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Investigation of the pathogenic potential and the possibility of cross-species transmission of H5 avian influenza viruses detected on the territory of the Russian Federation in 2018–2022

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Abstract

Introduction. The rapid evolution of highly pathogenic avian influenza (HPAI) viruses through antigenic drift and reassortment can lead to enhanced replication efficiency and cross-species transmission to mammals, as evidenced by recent outbreaks in various animal populations. Identifying mammalian pathogenicity markers in circulating HPAI viruses is crucial for evaluating their pathogenic potential and ability to cross species barriers.

The aim. This study analyzed genomic sequences of highly pathogenic H5 avian influenza virus (AIV) isolates collected in the Russian Federation between 2018 and 2022.

Materials and methods. We utilized original complete genome sequencing data alongside with nucleotide sequences of H5 AIV isolates and strains available in public databases.

Results. Analysis revealed a predominance of viruses with replication complexes adapted to avian cells. Examination of viral hemagglutinin amino acid sequences showed that most strains maintained receptor-binding sites of avian origin, with enhanced affinity for SAα-2,3-Gal receptors present in avian epithelial cells. However, we identified several mammalian virulence factors that have emerged and spread within the avian influenza virus population, including full-length active PB1-F2 protein, a 5-amino-acid insertion in the NS1 protein, and specific amino acid substitutions in the M1 protein.

Conclusion. The presence of mammalian pathogenicity factors in the avian influenza virus population may facilitate successful cross-species transmission through suppression of specific immune responses, followed by adaptation of viral hemagglutinin to mammalian cell receptors through antigenic drift and natural selection. The observed elimination of certain adaptive mutations from the avian influenza virus population validates the effectiveness of stamping-out policies and vaccination restrictions in industrial poultry farming as important measures to mitigate the zoonotic potential of avian influenza.

Keywords: *avian influenza, genetic analysis, amino acid substitutions, adaptive mutations, cross-species transmission of the virus*

Ethics approval. Authors confirm compliance with institutional and national standards for the use of laboratory animals in accordance with «Consensus Author Guidelines for Animal Use» (IAVES, 23 July, 2010). The research protocol was approved by the Ethics Committee of the Federal Centre for Animal Health (protocol No. 17, April 24, 2023).

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Оригинальное исследование
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Изучение патогенного потенциала и возможности межвидового перехода вирусов гриппа птиц подтипа H5, выявленных на территории России в 2018–2022 годах

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Аннотация

Введение. Высокая скорость эволюции вирусов высокопатогенного гриппа птиц (ВПГП), обусловленная антигенным дрейфом и реассортацией, может привести к устойчивой репликации и передаче вируса млекопитающим, что наблюдается в популяциях животных в последние годы. Исследование маркеров патогенности для млекопитающих у циркулирующих вирусов ВПГП даёт возможность оценить их патогенный потенциал и способность к межвидовому переходу.

Цель работы — провести анализ геномных последовательностей изолятов вируса гриппа птиц (ВГП) подтипа H5, выявленных на территории России в 2018–2022 гг.

Материалы и методы. В работе использованы результаты собственного полногеномного секвенирования и нуклеотидные последовательности изолятов и штаммов ВГП подтипа H5, опубликованные в открытых базах данных.

Результаты. Установлено, что преобладают вирусы с репликативным комплексом, адаптированным к размножению в клетках птиц. Анализ аминокислотной последовательности вирусного гемагглютинина выявил доминирование в рецептор-связывающем сайте белка аминокислот, характерных для ВГП и обеспечивающих повышенное сродство к рецепторам SAα-2,3-Gal эпителиальных клеток птиц. Показано появление и распространение в популяции ВГП факторов вирулентности для млекопитающих, таких как полноразмерный активный белок PB1-F2, дополнительная вставка из 5 аминокислот в белке NS1 и аминокислотные замены в белке M1.

Заключение. Наличие в популяции ВГП факторов патогенности для млекопитающих может способствовать успешному межвидовому переходу вируса за счёт подавления отдельных элементов иммунной защиты с последующей адаптацией вирусного гемагглютинина к клеточным рецепторам млекопитающих в результате антигенного дрейфа с дальнейшим закреплением приобретённых мутаций естественным отбором. Элиминация из популяции ВГП ряда адаптационных мутаций, способствующих размножению ВГП в клетках млекопитающих, подтверждает эффективность стратегии стемпинг аут и запрета на вакцинацию в промышленном птицеводстве в качестве сдерживающего фактора для гриппа птиц как зооантропонозного заболевания.

