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Genetic characteristics of influenza A and B viruses circulating in Russia in 2019–2023

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Abstract

Relevance. Influenza viruses have a high potential for genetic change. These viruses are monitored annually around the world, including Russia, to determine the dominant genetic groups and select the strains to be included in influenza vaccines.

Objectives of the study include: analysis of influenza virus circulation in Russia in 2019–2023, phylogenetic and molecular analysis of hemagglutinin (HA) sequences of influenza viruses, detection of mutations associated with drug resistance to neuraminidase (NA) inhibitors and M2-protein (M2) ion channel inhibitors.

Materials and methods. Biological samples containing RNA of influenza viruses were studied: 410 A(H1N1) pdm09, 147 A(H3N2) and 167 B(Victoria). Sequencing of the HA, NA, M fragments was performed on the 3500xL Genetic Analyzer (Applied Biosytems). Data processing and analysis were carried out using DNASTAR, Nextclade, FluSurver and BioNumerics v.6.6 software.

Results. Influenza A(H1N1)pdm09, A(H3N2), B(Victoria) viruses circulating in 2019-2023 were investigated. The highest variability of HA was observed in A(H3N2) viruses. All influenza A(H1N1)pdm09 viruses in the 2022–2023 season had a previously unknown mutation *E224A* in HA, which increases its affinity for α-2,3 sialic acids — receptors localized in the human lungs, to which the virus binds via HA. 2 and 3% of influenza A(H1N1)pdm09 viruses in 2019–2020 and 2022–2023, respectively, had the *D222N* mutation in the receptor-binding site of HA, which is associated with more severe disease. The oseltamivir and zanamivir resistance mutation *H275Y* in NA was detected in 2.3% of influenza A(H1N1)pdm09 viruses in 2022–2023. No oseltamivir and zanamivir resistance mutations in NA were detected in all tested influenza A(H3N2) and B viruses. Sequencing data revealed a mutation of adamantane resistance *S31N* in M2 in all studied influenza viruses A(H1N1)pdm09 and A(H3N2).

Conclusions. The detection of amino acid substitutions in HA antigenic sites and resistance mutations in NA and M2 confirms the evolution of influenza viruses and the necessity for continuous genetic surveillance. The vast majority of currently circulating viruses remain sensitive to NA inhibitors.

Keywords: *influenza viruses, sequencing, phylogenetic analysis, molecular genetic analysis*

Ethics approval. The study was conducted with the informed consent of the patients. The research protocol was approved by the Ethics Committee of the Central Research Institute for Epidemiology (protocol No. 3, March 27, 2020).

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ORIGINAL RESEARCHES

Оригинальное исследование
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Генетическая характеристика вирусов гриппа А и В, циркулировавших в России в 2019–2023 годах

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Аннотация

Актуальность. Вирусы гриппа обладают высоким потенциалом генетических изменений. Ежегодно по всему миру, в том числе в России, проводится мониторинг вирусов гриппа, чтобы определить доминирующие генетические группы и отобрать среди них штаммы, которые войдут в состав противогриппозных вакцин.

Цели исследования: анализ циркуляции вирусов гриппа в России в 2019–2023 гг., проведение филогенетического и молекулярного анализа последовательностей гемагглютинина (HA) вирусов гриппа, выявление мутаций резистентности к ингибиторам нейраминидазы (NA) и ингибиторам ионного канала M2-белка (M2).

Материалы и методы. Исследованы биологические образцы, содержащие РНК вирусов гриппа: 410 A(H1N1)pdm09, 147 A(H3N2) и 167 B(Виктория). Осуществляли секвенирование фрагментов сегментов HA, NA, M, проводили обработку и анализ данных.

Результаты. Исследованы нуклеотидные последовательности HA, NA, M вирусов гриппа A(H1N1)pdm09, A(H3N2) и B(Виктория), циркулировавших в 2019–2023 гг. Наибольшая вариабельность HA наблюдалась у вирусов A(H3N2). Все вирусы гриппа A(H1N1)pdm09 сезона 2022–2023 гг. имели не встречавшуюся ранее мутацию *E224A* в HA, которая увеличивает его сродство к α-2,3-сиаловым кислотам — рецепторам, локализованным в лёгких человека, с которыми связывается вирус посредством HA. У 2 и 3% вирусов A(H1N1)pdm09 в сезонах 2019–2020 и 2022–2023 гг. соответственно в рецептор-связывающем сайте HA обнаружена мутация *D222N*, которая ассоциирована с более тяжёлым заболеванием. Мутация устойчивости к осельтамивиру и занамивиру *H275Y* в NA выявлена у 2,3% вирусов гриппа A(H1N1)pdm09 в 2022–2023 гг. Во всех исследованных вирусах гриппа A(H3N2) и B мутации устойчивости к осельтамивиру и занамивиру в NA не обнаружены. По данным секвенирования, во всех исследованных вирусах гриппа A(H1N1)pdm09 и A(H3N2) имелась мутация устойчивости к адамантанам *S31N* в M2.

Выводы. Обнаружение мутаций, затрагивающих антигенные и рецептор-связывающие сайты HA, а также мутаций резистентности в NA и M2 подтверждает необходимость постоянного генетического надзора за вирусами гриппа. Подавляющее большинство циркулирующих в настоящее время вирусов сохраняют чувствительность к ингибиторам NA.

Ключевые слова: *вирусы гриппа, секвенирование, филогенетический анализ, молекулярно-генетический анализ*

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Источник финансирования. Авторы заявляют об отсутствии внешнего финансирования при проведении исследования.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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Introduction

Influenza viruses are enveloped viruses of the *Orthomyxoviridae* family, which are classified into 4 genera: influenza A, B, C and D viruses. Influenza A and B viruses pose the most significant danger to human health [1].

Influenza A viruses are the cause of most annual epidemics and all recurrent human pandemic diseases. They are subdivided into subtypes according to the combination of 2 surface glycoproteins located in the lipid membrane of virions: hemagglutinin (HA) and neuraminidase (NA). In birds, the main natural reservoir of influenza A viruses, 16 HA and 9 NA have been described; another 2 HA and NA have been found in bats [2]. Currently, influenza A viruses of the H1N1pdm09 and H3N2 subtypes cause the greatest number of epidemic diseases in humans[1].

Influenza B viruses are specific to humans (cases in seals have also been described [3]) and are divided into 2 evolutionary lineages with significantly different antigenic properties (B/Victoria and B/Yamagata).

Influenza viruses are in a state of continuous evolution in their reservoirs, facilitated by a high mutation rate due to the absence of the proofreading activity of RNA-dependent RNA polymerase [4]. The main reservoir for influenza A viruses is wild migratory birds and other mammals, including humans, while influenza B and C viruses have no reservoir in the wild. Cumulative changes in sequences encoding HA and NA lead to antigenic drift of influenza A and B viruses: the structure of antigenic surfaces recognized by specific antibodies changes, contributing to the annual epidemic [5]. Antigenic shift is also possible for influenza A viruses: segments encoding HA (and to a lesser extent NA) that have evolved in animal influenza viruses can combine with human influenza virus segments to form new reassortant strains capable of causing pandemics [4, 5]. Since 1889, there have been 5 known pandemics of influenza A viruses, the most serious of which in 1918 was caused by the H1N1 subtype and the most recent in 2009 by the A(H1N1)pdm09 subtype [4, 6]. The H3N2 subtype began circulating in the 1968 pandemic [7]. Since 1971, both subtypes of influenza A virus — H3N2 and H1N1 (since 2009 — H1N1pdm09) and both antigenic lineages of influenza B virus have circulated annually with greater or lesser intensity.

Influenza B virus was identified in 1940.Two antigenic virus lineages, B/Victoria/2/87-like (Victoria) and B/Yamagata/16/88-like (Yamagata), have been co-circulating since 1983 [8].

B/Victoria influenza viruses evolve more rapidly with greater positive selection pressure than B/Yamagata lineage viruses [9], and the latter have not circulated since March 2020: since then, there have been sporadic reports of B/Yamagata influenza viruses, but without confirmation by HA sequencing of the virus[2].

Under the auspices of WHO, influenza viruses are monitored worldwide, including their typing, genetic characterization and antigenic properties, to select among the predominant antigenic groups of virus strains to be included in influenza vaccines in the next epidemic season. The selection of vaccine strains with specific immunogenic properties is necessary to ensure an immune response against influenza viruses [10]. Large-scale genetic monitoring allows deep differentiation into clades and subclades and defines the trend of influenza virus evolution: the emergence, spread and disappearance from circulation of certain genetic variants.

