATGATGGAAAGT
GCCAGACCAGAAGATGTGTCTTTCCAGGGGCGGGGAGTCTTCGAGCTCTCGGACGAAAAGGCAACGGACCCGATC
GTGCCTTCCTTTGACATGAGTAATGAAGGATCTTATTTCTTCGGAGACAATGCAGAGGAGTATGACAATTAAAGAAA
AATACCCTTG

Figure 1 – Nucleotide sequence of the NP gene of Kazakhstan influenza virus strains A/northern shoveler/North-Kazakhstan/20/2018(H3N8) and A/garganey/North-Kazakhstan/45/2018(H3N8)

Analysis of the nucleotide sequence of the NP gene of Kazakhstan influenza virus strains A/northern shoveler/North-Kazakhstan/20/2018(H3N8) and A/garganey/North Kazakhstan/45/2018(H3N8) by the BLAST program showed that the sequence homology between them is 97% . The results are presented in Figure-2.

Further studies were aimed at conducting a comparative analysis of the NP gene of new strains of avian influenza virus with the available data in the international gene bank and compiling a phylogenetic tree. To determine the phylogenetic characteristics of two influenza virus strains A/northern shoveler/North-Kazakhstan/20/2018(H3N8) and A/garganey/North Kazakhstan/45/2018(H3N8), the sequenced region of the NP gene was aligned with the nucleotide sequences of influenza virus strains from the international data bank. The results are shown in Figure-4

Score	Expect	Identities	Gaps	Strand
2566 bits(1389)	0.0	1497/1551(97%)	0/1551(0%)	Plus/Plus

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Sbjct  1     ....................................A.........................  60

Query  61    CGATCTTATGAACAGATGGAAACTGGTGGAGAGCGCCAGAATGCCACTGAGATCAGAGCA  120
Sbjct  61    ................................A..................A.........  120

Query  121   TCTGTTGGAAGAATGGTTGGTGGAATTGGGAGGTTCTACATACAGATGTGCACTGAGCTC  180
Sbjct  121   ...........G.................................................A...  180

Query  181   AAACTCAGCGACTATGAAGGAAGGCTGATCCAGAACAGCATAACAATAGAGAGAATGGTT  240
Sbjct  181   ....................G.........................G...............  240

Query  241   CTATCTGCATTTGATGAAAGGAGGAACAAATATCTGGAAGAACATCCCAGTGCGGGGAAG  300
Sbjct  241   ..C.............................C............................  300

Query  301   GACCCGAAGAAAACTGGAGGTCCAATTTATCGGAGGAGAGATGGGAAATGGGTGAGAGAA  360
Sbjct  301   ................................A..................A.........  360

Query  361   CTGATCCTGTACGACAAAGAGGAGATCAGGAGGATCTGGCGTCAAGCGAACAATGGAGAA  420
Sbjct  361   ...........T..................A.............................G  420

Query  421   GACGCAACTGCTGGTCTCACTCACCTGATGATCTGGCATTCCAATCTAAATGATGCCACA  480
Sbjct  421   ............................................................  480

Query  481   TACCAGAGGACAAGAGCTCTCGTGCGTACTGGGATGGACCCCAGGATGTGCTCTCTTATG  540
Sbjct  481   ..............................................A...........G...  540

Query  541   CAAGGATCAACTCTCCCAAGGAGATCTGGAGCTGCTGGTGCAGCAGTAAAGGGAGTCGGG  600
Sbjct  541   ..................G.............................................A  600

Query  601   ACAATGGTGATGGAACTAATTCGGATGATAAAGCGAGGAATTAATGATCGGAACTTCTGG  660
Sbjct  601   ..G.............................A.....G........C.....T.......  660

Query  661   AGAGGTGAGAATGGACGAAGGACAAGGATTGCATATGAGAGAATGTGCAACATCCTCAAA  720
Sbjct  661   .....C..A..C.....G..A.....................................  720

Query  721   GGGAAATTCCAAACAGCAGCACAAAGAGCAATGATGGACCAGGTGCGTGAAAGCAGGAAT  780
Sbjct  721   ....................................T.....................  780

Query  781   CCTGGCAATGCTGAAATTGAAGATCTCATCTTTCTGGCACGGTCTGCACTCATCCTGAGA  840
Sbjct  781   ............................T.................................  840