Ключевые слова: *грипп птиц, генетический анализ, аминокислотные замены, адаптационные мутации, межвидовой переход вируса*

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Introduction

Avian Influenza Virus (AIV) is the pathogen of a dangerous highly contagious disease of domestic and wild birds, characterized mainly by respiratory and digestive tract damage. In case of infection with highly pathogenic avian influenza (HPAI) viruses of H5 or H7 subtypes, bird mortality reaches 100%. In 1996, an influenza virus of the H5N1 A/goose/Guangdong/1/1996 subtype was discovered in China, which was subsequently recognized as the founder of the HPAI genetic lineage Gs/Gd/96. Over time, virus isolates of this genetic lineage became widespread not only in Asian countries but also worldwide. Thus, from 2005 to 2007, outbreaks of disease caused by this virus subtype caused significant damage to the poultry industry in Russia. Since 2014, the H5N8 subtype has been detected in Russia, whereas the H5N5 subtype has been detected there since 2016 and the H5N6 subtype — since 2018. In 2018–2019, outbreaks of HPAI (subtypes H5N1, H5N6 and H5N8) were registered among wild and domestic poultry in Asian and African countries, on the territory of Russia – in the Central, Southern, Volga and Far Eastern Federal Districts, including in farm birds at poultry farms (subtype H5N8). In 2020, H5N8 subtype HPAI was spread widely in the countries of Europe and the Middle East, on the territory of Russia and Kazakhstan [1, 2]. Furthermore, in Omsk, Rostov and Astrakhan regions, H5N5 subtype was detected. At the end of 2020, the H5N8 virus was detected in people who came into contact with sick poultry at a poultry farm in the Astrakhan region [3, 4].

In 2021–2022, H5N1 subtype influenza became widespread, with outbreaks reported in Europe, Asia, Africa, and North America [4]. Influenza outbreaks among mammals such as mink, foxes, and fur seals were of great concern [5-9]. The isolated virus has been found to have substitutions that indicate adaptation to reproduction in mammals. At the moment, the H5N1 subtype is represented in the territory of Russia. In August 2023, a dead fur seal was found on the territory of Sakhalin Island[1], and examination of pathological material from the animal showed the presence of avian influenza virus of the H5N1 subtype.

In the spring of 2024, for the first time in the United States, HPAI H5N1 virus was detected in cows on a dairy farm. Clinical signs included mastitis, lethargy, decreased feed intake, diarrhea and nasal discharge. Since then, the virus has been detected on dairy farms in at least 13 U.S. states, and environmental release of the virus with milk has also been confirmed. The identified virus has been assigned to genetic clade 2.3.4.4b and genotype B3.13, circulating in wild and domestic birds in North American countries since 2021 [10, 11]. After a certain amount of time, the HPAI H5N1 virus was detected in sick and dead cats [12] and a human dairy farm worker. The virus identified in human samples had an amino acid substitution in the PB2 protein (E627K), which is associated with viral adaptation to mammalian hosts and has previously been found in humans and other mammals infected with H5N1 and other subtypes of H5N1 and other subtypes of type A viruses, including H7N9 and H9N2 [13]. The transmission of HPAI from birds to mammals and then proven cross-species transmission from cows to cats and humans indicates a significant threat to public health.

The presence in influenza type A virus of a polybasic proteolytic cleavage site of the hemagglutinin protein provides the possibility of an extensive infectious process affecting various organs and tissues in different animal species. The ability of the virus to replicate and counteract the host immune response is provided by other viral proteins. Previously, various groups of scientists have identified specific amino acid substitutions that enable replication in mammals, counteraction to the immune response, and a more severe course of the infectious process [14–54].

The aim of this study was to analyze the genome of HPAI detected in Russia for the presence of pathogenicity markers potentially contributing to overcoming the cross-species barrier from birds to mammals and to assess the pathogenic potential of circulating viruses as agents of zooanthroponotic disease.

Materials and methods

Biomaterial

In this study, isolates of H5 subtype HPAI isolated from biomaterial from birds at ARRIAH in 2018–2022 were investigated. Virus-containing allantois fluid of pathogen-free chicken embryos or, if it was impossible to isolate viruses on chicken embryos, pathologic material from birds (cloacal and tracheal flushes, 10–20% organ suspensions prepared on the basis of 0.9% solution of NaCl) were used as material. Authors confirm compliance with institutional and national standards for the use of laboratory animals in accordance with «Consensus Author Guidelines for Animal Use» (IAVES, 23 July, 2010). The research protocol was approved by the Ethics Committee of the Federal Centre for Animal Health (protocol No. 17, April 24, 2023).

RNA isolation

Total RNA was isolated using RIBO-prep reagent kit for RNA/DNA isolation from clinical material (Central Research Institute of Epidemiology of Rospotrebnadzor).