During evolution, the most significant changes affect the HA of influenza viruses. The HA glycoprotein of influenza viruses is synthesized as a single polypeptide chain, which is further proteolytically cleaved into 2 subunits: HA1 and HA2. HA1 is responsible for binding the virus to sialic acids (SAs) on the cell membrane surface, while HA2 ensures fusion of the virus and endosome membranes.

Antigenic sites located at the HA1 apex near the receptor-binding site are the main targets for human neutralizing antibodies. HA glycosylation is associated with many properties, including immunogenicity and receptor specificity, and plays an important role in protecting antigenic sites from neutralizing antibodies [11]. The glycosylation pattern is more variable in HA1 than in HA2, which is more conserved [12].

Amino acid substitutions in the receptor-binding domain of HA affect the ability to bind to the host cell surface, which alters the virulence of the virus [13]. On the surface of human upper respiratory tract cells, predominantly SA-α2,6 are located, whereas in the lower respiratory tract, SA-α2,3 are located. Increased affinity of the virus for α-2,3-SAs may increase the severity of the disease [14].

The evolution of influenza B/Victoria viruses, in addition to point mutations, is facilitated by insertions and deletions of amino acids in the HA receptor binding region, which leads to immune evasion [15].

Amino acid substitutions in NA (influenza A and B viruses) and M2 (influenza A viruses) reduce the efficacy of drugs: NA inhibitors (oseltamivir, zanamivir) and adamantanes (amantadine and rimantadine), inhibitors of the M2 ion channel. According to the U.S. Centers for Disease Control and Prevention, virtually 100% of influenza A viruses are currently resistant to amantadine and rimantadine [16].

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[1] WHO. Influenza (seasonal). URL: https://www.who.int/ru/news-room/fact-sheets/detail/influenza-(seasonal)

[2] ECDC. Influenza virus characterisation, Summary Europe, July 2021. Stockholm; 2021. URL: https://www.ecdc.europa.eu/en/publications-data/influenza-virus-characterisation-summary-europe-july-2021; ECDC. Influenza virus characterization: summary report, Europe, July 2022. Copenhagen; 2022. URL: https://www.ecdc.europa.eu/en/publications-data/influenza-virus-characterization-summary-europe-july-2022

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The objectives of this study are the molecular genetic analysis of HA, NA and M segments of influenza A(H1N1)pdm09, A(H3N2) and B/Victoria viruses circulating in Russia during the epidemic seasons 2019–2023, identification of amino acid substitutions in HA compared to vaccine strains, their possible impact on antigenic properties and strength of binding to specific receptors; phylogenetic analysis on the HA gene sequences, as well as analysis of NA and M2 for the presence of molecular markers of resistance to antiviral drugs.

Materials and methods

Detection of influenza A and B virus RNA in biological material (nasopharyngeal and oropharyngeal swabs, sputum, tracheal aspirates, bronchoalveolar lavage) obtained as a result of routine monitoring for influenza viruses was carried out in laboratories of the Hygiene and Epidemiology Centers of Rospotrebnadzor in 50 regions of the Russian Federation (Central, Northwestern, Southern, North Caucasus, Volga, Urals, and Far Eastern federal districts) by polymerase chain reaction (PCR) with hybridization-fluorescence detection of amplification products. The study was conducted with the informed consent of the patients. The research protocol was approved by the Ethics Committee of the Central Research Institute for Epidemiology (protocol No. 3, March 27, 2020). In case of unfavorable outcome of the disease, postmortem material (lung autopsy specimens) was examined.

For molecular genetic analysis by nucleic acid sequencing, biological material was submitted to the Reference Center for monitoring of upper and lower respiratory tract infections at the Laboratory of Molecular Diagnostics and Epidemiology of Respiratory Tract Infections of the Central Research Institute of Epidemiology.

Extraction of influenza virus RNA from biological material and the subsequent reverse transcription reaction were performed using the RIBO-prep and REVERTA-L reagent kits (AmpliSens). PCR for influenza virus RNA detection was performed using AmpliSens Influenza virus A/B, AmpliSens Influenza virus A-type-FL, AmpliSens Influenza virus A/H1-swine-FL and AmpliSens Influenza virus B-type-FL reagent kits (produced by the Central Research Institute of Epidemiology).

To amplify fragments of HA, NA, M genes of influenza viruses A/H1N1pdm09 and A/H3N2, HA and NA genes of influenza B viruses, PCR with electrophoresis detection was performed on thermocyclers (DNA-Technology) using AmpliSens reagents (Central Research Institute of Epidemiology).

Amplified fragments of individual segments of influenza viruses (NA, NA, M) were sequenced in the Scientific Group of Genetic Engineering and Biotechnology of the Central Research Institute of Epidemiology by the Sanger method using the BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems by Thermo Fisher Scientific) on the 3500xL Genetic Analyzer sequencer (Applied Biosystems).

Sequencing results were analyzed in the Reference Center for monitoring of upper and lower respiratory tract infections using the DNASTAR software block (SeqMan, EditSeq, MegAlign). The obtained nucleotide sequences were uploaded to the international database GISAID, can be filtered by CRIE Search patterns. Phylogenetic analysis was performed using the BioNumerics v. 6.6 program with the UPGMA method. Nextclade, FluSurver online platforms were used to track amino acid mutations. Amino acid numbering is given according to the corresponding influenza virus subtype.

Results

Biological samples containing RNA of influenza viruses: 410 A(H1N1)pdm09, 147 A(H3N2), and 167 B(Victoria) circulating in the epidemic seasons 2019–2020, 2020–2021, 2021–2022, 2022–2023 were analyzed. Influenza A(H1N1)pdm09 and influenza B virus were both detected in 1 sample (upper respiratory swabs) from the 2022–2023 season. **Figure 1** shows that the number of influenza viruses tested varied in different years, which is due to the different intensity of the influenza epidemic process in different seasons.

The nucleotide sequences of NA, HA, M genes of influenza A(H1N1)pdm09 and A(H3N2) viruses, and NA and HA genes of influenza B virus were obtained and analyzed (**Table 1**).

Phylogenetic and molecular genetic analysis of influenza viruses were performed. Clustering of influenza viruses into clades and subclades was carried out on the basis of HA nucleotide sequences; for comparison, the sequences of vaccine strains, recommended in each season for the Northern Hemisphere, cultured in chicken embryos were used, since such vaccines are widely used in Russia.

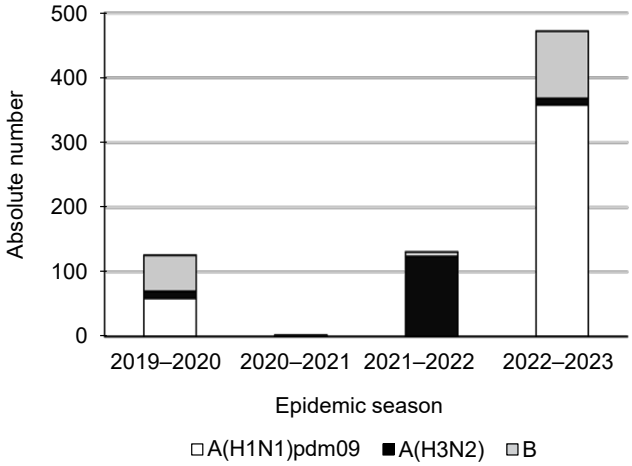

Fig. 1. Number of influenza viruses tested at the Reference center for monitoring upper and lower respiratory tract infections in 2019–2023.

Table 1. Number of sequenced segments

Influenza virus	Segment	Number (from the recovered/from the deceased)			
		2019–2020	2020–2021	2021–2022	2022–2023
(H1N1)pdm09	HA	59 (55/4)	–	–	351 (296/55)
	NA	59 (55/4)	–	–	87 (69/18)
	M	59 (55/4)	–	–	28 (16/2)
A(H3N2)	HA	11 (11/0)	2 (1/0)	124 (124/0)	10 (10/0)
	NA	2 (2/0)	1 (1/0)	44 (44/0)	1 (1/0)
	M	1 (1/0)	2 (1/0)	34 (34/0)	1 (1/0)
B	HA	56 (53/3)	–	7 (7/0)	104 (102/2)
	NA	35 (34/1)	–	1 (1/0)	36 (36/0)

Influenza A(H1N1)pdm09 viruses

The distribution of sequenced viruses into genetic clusters (clades, subclades and subgroups) and the results of comparison with the vaccine strain of each season are presented in **Table 2**. The homology of the nucleotide sequences of the HA gene of the studied viruses and vaccine strains varied in the range of 99.2–97.4% depending on the genetic cluster affiliation. The largest differences (up to 2.6%) were observed in the 2019–2020 season.

