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Genetic analysis of nucleotide sequences of neuraminidase gene of highly pathogenic avian influenza A/H5N8 virus isolates recovered in the Russian Federation in 2020

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SUMMARY

Avian influenza is a highly dangerous viral disease that causes huge economic damage to poultry farming. Currently, highly virulent influenza virus with N8 neuraminidase subtype is quite often detected in populations of domestic and wild birds in various countries of the world. The article provides data on complete nucleotide sequences of the neuraminidase gene of highly pathogenic avian influenza virus isolates recovered in the second half of 2020 from pathological material received from four regions of the Russian Federation. The conducted research showed that the subtype of the isolated virus was N8. According to the phylogenetic analysis, isolates of N8 virus belong to group 8C.4. During the phylogenetic analysis of the neuraminidase, we also took into account data on hemagglutinin classification, according to which H5N8 virus isolates belong to a widespread clade 2.3.4.4. Viruses of the clade were first registered in 2010 in China and they have been circulating up to now. The paper also provides data of a comparative analysis of nucleotide sequences of the studied isolates and the isolates from the international GenBank and GISAID databases, recovered in other countries from 2007 to 2020. During the analysis of the amino acid sequence of the studied isolates, no substitutions were found in the positions that affect resistance to neuraminidase inhibitors. The complete nucleotide sequences of the neuraminidase gene of the avian influenza virus subtype N8 (isolates A/domestic goose/OMSK/1521-1/2020, A/duck/Chelyabinsk/1207-1/2020, A/duck/Saratov/1578-2/2020, A/goose/Tatarstan/1730-2/2020) are published in the international GenBank and GISAID databases. Based on the analysis of the nucleotide sequences of the studied isolates, the article shows gradual evolution of the N8 subtype virus.

Keywords: avian influenza virus, RT-PCR, sequencing, N8 neuraminidase subtype, phylogenetic analysis, H5N8

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Генетический анализ нуклеотидных последовательностей гена нейраминидазы изолятов вируса высокопатогенного гриппа птиц A/H5N8, выделенных на территории Российской Федерации в 2020 году

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РЕЗЮМЕ

Грипп птиц является особо опасной болезнью вирусной этиологии, наносящей огромный экономический ущерб птицеводству. В настоящее время в популяциях домашних и диких птиц в различных странах мира достаточно часто выявляют высоковирулентный вирус гриппа с нейраминидазой подтипа N8. В статье представлены данные по изучению полных нуклеотидных последовательностей гена нейраминидазы изолятов вируса высокопатогенного гриппа птиц, выделенных во второй половине 2020 г. из патологического материала, поступившего из четырех регионов Российской Федерации. В результате проведенных исследований определен подтип выделенного вируса – N8. Согласно данным филогенетического анализа, изоляты вируса N8 относятся к группе 8С.4. При проведении филогенетического анализа по нейраминидазе также учитывали данные классификации по гемагглютинину, согласно которой изоляты вируса H5N8 относятся к широко распространенной кладе 2.3.4.4. Вирусы данной клады впервые зарегистрированы в 2010 г. в Китае и продолжают циркулировать до настоящего времени. Также в работе приведены данные сравнительного анализа нуклеотидных последовательностей исследуемых изолятов и изолятов из международных баз данных GenBank и GISAID, выделенных в других странах в период с 2007 по 2020 г. В ходе проведения анализа аминокислотной последовательности исследуемых изолятов в позициях, которые влияют на резистентность к ингибиторам нейраминидаз, замен не обнаружено. Полные нуклеотидные последовательности гена нейраминидазы вируса гриппа птиц подтипа N8 изолятов A/domestic goose/Omsk/1521-1/2020, A/duck/Chelyabinsk/1207-1/2020, A/duck/Saratov/1578-2/2020, A/goose/Tatarstan/1730-2/2020 опубликованы в международных базах GenBank и GISAID. На основании анализа нуклеотидных последовательностей изученных изолятов показана постепенная эволюция вируса подтипа N8.