Query  841   GGGTCAGTGGCCCATAAGTCCTGCTTGCCTGCTTGTGTGTACGGACTCGCTGTGGCCAGT  900
Sbjct  841   ..A.....................................A.....................  900

Query  901   GGATACGACTTTGAGAGAGAAGGGTACTCTCTAGTTGGAATAGATCCTTTCCGTCTGCTT  960
Sbjct  901   ............................................................  960

Query  961   CAAAACAGCCAGGTCTTCAGTCTCATTAGACCAAATGAGAATCCAGCACACAAGAGTCAA  1020
Sbjct  961   ........................................C...................  1020

Query  1021  TTGGTGTGGATGGCATGTCATTCTGCAGCATTCGAGGATCTGAGAGTCTCAAGTTTCATC  1080
Sbjct  1021  .....T...........C...A..........T..A..C.....................  1080

Query  1081  AGAGGGACAAGAGTAGTTCCAAGAGGACAACTATCCACCAGAGGAGTTCAAATTGCTTCA  1140
Sbjct  1081  ..............G..C.............................................  1140

Query  1141  AATGAGAATATGGAAACAATGGACTCCAGCACTCTTGAACTGAGAAGCAGATATTGGGCT  1200
Sbjct  1141  ..C.....C.............................T....G................  1200

Query  1201  ATAAGAACCAGGAGTGGAGGAAACACCAACCAACAAAGAGCATCTGCAGGACAAATCAGT  1260
Sbjct  1201  ....................................G.................G......  1260

Query  1261  GTACAGCCCACTTTCTCGGTACAGAGAAATCTTCCCTTTGAAAGAGCGACCATTATGGCG  1320
Sbjct  1261  ........................................C...................  1320

Query  1321  GCGTTCACAGGGAATACTGAGGGCAGAACATCCGACATGAGGACTGAAATCATAAGAATG  1380
Sbjct  1321  ...........A........A...........T.............................  1380

Query  1381  ATGGAAAGTGCCAGACCAGAAGATGTGTCTTTCCAGGGGCGGGGAGTCTTCGAGCTCTCG  1440
Sbjct  1381  ............................C.................................  1440

Query  1441  GACGAAAAGGCAACGGACCCGATCGTGCCTTCCTTTGACATGAGTAATGAAGGATCTTAT  1500
Sbjct  1441  ..............A.............................................  1500