Based on sequencing results, A(H1N1)pdm09 viruses from the 2019-2020 season belonged to subclade 6B.1A.5 subgroups 5a, 5a.1, 5a.2, and subclade 6B.1A.7. This season, viruses from subclade 6B.1A.5 subgroup 5a (66%), characterized by amino acid substitutions *N129D* and *T185A* in HA1, prevailed. Subgroup 6B.1A.5a.1, characterized by amino acid substitutions *D187A*, *Q189E*, included 31% of the studied viruses. One virus belonged to subgroup 6B.1A.5a.2 (amino acid substitutions *N156K*, *L161I*, *V250A*), 1 virus belonged to subclade 6B.1A.7 (amino acid substitutions *K302T* in HA1 and *I77M*, *N169S*, *E179D* in HA2).

The A/Brisbane/02/2018 strain recommended for inclusion in the vaccine for the 2019–2020 epidemic season for the Northern Hemisphere belonged to subclade 6B.1A.1.

In the 2020–2021 and 2021–2022 seasons, influenza A(H1N1)pdm09 viruses were not sent to the reference center because they were not circulating.

All tested A(H1N1)pdm09 viruses from the 2022–2023 season belonged to clade 6B.1A.5a.2a, characterized by amino acid substitutions *K54Q*, *A186T*, *Q189E*, *E224A*, *R259K*, and *K308R*. This season's vaccine strain A/Victoria/2570/2019 was assigned to subgroup 6B.1A.5a.2.

The results of phylogenetic analysis on the HA gene of influenza A subtype H1N1pdm09 viruses in the 2019–2023 seasons are shown in **Figure 2**. The dendrogram contains a set of selected sequences of HA, which represented genetic diversity of circulated influenza viruses.

Five antigenic sites are identified in the HA molecule of influenza A(H1N1)pdm09 virus, which include the following amino acid positions: Sa (121–122 and 150–162), Sb (184–195), Ca1 (163–167, 200–202 and 232–235), Ca2 (133–139 and 218–219), Cb (67–72) [17].

From 7 to 12 mutations were detected in the HA amino acid sequence of 2019-2020 viruses compared to the vaccine strain A/Brisbane/02/2018. Of these, 7 were located in antigenic sites: Sa — *S121I/N* ($n = 2$), *N156K* ($n = 1$), *L161I* ($n = 1$), Sb — *T185I* ($n = 57$), *D187A* ($n = 18$), *Q189E* ($n = 16$), *S190N* ($n = 1$). An *R205K* substitution was found near the Ca1 antigenic site ($n = 2$). The mutations *D187A* ($n = 18$), *R221K* ($n = 2$), and *D222N* ($n = 1$) were located in the receptor-binding site. One sample belonged to subclade 6B.1A7 and carried amino acid substitutions *E68D* (in Cb), *T120A*, *S121N* (in Sa1), *R223Q*, *K302T* (in HA1), and *I77M*, *N169S*, *E179D* (in HA2).

Table 2. Results of nucleotide sequence analysis of HA influenza A(H1N1)pdm09 viruses received by the Reference center in 2019–2023

Epidemic season, years	Vaccine strain (genetic cluster)	Number of samples studied	Genetic cluster	*HA* gene homology with the vaccine strain, %
2019–2020	A/Brisbane/02/2018 (6B.1A.1)	39	6B.1A.5a	98.4–99.2
		18	6B.1A.5a.1	98.2–98.6
		1	6B.1A.5a.2	99.2
		1	6B.1A.7	98.2
2022–2023	A/Victoria/2570/2019 (6B.1A.5a.2)	351	6B.1A.5a.2a	97.4–98.9

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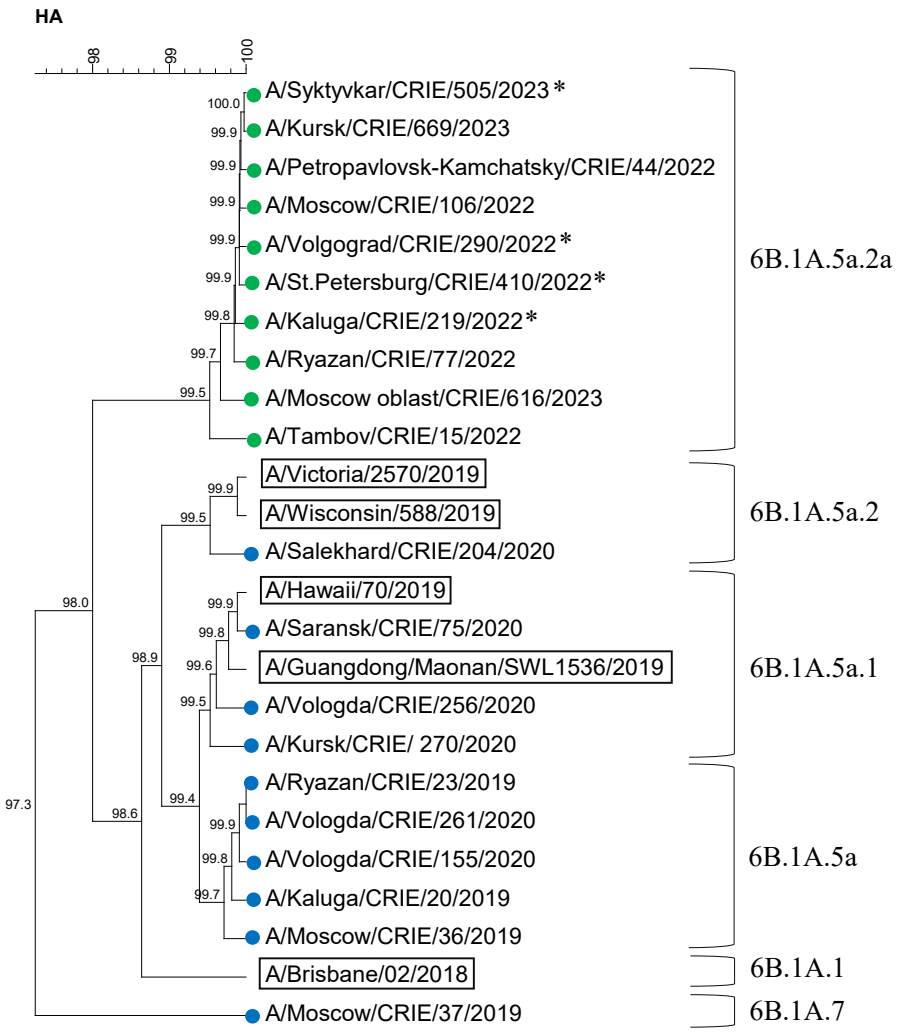

Fig. 2. Dendrogram of the HA gene of influenza A(H1N1)pdm09 viruses (data of the CRIE Reference center, 2019–2020, 2022–2023).

Vaccine strains are indicated by rectangles. Blue and green circles indicate the sequences of viruses circulating in 2019–2020 and 2022–2023 influenza seasons, respectively.
Fatal cases are marked with an asterisk.

Influenza A(H1N1)pdm09 2022–2023 viruses relative to the vaccine strain contained 6-12 amino acid substitutions. *R259K*, *K54Q*, *A186T*, *Q189E*, *E224A*, *K308R/G* substitutions in HA1 were detected more frequently. Some of the mutations affected antigenic sites: Sa — *S121N* (n = 1), *S122L* (n = 1), *K154R* (n = 1), *G155E* (n = 1), *N162S* (n = 1, loss of glycosylation site), Sb — *A186T* (n = 351), *D187V* (n = 1), *Q189E* (n = 351), *N194S* (n = 1), Ca1 – *V234I* (n = 1), Cb — *S69P* (n = 1), *L70F* (n = 1). Amino acid substitutions *N125H* (n = 1), *R205K* (n = 3) were found adjacent to antigenic sites. The *D187V* (n = 1) and *D222N* (n = 12) mutations were located in the receptor-binding site.The *D145N* mutation in HA2 resulting in an additional glycosylation site was found in 2 virus samples.

In 2019–2020, all influenza A(H1N1)pdm09 viruses lacked mutations conferring resistance to oseltamivir and zanamivir, whereas in 2022–2023 viruses with the H275Y mutation in the NA accounted for 2.3% and were detected in respiratory swabs of unvaccinated patients from the Arkhangelsk region in December 2022. All A(H1N1)pdm09 influenza viruses for which the M gene sequence was obtained were resistant to adamantanes (*S31N* mutation in M2).