Ключевые слова: вирус гриппа птиц, ОТ-ПЦР, секвенирование, подтип нейраминидазы N8, филогенетический анализ, H5N8

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INTRODUCTION

For many years, an unfavorable situation with highly pathogenic avian influenza (HPAI) has been observed around the world. Since 1996 highly virulent avian influenza virus subtype H5N1, isolated from domestic geese on one of the farms in Guangdong Province (China), has become epizootic [1]. Due to the disease introduction into the Russian Federation in 2005 resulting in significant damage to the national poultry industry, modern diagnostic methods were developed [2], primarily to detect A/H5N1 virus. From 2016 to 2020, outbreaks of HPAI caused by the H5N8 subtype were regularly recorded in Russia and in many countries of Asia, Africa and Europe.

One of the first HPAI H5N8 cases was reported in 1983, when the virus was detected in turkeys in Ireland [3, 4]. In 2001, the next H5N8 case was detected in wild birds in the State of New Jersey (the USA) during environmental monitoring, and since then several sporadic cases of HPAI have been reported in the country. After three HPAI isolates were detected in Korea in 2014: A/breeder duck/Korea/Gochang1/2014 (H5N8), A/duck/Korea/Buan2/2014 (H5N8) and A/Baikal Teal/Korea/Donglim3/2014 (H5N8), H5N8 subtype has spread widely across the USA, and then throughout other Asian and European countries [5–8]. The pathogenicity index of this virus is significantly higher than that of the virus isolated in Ireland [5]. According to Y. J. Lee et al., highly virulent avian influenza viruses of H5N8 subtype detected in Korea in 2014 occurred as a result of reassortment of the A/duck/Jiangsu/k1203/2010 (H5N8) virus with viruses of other subtypes that circulated in China from 2009 to 2012 [9]. The exact origin of the virus discovered in 2010 in China

remains unknown. K. Zhao et al. note in their studies that this strain was very likely a new reassortant of three subtypes: H5N1 (high identity in the PB1, PA, M, NS genes), H3N8 (in NA gene), H6N2 (NP gene) [10].

In late 2016, HPAI epidemic situation in the Russian Federation deteriorated. Thus, from November to December 2016, HPAI H5N8 outbreaks in domestic birds were recorded in the Astrakhan and Rostov Oblasts, in the Krasnodar Krai and the Republic of Kalmykia. In 2017, more than 30 cases of highly virulent avian influenza virus subtype H5N8 were detected in poultry herds in 8 regions: the Rostov, Moscow, Nizhny Novgorod and Samara Oblasts, the Republics of Tatarstan, Mari El, as well as in the Udmurt and Chechen Republics. Influenza cases were detected in wild migrating birds in the Krasnodar Krai and in the Kaliningrad Oblast, and in zoo birds in the Voronezh Oblast. The disease outbreaks resulted in huge economic losses on commercial farms of the Astrakhan, Rostov, Moscow Oblasts, and in the Republic of Tatarstan [11].

In 2018, HPAI H5N8 was found in domestic birds from the Kursk, Orel, Voronezh, Smolensk, Saratov, Samara, Ulyanovsk, Penza, Nizhny Novgorod, Rostov Oblasts, the Republics of Udmurtia, Mari El, Chuvashia and Tatarstan (more than 80 cases) [12].

Thus, H5N8 influenza viruses, closely related to the isolates originally recovered in South Korea in early 2014, have globally spread. According to the information published by the OIE on November 12, 2020, 265 outbreaks of highly pathogenic avian influenza of H5N8 subtype (111 in domestic, 154 in wild birds) were registered in Europe, Asia and Africa [13], including outbreaks in 12 regions of the Russian Federation: Kostroma, Kurgan, Omsk, Rostov, Samara, Saratov, Tomsk, Tyumen, Chelyabinsk Oblasts, Khanty-Mansi Autonomous Okrug – Yugra, the Republic of Tatarstan, the Karachay-Cherkess Republic.

The purpose of this work was to genetically analyze nucleotide sequences of N8 neuraminidase gene in HPAI virus isolates, recovered in the Russian Federation in 2020, in order to get up-to-date information on the genetic relatedness of HPAI isolates.