Query  1501  TTCTTCGGAGACAATGCAGAGGAGTATGACAATTAAAGAAAAATACCCTTG  1551
Sbjct  1501  ..................A................................  1551
```

Figure 2 – Comparative analysis of the nucleotide sequence of the NP gene of Kazakhstan influenza virus strains ON682725 A/northern shoveler/North-Kazakhstan/20/2018(H3N8) and MT126635 A/garganey/North azakhstan/45/2018(H3N8)

Range 1: 1 to 498 GenPept Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Method	Identities	Positives	Gaps
1028 bits(2657)	0.0	Compositional matrix adjust.	494/498(99%)	498/498(100%)	0/498(0%)

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Query  1    MASQGTKRSYEQMETGGERQNATEIRASVGRMVGGIGRFYIQMCTELKLSDYEGRLIQNS  60
Sbjct  1    ............................................................  60

Query  61   ITIERMVLSAFDERRNKYLEEHPSAGKDPKKTGGPIYRRRDGKWVRELILYDKEEIRRIW  120
Sbjct  61   ...........................................M................  120

Query  121  RQANNGEDATAGLTHLMIWHSNLNDATYQRTRALVRTGMDPRMCSLMQGSTLPRRSGAAG  180
Sbjct  121  ............................................................  180

Query  181  AAVKGVGTMVMELIRMIKRGINDRNFWRGENGRRTRIAYERMCNILKGKFQTAAQRAMMD  240
Sbjct  181  ............................................................  240

Query  241  QVRESRNPGNAEIEDLIFLARSALILRGSVAHKSCLPACVYGLAVASGYDFEREGYSLVG  300
Sbjct  241  ............................................................  300

Query  301  IDPFRLLQNSQVFSLIRPNENPAHKSQLVWMACHSAAFEDLRVSSFIRGTRVVPRGQLST  360
Sbjct  301  .................................T..........................  360

Query  361  RGVQIASNENMETMDSSTLELRSRYWAIRTRSGGNTNQQRASAGQISVQPTFSVQRNLPF  420
Sbjct  361  ............................................................  420

Query  421  ERATIMAAFTGNTEGRTSDMRTEIIRMMESARPEDVSFQGRGVFELSDEKATDPIVPSFD  480
Sbjct  421  ...............................................N........  480

Query  481  MSNEGSYFFGDNAEEYDN  498
Sbjct  481  .............K....  498
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Figure 3 – Comparative analysis of the amino acid sequence of the NP gene of the Kazakh strains of the influenza virus ON682725 A/northern shoveler/North-Kazakhstan/20/2018(H3N8) and MT126635 A/garganey/North Kazakhstan/45/2018(H3N8)

Figure 4 – Phylogenetic tree of nucleotide sequences of the NP gene of strains ON682725 A/northern shoveler/North-Kazakhstan/20/2018(H3N8) is marked with a square and MT126635 A/garganey/North Kazakhstan/45/2018(H3N8) is marked with a circle with influenza virus strains from international data bank GenBank

Kazakhstan, as the largest country in Central Asia, is the site of transcontinental migration routes connecting East Asian migration routes with European ones and vice versa [22]. Genetic analysis in the avian influenza monitoring system makes it possible to determine the genetic characteristics of influenza virus strains isolated from wild birds, identify strains with unusual biological properties and strains with high virulence, as well as predict epizootic situations and justify timely interventions in the epizootic process. The study of phylogenetic similarities of circulating avian influenza virus strains around the world is necessary to identify the mechanisms of their spread [19].

The study of phylogenetic similarities of the genetic relationship of Kazakhstani strains of avian influenza virus in the NP gene of European A/northern shoveler/North-Kazakhstan/20/2018(H3N8) and Asian origin A/garganey/North Kazakhstan/45/2018(H3N8) with reference viruses from the international database showed (Figure 4) that the strain of European origin A/northern shoveler/North-Kazakhstan/20/2018(H3N8) showed the greatest relationship with the strain from Russia (A/duck/Moscow/4298/2010(H3N8) and the Netherlands (A/Mallard/Netherlands/8/2013(H3N8) through which the migratory route of wild geese runs, the homology between strains was 99%. with strains from Mongolia, Bangladesh and South Korea, identity between strains varies between 96%-97% There are 4 major transcontinental migration routes in Mongolia alone (East Asia/Australia, Central and Asia/India, Western Asia/Africa and the Mediterranean/Black Sea), outbreaks of various subtypes of avian influenza virus have also been detected in these countries [20].

The presence of similar segments of identified low pathogenic H3N8 strains in wild waterfowl from Russia and Europe also suggests a link between new reassortants and migratory birds from Asian countries of the African-Eurasian flyway as molting and resting sites along the Russian-Kazakhstan, Mongolian and Chinese borders [21]. According to the World Organization for Animal Health, the deterioration of the epizootic picture in 2018-2019 led to outbreaks of highly pathogenic H5N8 strains around the world in 2020. Significant deaths were recorded among poultry in Kazakhstan, China, Mongolia and Russia [23].

An analysis of various literature shows a significant spread of the H3N8 subtype avian influenza virus on all continents of the world; the availability of such data allows us to conclude that there is a global threat to human and animal health. The avian influenza virus is distinguished by the peculiarity of interspecies transfer of the host, thereby creating a great danger for new epizootic outbreaks. Observation and study of the molecular genetic properties of circulating new strains of avian influenza virus in the territory of the Republic of Kazakhstan is necessary for preventive measures and the prevention of new outbreaks.

The study of the origin of hemagglutinin (HA) of these strains by phylogenetic analysis differentiated the strain A/northern shoveler/North-Kazakhstan/20/2018(H3N8) into the group of the European line, the strain A/garganey/North Kazakhstan/45/2018(H3N8) belonged to the group Asian line [24].

Thus, the phylogenetic tree of the NP gene showed that two genetically different H3 viruses of Asian and European origin circulate in Kazakhstan. The circulation of these strains is due to the location of Kazakhstan on the main migratory routes of wild birds, which are reservoirs of the avian influenza virus. Recombination of H3 subtype influenza viruses requires constant monitoring in Kazakhstan.

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