Influenza A(H3N2) viruses

The distribution of the studied influenza A(H3N2) viruses by genetic clusters and the results of comparison with the vaccine strain according to each season are presented in **Table 3**. The homology of the nucleotide sequences of the *HA* gene of the studied viruses and vaccine strains varied in the range of 99.0–95.8% depending on the genetic cluster affiliation. Maximum differences (3.6–4.2%) were observed in the 2019–2020 season.

Influenza A(H3N2) viruses from the 2019–2020 season were categorized as group 3C.2a1b, clusters 3C.2a1b.1b and 3C.2a1b.2a. Vaccine strain A/Kansas/14/2017 was assigned to cluster 3C.3a.1 (characterized by amino acid substitutions at positions *S91N*, *N144K*, the latter resulting in the loss of a potential glycosylation site, *F193S* in HA1 and *D160N* in HA2). Cluster 3C.2a1b.1b, characterized by amino acid substitutions at positions *S137F*, *A138S*, and *F193S* in HA1, included 64% of the samples. The cluster 3C.2a1b.2a, characterized by amino acid substitutions at positions *K83E*, *Y94N* in HA1 and *I193M* in HA2, included 36% of the samples.

In the hemagglutinin molecule of influenza A(H3N2) virus, the following amino acid positions are considered to be antigenic sites:

- A (122, 124, 126, 130–133, 135, 137, 138, 140, 142–146, 150, 152, 168);
- B (128, 129, 155–160, 163–165, 186–190, 192–194, 196–198);
- C (44–48, 50, 51, 53, 54, 273, 275, 276, 278–280, 294, 297, 299, 300, 304, 305, 307–312);
- D (96, 102, 103, 117, 121, 167, 170–177, 179, 182, 201, 203, 207–209, 212–219, 226–230, 238, 240, 242, 244, 246–248);
- E (57, 59, 62, 63, 67, 75, 78, 80–83, 86–88, 91, 92, 94, 109, 260–262, 265) [18].

Three antigenic sites overlap with the receptor-binding site: site A with loop 130 (135, 136, 137, 138, 153); site B with helix 190 (186, 190, 194, 195); and site D with loop 220 (226 and 228) [19].

Both influenza A(H3N2) viruses of the 2020–2021 season belonged to subclade 2a.2 of subgroup 3C.2a1b.2a.2a.2a.2, which is characterized by amino acid substitutions *Y159N*, *T160I* (loss of glycosylation site, *L164Q*, *G186D*, *D190N*) in HA1, whereas vaccine strain A/Hong Kong/2671/2019 belonged to cluster 3C.2a1b.1b.

In the 2021–2022 season, as in the previous season, all influenza A(H3N2) viruses belonged to subcluster 3C.2a1b.2a.2. In the 2022–2023 classification, viruses were assigned to 4 subclades: viruses of 3C.2a1b.2a.2a.2 (amino acid substitutions *D53G*, *R201L*, *S219Y*) prevailed, 3C.2a1b.2a.2c (amino acid substitutions *S205F*, *A212T*) were less frequently found, and 3C.2a1b.2a.2a.2 and 3C.2a1b.2a.2a.2a.1 (amino acid substitutions *D53G*, *D104G*, *K276R*) were even less frequently found. The vaccine strain A/Cambodia/e0826360/2020 belonged to group 3C.2a1b.2a, but to a different genetic subgroup 3C.2a1b.2a.1a, characterized by amino acid substitutions *L157I*, *K220R*.

Influenza A(H3N2) viruses from the 2022-2023 season belonged to clade 3C.2a1b.2a.2a.2, as did the vaccine strain A/Darwin/9/2021, which was in the vaccine administered in the Northern Hemisphere in 2022–2023. Within the clade, the viruses were differentiated into subclades: 3 belonged to 3C.2a1b.2a.2a.3a.1 (amino acid substitution *I140K*), 3 belonged to 3C.2a1b.2a.2b (characterized by amino acid substitutions *E50K*, *F79V*, *I140K*), and 2 belonged to 3C.2a1b.2a.2a.2a.1b (amino acid substitutions *I140K*, *R299K*). Only 2 HAs were close to the vaccine strain A/Darwin/9/2021 (subclade 3C.2a1b.2a.2a, characterized by amino acid substitution *H156S*).

The results of phylogenetic analysis on the HA gene of influenza A subtype H3N2 viruses are presented in **Fig. 3**. In the 2019–2020 season, 21–23 mutations were detected in the HA sequence of viruses compared to the vaccine strain A/Kansas/14/2017, 19 of these mutations located in antigenic sites:

- A — *T131K* (n = 3), *T135K* (n = 7), *S137F* (n = 7), *I140K* (n = 7), *K144S* (n = 10);
- B — *A128T* (n = 3), *S159Y* (n = 10), *K160T* (n = 10), *N190D* (n = 10), *S193F* (n = 3);
- D — *N121K* (n = 10), *N171K* (n = 10), *V230I* (n = 1), *T246N* (n = 10);
- E — *E62G* (n = 10), *K83E* (n = 3), *N91S* (n = 10), *K92R* (n = 10), *Y94N/S* (n = 3).

Three substitutions were localized in the receptor-binding site: in loop 130, *T135K*, *S137F*, and in helix 190, *N190D*. The *I77V*, *M149I*, and *G155E* mutations in HA2 of 11 viruses were found.

Mutations that lead to the appearance of new potential N-glycosylation sites were found: *A128T* (in 3 viruses of subgroup 3C.2a1b.2a), *K160T* (in all 10 viruses), *T246N* (in all 10 viruses). Seven viruses of subgroup 3C.2a1b.1b had a *T135K* mutation that results in loss of the N-glycosylation site.

A(H3N2) viruses examined in the 2020–2021 season, there were 22–24 substitutions in the HA se-

Table 3. Results of HA nucleotide sequence analysis of influenza A(H3N2) viruses received by the Reference center in 2019–2023

Epidemic season. years	Vaccine strain (genetic cluster)	Number of samples studied	Genetic cluster	HA gene homology with the vaccine strain. %
2019–2020	A/Kansas/14/2017 (3C.3a.1)	7	3C.2a1b.1b	96.2–96.4
		4	3C.2a1b.2a	95.8–96.0
2020–2021	A/Hong Kong/2671/2019 (3C.2a1b.1b. previously 3C.2a1b + T135K-B)	2	3C.2a1b.2a.2a.2	97.3–97.4
2021–2022	A/Cambodia/e0826360/2020 (3C.2a1b.2a.1a)	3	3C.2a1b.2a.2	98.6–98.7
		3	3C.2a1b.2a.2a.1	98.6–98.7
		105	3C.2a1b.2a.2a.2	98.1–98.9
		13	3C.2a1b.2a.2c	98.6–98.9
2022–2023	A/Darwin/9/2021 (2a. previously 3C.2a1b.2a.2a)	2	3C.2a1b.2a.2a	99.4
		2	3C.2a1b.2a.2a.1b	98.9–99.0
		3	3C.2a1b.2a.2a.3a.1	98.5–98.6
		3	3C.2a1b.2a.2b	98.5

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quences compared to the vaccine strain A/Hong Kong/2671/2019, 16 of which were in the antigenic sites of both viruses: A — *T131K*, *K135T*, *F137S*, *S138A*; B — *A128T*, *H156S*, *Y159N*, *L164Q*, *V186D*, *D190N*; C — *D53G*; D — *R201K*, *S219Y*; E — *K83E*, *Y94N*. One virus had an *I214V* mutation in site D; 5 substitutions were localized in the receptor-binding site: in loop 130, *K135T*, *F137S*, *S138A*; and in helix 190, *V186D*, *D190N*. Additionally, *N225D*, *A128T*, and *K135T* mutations were found outside the antigenic sites in both viruses, the latter resulting in new potential N-glycosylation sites.

In the 2021–2022 season, HA amino acid sequences contained 9–12 mutations compared to the vaccine strain A/Cambodia/e0826360/2020.

The following substitutions in antigenic sites were detected in 124 viruses of all subclades: B, Y159N (1 had *Y159S*), *K160I*, *L164Q*, *R186D*, *D190N*, *P198S*; D, *N171K*. The *R186D*, *D190N* substitutions were in the receptor-binding site. Three viruses of subclade 3C.2a1b.2a.2a.2 had mutations: A — *I140K*, D — *R201I*. One virus had the *S219Y* mutation in the antigenic site D.