MATERIALS AND METHODS

Viruses. The following isolates of the avian influenza virus subtype H5N8 were used in the work:

– A/duck/Chelyabinsk/1207-1/2020 – it was isolated from a domestic duck in the village of Peschanoe, the Uvelsky Raion of the Chelyabinsk Oblast in late July 2020;

– A/domestic goose/Omsk/1521-1/2020 – it was isolated from a domestic goose in the village of Irtyshsky, the Omsk Raion, the Omsk Oblast in September 2020;

– A/duck/Saratov/1578-2/2020 – it was isolated from a domestic duck in the Engels Raion of the Saratov Oblast in September 2020;

– A/goose/Tatarstan/1730-2/2020 – it was isolated from a domestic goose in the village of Meshcheryakovo, the Buinsky Raion of the Republic of Tatarstan in October 2020.

The virus was isolated in 10–11-day-old SPF chicken embryo eggs.

Primers. Several primer systems were used in the work, designed to detect RNA of HPAI subtype N8 and to determine complete nucleotide sequence of the neuraminidase (NA) gene of HPAI subtype N8 isolates (ZAO "Syntol", Russia).

Extraction of RNA. Viral RNA was extracted from the allantoic fluid of SPF chicken embryo eggs infected with the corresponding isolates, using the AmpliPrime RIBO-sorb kit (NextBio LLC, Russia) in accordance with the instructions for use.

Reverse transcription polymerase chain reaction (RT-PCR). Reverse transcription followed by amplification of NA gene fragments to determine the complete nucleotide sequence of HPAI isolates of N8 subtype was performed in BioRad programmable amplifiers (Bio-Rad Laboratories, USA). Synthesis of the first chain of complementary DNA on viral RNA was carried out using RNA-dependent DNA polymerase from avian myeloblastosis virus (AMV Reverse Transcriptase). The reaction was performed in 25 µl of reaction mixture containing 1 µl of 10 mM dNTP, 5 µl of 5× buffer, 3 µl of 25 mM $MgCl_2$, 1 µl of 10 µM forward and reverse primer, 0.125 µl of reverse transcriptase (Promega, USA), and 0.25 µl of Taq polymerase, 8.625 µl of RNase-free water and 5 µl of total RNA. To prevent evaporation of the mixture, 15 µl of Mineral Oil Light White (MP Biomedicals, France) was layered on top. The reaction mixture was incubated in an amplifier at 50 °C for 25 minutes to develop the first cDNA chain. Then they were heated at 95 °C for 10 minutes. The next 39 cycles of conventional PCR were carried out at the following temperature conditions: denaturation at 95 °C – for 50 seconds, annealing of primers at 55 °C – for 50 seconds, elongation at 72 °C – for 1 minute.

The amplification products were analyzed by electrophoresis in 1% agarose gel stained with ethidium bromide.

Purification of PCR products. cDNA fragments amplified in PCR were purified using the Wizard® SV Gel and PCR Clean-Up System kit (Promega, USA) in accordance with the manufacturer's instructions.

DNA sequencing. The primary nucleotide sequence of AIV NA gene fragments was determined in an automatic sequencer ABI Prism 3100 Genetic Analyzer (Applied Biosystems, USA) using the BigDye™ Terminator Cycle Sequencing kit (Applied Biosystems, USA) according to the manufacturer's instructions.

Phylogenetic analysis of nucleotide sequences was performed using the BioEdit Software, Version 7.0.5.3 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). To compare the complete sequences of NA gene of the recovered isolates we used NA gene sequences of the AIV/N8 strains from the international GenBank (http://www.ncbi.nlm.nih.gov/genomes/FLU/FLU.html) and GISAID databases.

The phylogenetic tree was constructed using the NJ algorithm (including the resampling method "bootstrap") in the implementation of the MEGA package, Version 7.0.26.

RESULTS AND DISCUSSION

One of the main objectives of this research was to obtain up-to-date information about the genetic relatedness of AIV isolates recovered in 2020 in the Russian Federation, as well as their comparison with other AIVs of subtype N8.

At the first stage of the research, we determined nucleotide sequences of the neuraminidase gene of the four influenza virus isolates and compared them with the sequences of AIV N8 subtype from Eurasia and Africa stored in the international databases.

As a rule, the genetic characterization of avian influenza viruses is based on the hemagglutinin classification, which was implemented by the WHO/OIE/FAO working

group and is widely used by the research community. Judging by the available publications, there is no officially regulated classification by neuraminidase. The only publication detailing the classification of neuraminidase subtypes was prepared in 2012 by American scientists from the University of Nebraska [14]. However, since then there have been significant genetic changes in avian influenza viruses, a large mass of new data has been obtained, which raises the question of adding new information to the proposed classification system [15–17]. The introduction of an official virus classification system is not an easy task and it requires a thorough analysis of all the nucleotide sequences of isolate genes available in the database, alignment, construction of phylogenetic trees and, based on them, designation of clades and subclades. An even more difficult question is whether the new system of virus nomenclature will be adopted and used by scientists studying the influenza virus.

According to J. Xu et al., neuraminidase of subtype N8 is divided into three lines, each line has a letter designation: 8A, 8B, 8C [14]. Line 8A is "North American", line 8B is represented by viruses isolated from horses, and the "Eurasian" line belongs to 8C. However, it is clear now that the "line division" approach is not enough to describe the viruses identified over the past few years. For example, this classification does not include viruses of the H5N8 subtype, which were first isolated in China in 2010 and subsequently became widespread in Southeast Asia, and then in Russia, Europe and North America. Therefore, when conducting a phylogenetic analysis of the Russian isolates recovered in 2020 we took into account classification data for both neuraminidase and hemagglutinin described in publications.

For comparative and phylogenetic analyses of nucleotide sequences of NA gene of AIV N8 isolates obtained in this study, we used sequences of known strains of this subtype belonging to different genetic groups from GenBank and GISAID databases. 92 nucleotide sequences of the neuraminidase gene of 1,370 nucleotide bases in size were considered in the work.

A dendrogram was constructed based on the complete sequences of the NA gene of AIV N8 (Fig.), including viruses isolated in the Russian Federation, China, Thailand, Vietnam, Korea, England and other countries from 2007 to 2020. The statistical reliability of the topology of phylogenetic trees was checked using bootstrap analysis; calculations were performed for 500 repetitions.

The Russian isolates of AIV H5N8 presented in the work are highlighted in red font in the figure. The phylogenetic tree constructed on the basis on the nucleotide sequences of NA gene demonstrated four different groups. Based on the classification of neuraminidase developed by J. Xu et al., it can be concluded that all the isolates used in the study belong to the "Eurasian" line 8C, which over time has divided into several separate groups. The identification of groups and subgroups clearly indicated on the tree was performed independently using as an example classification of neuraminidase of other subtypes, also developed by J. Xu et al. [14]. As a result, each group was given a number. According to number Group One was named 8C.1, Group Two – 8C.2, Group Three and Four – 8C.3 and 8C.4 respectively. Groups 8C.1, 8C.2, and 8C.3 included isolates of low pathogenic avian influenza virus.

Group 8C.4 mainly included isolates of highly pathogenic avian influenza virus, i. e. A/domestic goose/Omsk/1521-1/2020, A/duck/Saratov/1578-2/2020, A/duck/Chelyabinsk/1207-1/2020, A/goose/Tatarstan/1730-2/2020.

According to the classification by hemagglutinin, the isolate Group 8C.4 is comparable to Subclade 2.3.4.4.b of Clade 2.3.4.4.

The analysis of avian influenza virus isolates of H5N8 subtype recovered in 2020 demonstrates significantly greater heterogeneity, as compared to the group of isolates detected in 2016–2017. Thus, isolates of A/mute swan/Kazakhstan/1-267-20-B/2020, A/barnacle goose/Germany-SH/AI02167/2020, A/chicken/England/030720/2020, A/chicken/Netherlands/20016597-026030/2020, A/domestic goose/Omsk/1521-1/2020, A/duck/Saratov/1578-2/2020, A/duck/Chelyabinsk/1207-1/2020 form a very homogeneous group of isolates which, presumably, spread due to the migration of wild birds. The difference between these isolates is 0.1–0.6%. However, it is worth noting that isolates A/domestic duck/Poland/285/2020 and A/Mandarin duck/Korea/H242/2020 recovered in Poland and South Korea show significantly greater differences (4.1–4.9%). In addition, detection of isolate A/goose/Tatarstan/1730-2/2020 is of great interest, as it demonstrates a high similarity with avian influenza virus isolates of the same H5N8 subtype isolated in 2017 and differs from most isolates of 2020. The level of differences of A/goose/Tatarstan/1730-2/2020 isolate from other isolates identified in 2020 was 3.3%, and its genetic similarity with A/swan/Voronezh/2/2017 isolate was 0.6%.