Three viruses of subclade 3C.2a1b.2a.2a.1 contained mutations: C — *D53G*, *K276R*; B — *H156S*; outside antigenic sites — *D104G*; 105 viruses of subclade 3C.2a1b.2a.2a.2a.2 had mutations in antigenic site D — *R201K*, *S219Y*. Total 104 viruses had an *I25V* substitution outside the antigenic sites. Four viruses had mutations that lead to loss of the glycosylation site: 3 viruses had *N122D*, 1 had *N165K*. Thirteen viruses of

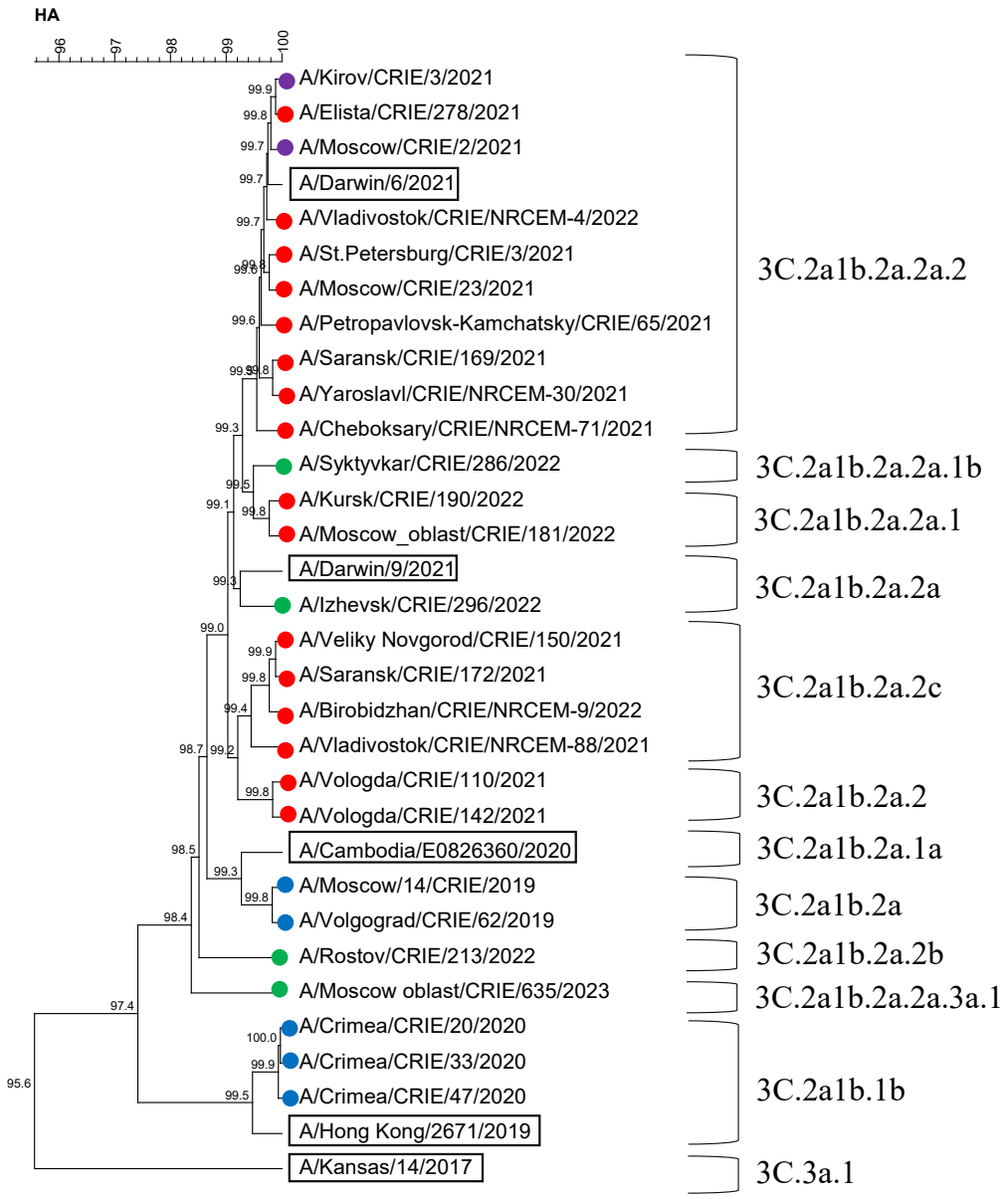

Fig. 3. Dendrogram of the HA gene of influenza A(H3N2) viruses (data from the CRIE Reference center, 2019–2023).
Vaccine strains are indicated by rectangles. Blue, violet, red and green circles indicate the sequences of viruses circulating in 2019–2020, 2020–2021, 2021–2022 and 2022–2023 influenza seasons, respectively. Fatal cases are marked with an asterisk.

subclade 3C.2a1b.2a.2c had an *A212T* mutation in antigenic site D. Twelve viruses had an *S205F* substitution outside the antigenic sites. Two viruses had an *S124N* mutation resulting in loss of the glycosylation site.

In the 2022–2023 season, the A(H3N2) viruses studied had 2–9 amino acid substitutions in HA compared with vaccine strain A/Darwin/9/2021 and 3–9 substitutions compared with A/Darwin/6/2021 (the sequence of strain A/Darwin/9/2021 differs from A/Darwin/6/2021 by the *G53D* mutation). Two viruses of subclade 3C.2a1b.2a.2a had a mutation in the D site, *I217V*. Two viruses of subclade 3C.2a1b.2a.2a.1b had mutations localized in site A — *I140K*, C — *K276R* and *R299K*. Three viruses of subclade 3C.2a1b.2a.2a.3a.1 had mutations in the C site — *E50K*, *D53N*, A — *I140K*, B — *I192F*; 3 viruses of subclade 3C.2a1b.2a.2b had *E50K* mutations in the C site and *I140K* mutations in site A. Two viruses had an amino acid substitution of *N96S* in HA1 leading to the appearence of N-glycosylation site, and one had an *N122D* mutation in HA1 leading to loss of the N-glycosylation site.

No resistance mutations to oseltamivir and zanamivir were detected in the NA of influenza A(H3N2) viruses tested for the 2019–2023 seasons, while all had the *S31N* adamantane resistance mutation in M2.

Influenza B viruses

All influenza B viruses received for study in 2019–2023 belonged to the B/Victoria lineage (according to PCR and sequencing results).

The distribution of investigated influenza B viruses by genetic clusters and the results of comparison with the vaccine strain of each season are presented in **Table 4**. The homology of the nucleotide sequences of the HA gene of the studied viruses and vaccine strains varied in the range of 99.7–98.2% depending on the genetic cluster affiliation. Maximum similarity (99.0–99.7%) was observed in the season 2022–2023.

In the 2019–2020 season, 98% of influenza B viruses belonged to the Victoria lineage subclade V1A.3 (1A(Δ3)B), characterized by triple deletion of amino acid residues 162–164 and amino acid substitutions *K136E*, *G133R* in HA; the remainder belonged to the subgroup V1A.3a.1, characterized by amino acid substitutions in HA1 *V117I*, *V220M*. The vaccine strain B/Colorado/06/2017 for the 2019–2020 Northern Hemisphere epidemic season belonged to subclade V1A.1 (1A(D 2)B) of the Victoria lineage, characterized by double deletion of amino acid residues 162–163 and amino acid substitutions at positions *D129G*, *I180V* in HA1, *R151K* in HA2.

In the 2021–2022 season, 7 influenza B viruses belonged to the Victoria lineage subclade V1A.3, subgroup V1A.3a.2 (characterized by amino acid substitutions at HA1 positions *A127T*, *P144L*, *K203R*). Vaccine strain B/Washington/02/2019, which was included in the 2021–2022 Northern Hemisphere vaccines, belonged to the Victoria lineage of subclade V1A.3.

In the 2022–2023 season, all influenza B viruses belonged to lineage Victoria subclade V.1A.3a.2. Vaccine strain B/Austria/1359417/2021, which was included in vaccines in Russia in 2022–2023, also belonged to lineage Victoria subclade V.1A.3a.2. The results of phylogenetic analysis on the HA gene of influenza B viruses of lineage Victoria are presented in **Fig. 4**.

There are 4 antigenic sites in the HA molecule of influenza B virus: loop 120 and adjacent regions (116–137), loop 150 (141–150), loop 160 (162–167), helix 190 and its surrounding regions (194–202) [20]. The receptor-binding site is formed by helix 190 (193–202), loop 240 (237–242), loop 140 (136–143) [21].