A comparative analysis of AIV H5N8 isolates demonstrated that the maximum level of differences in the nucleotide sequence of the NA gene in Group 8C.4 was 9%.

The amino acid sequence of the studied isolates was also analyzed. As indicated in the literature, there are several amino acids, substitutions of which may affect the AIV resistance to drugs inhibiting neuraminidase activity (zanamivir, oseltamivir): E119Q (number 117 based on N8), R292K (291 based on N8), V116D (114 based on N8), or potential sensitivity to oseltamivir and peramivir: H274Y (273 based on N8) [18–20]. There were no substitutions in the predicted amino acid sequence for the above-mentioned positions. Amino acid residues directly involved in the catalytic activity of an enzyme remained unchanged: R118 (116 based on N8), D151 (149 based on N8), R152 (150 based on N8), R292 (291 based on N8).

Comparative and phylogenetic analyses of AIV/N8 isolates indicate the ongoing evolution of the highly pathogenic avian influenza virus of H5N8 subtype in various geographical regions.

The obtained nucleotide sequences of neuraminidase gene of the following AIV N8 isolates A/domestic goose/Omsk/1521-1/2020, A/duck/Chelyabinsk/1207-1/2020, A/duck/Saratov/1578-2/2020, A/goose/Tatarstan/1730-2/2020 were published in international databases GenBank and GISAID (MW276113, EPI1812535, EPI1811679, EPI1815193).

CONCLUSION

The research undertaken helped to determine nucleotide sequences of the NA gene of AIV A/H5N8 isolates: A/domestic goose/Omsk/1521-1/2020, A/duck/Chelyabinsk/1207-1/2020, A/duck/Saratov/1578-2/2020,

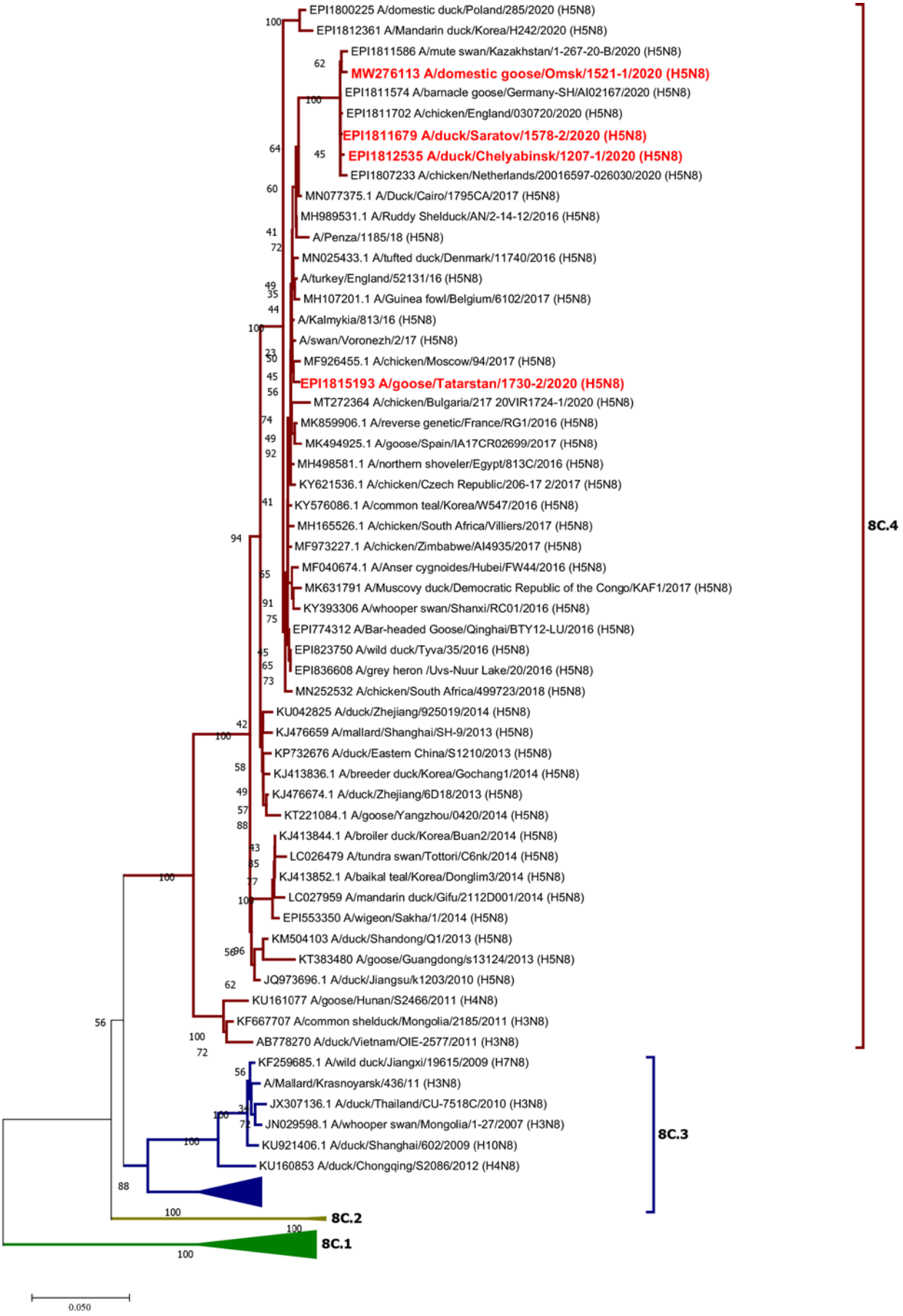

Fig. Phylogenetic tree based on the nucleotide sequences of NA gene of HPAI subtype N8

A/goose/Tatarstan/1730-2/2020. Up-to-date information was obtained on genetic relatedness based on NA gene of the studied AIV N8 isolates and isolates recovered from 2007 to 2020 in the Russian Federation and other countries. It was demonstrated that more information shall be added to the current N8 subtype-based classification of avian influenza viruses, due to the wide and rapid spread of genetically similar avian influenza viruses of H5N8 subtype in Europe, Asia, and Africa during 2014–2017.

REFERENCES

1. Swayne D. E., Pantin-Jackwood M. Pathobiology of avian influenza virus infections in birds and mammals. *In: Avian Influenza. Ed. by D. E. Swayne*. Ames: Blackwell; 2008; 87–122.

2. Mel'nov S. B., Lebed'T. L., Kipen'V. N. Osnovy molekulyarno-geneticheskogo analiza = Basics of molecular genetic analysis. *In: Sovremennye problemy biokhimii. Metody issledovanii = Modern problems of biochemistry. Test methods. E. V. Barkovskii et al.; ed. by prof. A. A. Chirkin*. Minsk: Vysheishaya shkola; 2013; 404–438. (in Russ.)

3. Murphy T. M. The control and epidemiology of an influenza A outbreak in Ireland. *In: Acute Virus Infections of Poultry. Current Topics in Veterinary Medicine and Animal Science. Eds. J. B. McFerran, M. S. McNulty*. Dordrecht: Springer; 1986; 37: 23–28. DOI: 10.1007/978-94-009-4287-5_2.

4. El-Shesheny R., Barman S., Feeroz M., Hasan M., Jones-Engel L., Franks J., et al. Genesis of influenza A (H5N8) viruses. *Emerg. Infect. Dis*. 2017; 23 (8): 1368–1371. DOI: 10.3201/eid2308.170143.

5. Marchenko V. Yu., Susloparov I. M., Kolosova N. P., Goncharova N. I., Shipovalov A. V., Durymanov A. G., et al. Isolation of highly pathogenic influenza A (subtype H5N8) virus on the territory of Republic of Sakha (Yakutia). *Dal'nevostočnyj* žurnal *infekcionnoj patologii*. 2015; (28): 38–43. Available at: https://infectpataog.elpub.ru/jour/article/view/127/128. (in Russ.).

6. Lee D. H., Torchetti M. K., Winker K., Ip H. S., Song C. S., Swayne D. E. Intercontinental spread of Asian-origin H5N8 to North America through Beringia by migratory birds. *J. Virol*. 2015; 89 (12): 6521–6524. DOI: 10.1128/JVI.00728-15.