In the 2019–2020 season, the HA amino acid sequences of viruses had 8–12 mutations compared to the vaccine strain B/Colorado/06/2017, as well as an additional amino acid deletion at position 164. There were 11 substitutions in antigenic sites: *I117V* (n = 2), *R118K* (n = 2), *N126K* (n = 5), *A127T* (n = 1), *E128K* (n = 1), *D129N* (n = 55), *G133R* (n = 50), *Y135D* (n = 1), *K136E* (n = 56) in loop 120; *N166D* (n = 1) in loop 160; N197D (n = 1) in helix 190. Three substitutions were in the receptor-binding site: in helix 190, *N197D*; in loop 240, *P241Q* (n = 1); in loop 140, *K136E*. One virus each had *N166D*, *N197D*, and *N233S* substitutions that result in loss of the glycosylation site.

Influenza B viruses in the 2021–2022 season had 8–9 amino acid substitutions in HA compared to the vaccine strain B/Washington/02/2019. Amino acid substitutions were located in 5 positions of antigenic sites: in loop 120, *A127T* (n = 7), *R133G* (n = 7); in

Table 4. Results of HA nucleotide sequence analysis of influenza B viruses of the Victoria lineage received by the Reference center in 2019–2023

Epidemic season	Vaccine strain (genetic cluster)	Number of samples studied	Genetic cluster	*HA* gene homology with the vaccine strain. %
2019–2020	B/Colorado/06/2017 (V1A.1. previously 1A(Δ2)B)	55	V1A.3	98.2–98.8
		1	V1A.3a.1	98.6
2021–2022	B/Washington/02/2019 (V1A.3. previously 1A(Δ3)B)	7	V1A.3a.2	98.2–98.7
2022–2023	B/Austria/1359417/2021 (V1A.3a.2)	104	V1A.3a.2	99.0–99.7

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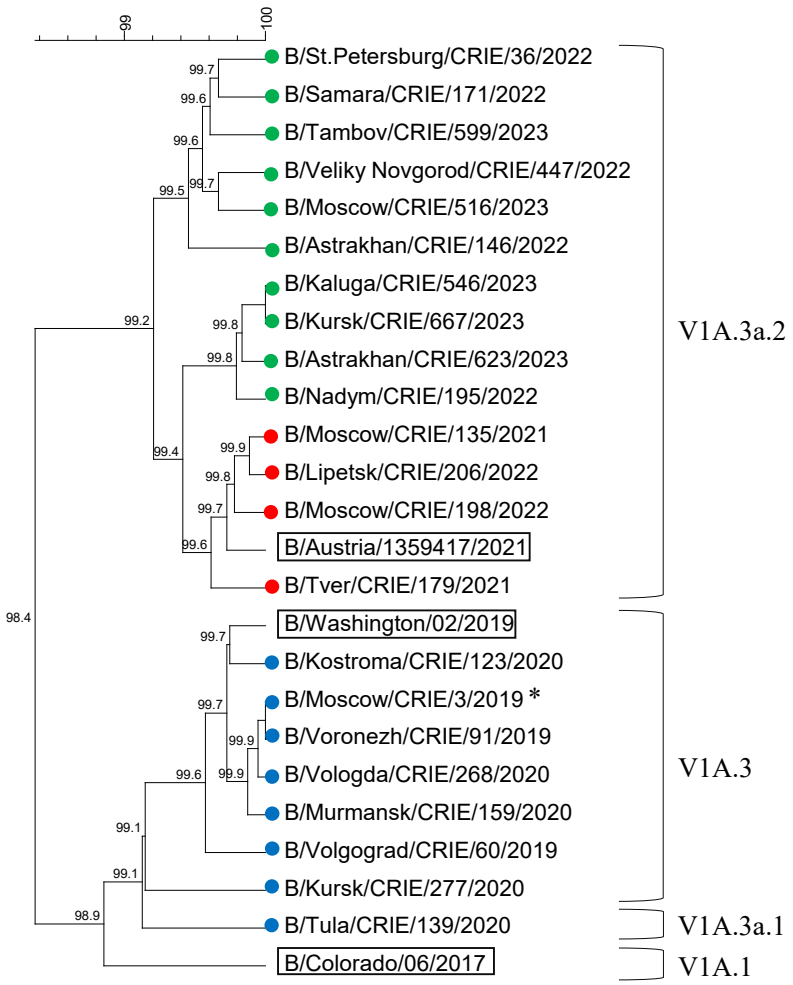

Fig. 4. Dendrogram of the HA gene of influenza B viruses of the Victoria lineage.

Data from the CRIE Reference center, 2019–2020, 2020–2021, 2022–2023. Vaccine strains are indicated by rectangles. Blue, red and green circles indicate the sequences of viruses circulating in 2019–2020, 2021–2022 and 2022–2023 influenza seasons, respectively.
Fatal cases are marked with an asterisk.

loop 150, *P144L* (*n* = 7), *N150K* (*n* = 7); and in helix 190, *N197D/E* (*n* = 7). One substitution was in the receptor-binding site, in helix 190 – *N197D/E*. Mutations *N197D* observed in 6 viruses and *N197E* identified in 1 virus result in loss of the glycosylation site.

There were 1–6 mutations in the HA of influenza B viruses in the 2022–2023 season compared to the vaccine strain B/Austria/1359417/2021. Seven substitutions were in antigenic sites: in loop 120 — *T121N* (*n* = 25), *H122N* (*n* = 3), *E128K* (*n* = 67), in loop 150 — *G149E* (*n* = 1), in helix 190 — *D197E* (*n* = 40), *T199A/I* (*n* = 100). Two substitutions were in the receptor-binding site, in helix 190 — *D197E*, *T199A/I*. In one virus, a *T196I* substitution was found in HA2 that resulted in loss of the glycosylation site.

No mutations in the NA gene that reduce sensitivity to oseltamivir and zanamivir were found in the examined influenza B viruses of the 2019–2023 seasons.

Discussion

The etiologic structure of influenza varied in different epidemic seasons in Russia and globally. The samples containing influenza viruses submitted to the Reference Center for study were randomly selected in Russian regions during patient screening, so we can assume that their spectrum and genetic characteristics reflect general patterns across the country and allow us to judge the structure of influenza and genetic diversity of circulating viruses in Russia.

In the 2019–2020 season, the Reference Center received mainly influenza A(H1N1)pdm09 and B viruses: 46.8% of A(H1N1)pdm09, 8.7% of influenza A(H3N2) , and 44.5% of B/Victoria. According to the WHO National Influenza Center, all three influenza subtypes were present in the Russian population this season, with influenza B/Victoria virus predominating (43.7%) [22]. According to the European Center for Disease Prevention and Control (ECDC), in the European region, among the viruses that were typed, 51% A(H1N1)pdm09, 40.1% A(H3N2), 8.7% B/Victoria, 0.2% B/Yamagata were detected[3]. A(H3N2) viruses were the second most abundant, but not B/Victoria influenza vi-

[3] ECDC. Influenza virus characterisation, summary Europe, July 2020. Stockholm; 2020. URL: https://www.ecdc.europa.eu/en/publications-data/influenza-virus-characterisation-summary-europe-july-2020

ruses as in Russia, which may be due to the different patterns of spread of these virus types in different countries.

In the 2020–2021 season, only 2 influenza viruses (A(H3N2)) were tested in the Reference Center. According to the WHO National Influenza Centre, in Russia, only 37 positive materials were found during the entire season during PCR-based influenza screening, and only 2 influenza B/Victoria viruses were isolated in culture and antigenically characterized [22]. In the European region, this season also saw a 99.4% decrease in the number of confirmed influenza cases compared to the 2019–2020 season [22]. Influenza viruses were distributed as follows: 14.2% A(H1N1)pdm09, 80.6% A(H3N2), 4.9% B/Victoria, 0.3% B/Yamagata [9], i.e., just as in Russia, A(H3N2) virus predominated.

The low activity of influenza viruses worldwide was caused by the emergence of the novel betacoronavirus SARS-CoV-2 in December 2019 in China and subsequent preventive and protective measures [23]. These included restrictions on the movement of people: closing countries' borders, suspending international flights, quarantining those arriving in the country and isolating those who became ill. In addition, personal hygiene practices (frequent hand washing, use of disinfectants and personal protective equipment) were monitored to reduce transmission of influenza viruses. The timing of these measures correlates directly with the sharp drop in influenza incidence in 2020–2021.