7. Pohlmann A., Starick E., Harder T., Grund C., Höper D., Globig A., et al. Outbreaks among wild birds and domestic poultry caused by reassorted influenza A(H5N8) clade 2.3.4.4 viruses, Germany, 2016. *Emerg. Infect. Dis*. 2017; 23 (4): 633–636. DOI: 10.3201/eid2304.161949.

8. Dalby A. R., Iqbal M. The European and Japanese outbreaks of H5N8 derive from a single source population providing evidence for the dispersal along the long distance bird migratory flyways. *Peer J*. 2015; 3:e934. DOI: 10.7717/peerj.934.

9. Lee Y. J., Kang H. M., Lee E. K., Song B. M., Jeong J., Kwon Y. K., et al. Novel reassortant influenza A(H5N8) viruses, South Korea, 2014. *Emerg. Infect. Dis*. 2014; 20 (6): 1087–1089. DOI: 10.3201/eid2006.140233.

10. Zhao K., Gu M., Zhong L., Duan Z., Zhang Y., Zhu Y., et al. Characterization of three H5N5 and one H5N8 highly pathogenic avian influenza viruses in China. *Vet. Microbiol*. 2013; 163 (3–4): 351–357. DOI: 10.1016/j.vetmic.2012.12.025.

11. Volkov M. S., Irza V. N., Varkentin A. V. History of highly pathogenic avian influenza eradication in Russian Federation in 2016–2017. *Veterinary Science Today*. 2018; (1): 3–10. DOI: 10.29326/2304-196X-2018-1-24-3-7.

12. Volkova M. A., Chvala Ir. A., Yaroslavtseva P. S., Sosipatorova V. Yu., Osipova O. S., Chvala I. A. Serological monitoring for avian influenza in the Russian Federation in 2017–2018. *Veterinary Science Today*. 2019; (2): 3–7. DOI: 10.29326/2304-196X-2019-2-29-3-7.

13. World Animal Health Information and Analysis Department. Highly Pathogenic Avian Influenza (HPAI). Report No. 17: October 23 to November 12, 2020. Available at: https://www.oie.int/app/uploads/2021/03/hpai-asof12112020.pdf.

14. Xu J., Davis C. T., Christman M. C., Rivailler P., Zhong H., Donis R. O., Lu G. Evolutionary history and phylodynamics of influenza A and B neuraminidase (NA) genes inferred from large-scale sequence analyses. *PLoS One*. 2012; 7 (7):e38665. DOI: 10.1371/journal.pone.0038665.

15. Kim Y. I., Pascua P. Q., Kwon H. I., Lim G. J., Kim E. H., Yoon S. W., et al. Pathobiological features of a novel, highly pathogenic avian influenza A(H5N8) virus. *Emerg. Microbes Infect*. 2014; 3 (10):e75. DOI: 10.1038/emi.2014.75.

16. Lee D. H., Sharshov K., Swayne D. E., Kurskaya O., Sobolev I., Kabilov M., et al. Novel reassortant clade 2.3.4.4 avian influenza A(H5N8) virus in wild aquatic birds, Russia, 2016. *Emerg. Infect. Dis*. 2017; 23 (2): 359–360. DOI: 10.3201/eid2302.161252.

17. Liu M., Li X., Yuan H., Zhou J., Wu J., Bo H., et al. Genetic diversity of avian influenza A (H10N8) virus in live poultry markets and its association with human infections in China. *Sci. Rep*. 2015; 5:7632. DOI: 10.1038/srep07632.

18. Bialy D., Shelton H. Functional neuraminidase inhibitor resistance motifs in avian influenza A(H5Nx) viruses. *Antiviral Res*. 2020; 182:104886. DOI: 10.1016/j.antiviral.2020.104886.

19. Oladejo B. O., Bi Y., Vavricka C. J., Li C., Chai Y., Xu K., et al. Structural insight into the mechanism of neuraminidase inhibitor-resistant mutations in human-infecting H10N8 influenza A virus. *bioRxiv*. 2018; 378075. DOI: 10.1101/378075.

20. Choi W. S., Jeong J. H., Kwon J. J., Ahn S. J., Lloren K. K. S., Kwon H. I., et al. Screening for neuraminidase inhibitor resistance markers among avian influenza viruses of the N4, N5, N6, and N8 neuraminidase subtypes. *J. Virol*. 2017; 92 (1):e01580-17. DOI: 10.1128/JVI.01580-17.

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