In the 2021–2022 season, the number of viruses tested at the Reference Center increased significantly, with influenza A(H3N2) virus predominating (94.7%,) and influenza B viruses accounting for 5.3%. No samples containing influenza A(H1N1)pdm09 virus were received. According to the WHO National Influenza Center, the A(H3N2) subtype was predominantly detected in Russia, with minor involvement of influenza B/Victoria and complete absence of A(H1N1)pdm09 virus [21]. Thus, the etiologic structure of influenza this season in Russia was represented by influenza A(H3N2) virus with a minor contribution of influenza B virus.

According to ECDC data, influenza A(H3N2) virus was also predominant in the European region, with A(H1N1)pdm09 (8.7%) and B being detected much less frequently: A(H3N2) 90.9%, B/Victoria 0.4%, B-Yamagata < 0.1%[4]. It should be noted that despite reports of isolated findings of B/Yamagata influenza viruses, available databases of genetic information do not contain the HA nucleotide sequences of B/Yamagata influenza viruses detected after March 2020, which means that their affiliation with the Yamagata lineage has not been confirmed by sequencing.

The spectrum of influenza viruses examined in the 2022–2023 season at the Reference Center was as follows: 75.5% influenza A(H1N1)pdm09 viruses, 2.1% influenza A(H3N2) viruses, 22.4% influenza B/Victoria viruses. Among the viruses admitted and examined, 12%, predominantly influenza A(H1N1)pdm09 and to a lesser extent influenza B/Victoria, were viruses found in autopsy material, indicating an increase in influenza severity compared with the 2021–2022 season, when influenza A(H3N2) virus predominated and no autopsy material was admitted (Table 1). The increased number of influenza deaths during seasons of active circulation of influenza A(H1N1)pdm09 virus can be explained by the greater affinity of this influenza A virus subtype for SA-α2,3, which facilitates its spread to the lungs and leads to the development of pneumonia more often. In addition, a lower level of immunity in the population due to lack of exposure to influenza A(H1N1)pdm09 virus during the 2020–2021 and 2021–2022 seasons could have contributed to a more severe epidemic.

According to the WHO National Influenza Center, in Russia as a whole, at the peak of the influenza and acute respiratory viral illness epidemic, influenza viruses were detected in 30% of patient samples examined during PCR screening, with the A(H1N1)pdm09 subtype predominating[5]. In the 2022–2023 season, according to ECDC data, the structure of influenza in the European region differed from that in Russia: 47.3% — A(H1N1)pdm09, 47.9% — A(H3N2), 4.8% — B/Victoria, 0% — B/Yamagata[6], which could be due to the various epidemiological features of the spread of these types of viruses in different countries.

Our data on the distribution of A(H1N1)pdm09 viruses by genetic groups coincided with the data of the A.A. Smorodintsev Research Institute of Influenza: in the 2019–2020 season, the genetic subgroup 6B.1A5 (reference strain A/Norway/3433/2018) prevailed[7], in the 2022–2023 season, the genetic subgroup 6B.1A.5a.2 (reference virus A/Sydney/5/2021) prevailed[8].

The significance of the detected mutations leading to amino acid substitutions was assessed according to the literature data confirmed experimentally. It should

[4] ECDC. Influenza virus characterisation, Summary Europe, July 2021. Stockholm; 2021. URL: https://www.ecdc.europa.eu/en/publications-data/influenza-virus-characterisation-summary-europe-july-2021

[5] A.A. Smorodintsev Research Institute of Influenza. Weekly national influenza and ARVI bulletin for the 39th week of 2023. URL: https://influenza.spb.ru/system/epidemic_situation/laboratory_diagnostics/?year=2023&week=39

[6] ECDC. Influenza virus characterization: summary report, Europe, February 2023. Copenhagen–Stockholm; 2023. URL: https://www.ecdc.europa.eu/en/publications-data/influenza-virus-characterization-summary-europe-february-2023

[7] A.A. Smorodintsev Research Institute of Influenza. Weekly national influenza and ARVI bulletin for the 39th week of 2020. URL: https://www.influenza.spb.ru/system/epidemic_situation/laboratory_diagnostics/?year=2020&week=39

[8] A.A. Smorodintsev Research Institute of Influenza. Weekly national influenza and ARVI bulletin for the 39th week of 2023. URL: https://influenza.spb.ru/system/epidemic_situation/laboratory_diagnostics/?year=2023&week=39

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be taken into account that different amino acids differ in physicochemical properties (nonpolar, polar charged and uncharged), they can change the spatial configuration of the protein in different ways, even if the substitution occurred in the same position of the amino acid chain. The functional significance of each mutation must be proven experimentally. In some cases, it is acceptable to draw an analogy, for example, between HAs of subtypes H1 and H5, because HAs of influenza A H1 and H5 viruses belong to the same group (H1/H2/H5/H6/H11/H13), which, according to the predicted amino acid sequence of HAs, may have similar HA spatial conformational structures [25].

N156K and *L161I* mutations were detected in the antigenic Sa site of influenza A(H1N1)pdm09 virus, both in 1 virus. The *S159N* mutation in the HA of influenza A(H5N1) virus (position equivalent to *N156K* in the A(H1N1)pdm09 influenza viruses examined) results in enhanced binding to α-2,6-SA of the ferret respiratory tract mucosa [26]. For the *N156K* mutation in A(H1N1)pdm09, a change in antigenic properties was predicted in ferret experiments [27].

T185I, *D187A*, *Q189E*, and *S190N* mutations were found in the antigenic site Sb of influenza A(H1N1) pdm09 virus. In HA of influenza A(H1N1) virus, the *D187A* substitution (190 by H3 numbering) results in decreased binding to α-2,6- and increased affinity for α-2,3-SA [28]. In HA of epidemic influenza A(H1N1) virus, substitution of *S190N* (193 by H3 numbering) changes the conformation of the Sb antigenic site [29]. An *R205K* mutation was found near the Ca1 antigenic site of A(H1N1)pdm09 virus. In HA of H5N1 virus, substitution of *N224K* (numbered H3) at an equivalent position leads to enhanced binding to α-2,6-SA [30].

Near the antigenic Ca2 site of A(H1N1)pdm09 virus, the *R221K* mutation was found to be part of the HA A(H1N1)pdm09 receptor-binding site. It was shown that changes in this HA can affect the antigenic properties of viruses [31].

The amino acid arginine (R) at position 223 enhances the affinity of A(H1N1)pdm09 for avian-type receptors (α-2,3-SAs). Given the fact that α-2,3-SAs are part of the glycocalyx of the epithelial cells lining human lung alveoli, such mutant strains can cause pneumonia, and further generalized inflammation. By 2020, the majority of circulating influenza A(H1N1)pdm09 viruses (99.80%) had the *R223Q* mutation, including all the viruses we sequenced, whereas at the early stage of the 2009 pandemic, strains with an arginine (R) in this position were still circulating in a minor population [32]. Apparently, the elimination of mutations that may reduce the spread of viruses due to the high danger of such strains for host life is one of the mechanisms of influenza viruses evolution.

Outside the antigenic sites, 2 viruses of the A(H1N1)pdm09 subtype had the *D94N* mutation and 1 had the *D94E* mutation. In HA of influenza A(H5N1) virus, the *D94N* substitution results in decreased binding to α-2,6-SA and increased affinity for α-2,3-SA, and enhances HA-mediated fusion to the membrane of mammalian cells [33].

The *E224A* mutation in the receptor-binding site of A(H1N1)pdm09 increases the affinity for α-2,3-SAs (bird-type receptors) localized in human lungs [34].

The *D222N* mutation in the receptor-binding site also enhances binding to α-2,3-SA. The *D222N* and *D222G* mutations are associated with a severe course of influenza, including pneumonia and acute respiratory distress syndrome [35]. Our earlier molecular genetic analysis of influenza A(H1N1)pdm09 viruses circulating in Russia from 2009 to 2014 showed that amino acid substitution of D for G or N at position 222 was statistically significantly more frequently found in the lungs of deceased patients than in respiratory swabs of recovered patients ($p < 0.0001$ and $p = 0.007$) [36]. In the 2019–2020 season, the *D222N* mutation was detected in 1 (2%) virus from bronchoalveolar lavage of a patient with severe community-acquired pneumonia. In the 2022–2023 season, the *D222N* mutation was present in influenza A(H1N1)pdm09 viruses found in autopsy specimens from 12 patients (22% of viruses from autopsy material; 3% of total).

Our data on the distribution of A(H3N2) viruses by genetic groups matched the data of the Smorodintsev Research Institute of Influenza. In the 2019–2020 season, viruses of genetic subgroups 3C.2 a1b + *T131K* (reference strain A/South Australia/34/2019) and 3C.2a1b + T135K-B (reference strain A/Hong Kong/2675/2019)[9] circulated in the ratio of 2 : 1; in 2021–2022, the genetic subgroup 3C.2a1b.2a.2 prevailed[10], and in 2022–2023 — subgroup 3C.2a1b.2a.2 (reference virus Bangladesh/4005/2020)[11].

In the 2020–2021 season, no influenza A(H3N2) viruses were isolated at the Smorodintsev Influenza Research Institute[12], and the viruses we examined belonged to group 3C.2a1b.2a.2, which was consistent with the ECDC data: influenza A(H3N2) viruses circulating in the influenza A(H3N2) virus population were group 3C.2a1b viruses, most of which were Cambodia-

[9] A.A. Smorodintsev Research Institute of Influenza. Weekly national influenza and ARVI bulletin for the 39th week of 2020. URL: https://www.influenza.spb.ru/system/epidemic_situation/laboratory_diagnostics/?year=2020&week=39

[10] A.A. Smorodintsev Research Institute of Influenza. Weekly national influenza and ARVI bulletin for the 24th week of 2022. URL: https://www.influenza.spb.ru/system/epidemic_situation/laboratory_diagnostics/?year=2022&week=24

[11] A.A. Smorodintsev Research Institute of Influenza. Weekly national influenza and ARVI bulletin for the 39th week of 2023. URL: https://influenza.spb.ru/system/epidemic_situation/laboratory_diagnostics/?year=2023&week=39

[12] A.A. Smorodintsev Research Institute of Influenza. Weekly national influenza and ARVI bulletin for the 39th week of 2021. URL: https://www.influenza.spb.ru/system/epidemic_situation/laboratory_diagnostics/?year=2021&week=39

(3C.2a1b.2a.1) and Bangladesh-like (3C.2a1b.2a.2) viruses[13].

From 1968 to 2003, antigenic drift of influenza A(H3N2) virus was mainly caused by single mutations in 7 amino acid positions in HA (145 in site A, 155, 156, 158, 159, 189, 193 in site B) near the receptor-binding site [37].

In HA of influenza A(H3N2) virus, substitution *A131D* (*T131K* in our samples) increases the charge of the HA molecule and results in decreased neutralization by a monoclonal antibody [38]. The *S193R* and *S193K* mutations (*S193F* in our samples) have been shown to affect the preferential binding of the virus to α2,6 and α2,3 SAs, respectively [39].

In the HA of swine influenza A(H3N2) virus, the S138A substitution (similar to that found in the samples we examined) leads to reduced virus replication in swine respiratory tract epithelial cells that express α2,6 and α2,3 SA receptors [40].

In HA of influenza A(H3N2) virus, substitution *K156Q* (*H156S* in our samples) leads to a decrease in the neutralizing antibody activity. This is due to the fact that this amino acid molds the globular head of HA, where it forms a new epitope adjacent to the receptor-binding domain [30].

The data we obtained on the distribution of influenza B viruses by genetic groups also corresponded to the data of the Smorodintsev Research Institute of Influenza: in 2019–2020, the absolute majority of sequenced influenza B viruses belonged to the V1A (del162-164) clade of the Victorian lineage (reference virus B/Washington/02/2019)[14]; in the 2021–2022 season, influenza B/Victoria viruses of the genetic subgroup V1A.3 a.2[15] were found, in the 2022–2023 season, influenza type B viruses were assigned to genetic subgroup V1A.3a.2 and similar to the reference virus B/Austria/1359417/2021.

A D197E mutation in the HA receptor-binding site was identified in 40 viruses. The significance of amino acid substitutions in this position of HA was experimentally proved by a group of researchers by passaging strain B/Brisbane/60/2008 in human lung epithelial cell line Calu-3: after 10 consecutive passages, mutation D197T appeared in HA and it was shown that strains with this substitution had significantly lower affinity to SA-α2,3 (bird type). This could be explained by the fact that α2,3-linked glycan forms 2 hydrogen bonds with the amino acid at position 197, and any substitution at this position could affect the binding of HA to receptors [41].

Conclusion

The article presents the results of genetic monitoring of influenza viruses A(H1N1)pdm09, A(H3N2) and B detected in 50 regions of Russia from 2019 to 2023. The results reflect general patterns and allow us to judge the etiologic structure of influenza, the intensity of the epidemic process and the genetic diversity of viruses circulating in Russia.

In the Rospotrebnadzor system, influenza viruses are monitored annually; even in the 2020–2021 season, against the background of the COVID-19 pandemic, screening of patients for influenza by PCR with hybridization-fluorescence detection of amplification products, typing and sequencing of detected influenza viruses continued in the same volume. That is why it can be stated that in Russia against the background of the COVID-19 pandemic in the season 2020–2021 influenza viruses practically disappeared from circulation and appeared again in the season 2021–2022.

The analysis demonstrated the phenomena of continuous evolution with the appearance in each season of genetic variants of influenza viruses A(H1N1)pdm09, A(H3N2) and B that had changes in the HA gene compared to the vaccine strain. Mutations leading to HA amino acid substitutions were recorded in antigenic sites, in the receptor binding region, some of them leading to the formation of new potential glycosylation sites or to their loss.

When comparing influenza A(H1N1)pdm09 viruses circulating in 2022–2023 in Russia with the 2019–2020 vaccine strain A/Brisbane/02/2018, the degree of differences in the nucleotide sequences of the HA gene was 2.7–3.1%, and with the first vaccine strain of influenza A(H1N1)pdm09 virus A/California/07/2009 — 5.0–5.3%.

The degree of difference in the nucleotide sequences of the HA gene of influenza A(H3N2) viruses circulating in 2022–2023 in Russia with the 2019–2020 vaccine strain A/Kansas/14/2017 amounted to 5.3–6.0%.

The level of difference in the nucleotide sequences of the HA gene of influenza B/Victoria viruses circulating in 2022–2023 in Russia with the 2019–2020 vaccine strain B/Colorado/06/2017 was 2.2–2.8%. Influenza B/Yamagata viruses were not identified during the study period.

The highest HA variability was observed for A(H3N2) viruses, which necessitated changing the vaccine strain 3 times in 4 seasons.

Of particular note, all influenza A(H1N1)pdm09 viruses from the 2022–2023 season had the previously unknown *E224A* mutation in HA, which increases affinity for SA-α2,3 localized in the human lung, which may contribute to complications. The *D222N* mutation,

[13] ECDC. Influenza virus characterisation, Summary Europe, July 2021. Stockholm; 2021. URL: https://www.ecdc.europa.eu/en/publications-data/influenza-virus-characterisation-summary-europe-july-2021

[14] A.A. Smorodintsev Research Institute of Influenza. Weekly national influenza and ARVI bulletin for the 39th week of 2020. URL: https://www.influenza.spb.ru/system/epidemic_situation/laboratory_diagnostics/?year=2020&week=39

[15] A.A. Smorodintsev Research Institute of Influenza. Weekly national influenza and ARVI bulletin for the 24th week of 2022. URL: https://www.influenza.spb.ru/system/epidemic_situation/laboratory_diagnostics/?year=2022&week=24

which is associated with more severe disease, was found in HA in 2% and 3% of influenza A(H1N1)pdm09 viruses in 2019–2020 and 2022–2023, respectively.

Almost all influenza viruses were sensitive to oseltamivir and zanamivir, only 2.3% of influenza A(H1N1)pdm09 viruses in 2022–2023 showed resistance mutation *H275Y* in NA. In all influenza A(H1N1) pdm09 and A(H3N2) viruses studied, an adamantane resistance mutation *S31N* in M2 was found.

Our results may help to understand the direction of evolution of influenza viruses. The continuous emergence of mutations in influenza viruses poses a global public health challenge because some mutations provide a selective advantage for viral replication in the upper respiratory tract and human-to-human transmission, and reduce sensitivity to antiviral drugs. Some mutations contribute to a more severe course of influenza and the development of complications. Mutations in antigenic sites allow influenza viruses to evade anamnestic and vaccine-induced antibodies.

Therefore, it is necessary to continue to monitor influenza viruses using molecular genetic analysis, which allows deep differentiation of influenza viruses and determines the trend in the evolution of influenza viruses: the emergence, spread and disappearance from circulation of certain genetic variants.

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