Reverse transcription and polymerase chain reaction

Real-time polymerase chain reaction with reverse transcription (RT-PCR-RT) were performed in one stage

[1] World Organisation for Animal Health. Event 5191. https://wahis.woah.org/#/in-event/5191/dashboard

using amplification reagents (Syntol) and primers and probes for amplification of *MP* and *HA* gene fragments.

The RT reaction was performed in two steps (primer annealing and RT itself) using the Maxima H Minus Reverse Transcriptase reagent kit (includes RT buffer and Maxima H revertase; Thermo Fisher Scientific), RiboLock RNase Inhibitor (Thermo Fisher Scientific), dNTPs solution (Syntol), RNase-free bidistilled water, and a solution of direct specific segment-universal primers for amplification of all segments of type A HPAI. Classical PCR was performed using amplification reagents (Syntol) and specific segment-universal primers for amplification of all segments of type A HPAI. PCR products were purified from the PCR mixture using the Wizard(R) SV Gel and PCR Clean-Up System kit (Promega).

Sequencing

Whole-genome sequencing was performed using a MiSeq genetic analyzer (Illumina) according to the instructions for the instrument. Nextera XT and Nextera XT Index Kit commercial kits (Illumina) were used for library preparation.

Nucleotide sequences

The results of in-house whole genome sequencing and nucleotide sequences of isolates and strains of H5 subtype HPAI from Russia, published in the GenBank database of the NCBI electronic resource[2] and EpiFlu platform[3] (see Appendix on the journal's website https://microbiol.crie.ru/jour) were used in this study.

The nucleotide and corresponding amino acid sequences were analyzed using the BioEdit v. 7.0.5.3 program. Sequences were aligned using the ClustalW multiple alignment program. The phylogenetic tree was constructed using the NJ algorithm in the implementation of the MEGA v. 7.06 package.

Results

As a result of studies conducted in 2018-2022 and covering all federal districts of the Russian Federation, ARRIAH specialists identified 1,082 samples that contained genetic material of H5 subtype HPAI (**Table 1**).

H5 subtype HPAI were detected predominantly in samples from poultry throughout the study period (Table 1). Some viruses (45) were subjected to whole-genome sequencing to study virus evolution and characterize their biological properties; the sample was compiled on the basis of geographical distribution and differences in virus subtypes by neuraminidase. To expand the study sample, whole-genome sequences of H5 subtype HPAI detected in Russia from 2018 to 2022 (134 isolates) available in public databases were retrieved. It is necessary to emphasize that the use of the terms "population", "virus circulation" is not correct for the avian influenza virus, which is capable of spreading over vast territories during one season of wild bird migration. In this case, the term "avian influenza virus population" will be understood as a set of 190 viruses detected in Russia in 2018–2022, for which whole-genome sequences were obtained. The term "HPAI population" does not imply the presence of foci of persistent illness and long-term circulation of avian influenza viruses on the territory of Russia.

Based on the analysis of the predicted amino acid sequence, the cleavage site of the viral hemagglutinin of the compared isolates was determined. For all viruses it had a similar structure containing 6 basic amino acids with a variation at position 342 — RE(K/R) RRKR. The exception was A/dalmatian pelican/Astrakhan/417-1/2021 (H5N5) virus, whose cleavage site contained 7 basic amino acids RKKRRKR. The amino acid motif $G_{225}QRG_{228}$ (according to H3 subtype numbering) was detected in the receptor-binding part of the viral protein in all viruses.

H5 subtype HPAI are capable of infecting mammals, including humans, despite the fact that their hemagglutinin predominantly interacts with cellular SAα-2,3-Gal receptors. However, in the case of successful reproduction of HPAI in mammalian cells, researchers have identified mutations in other viral genes that are considered markers of HPAI adaptation for reproduction in mammals. According to their phenotypic manifestation, the marker substitutions can be divided into two main groups: mutations associated with increased activity of the viral polymerase complex in mammalian cell culture (CC), and mutations that enhance the virulent properties of the virus during experimental infection of laboratory mice and cause changes in metabolism at the organismal level associated with modification of the immune response in the host organism. Amino acid substitutions for which a change in the biological properties of the virus has been experimentally demonstrated and a link between the mutation and its phenotypic manifestation was established were included in the analysis. **Table 2** shows the positions of amino

Table 1. Results of samples testing for the presence of the H5 subtype HPAI genome in Russia in 2018–2022

Year	Number of samples analyzed	Number of samples containing HPAI/H5	
		from domestic birds	from wild birds
2018	2749	208	0
2019	5558	2	0
2020	6288	222	27
2021	6418	297	56
2022	6087	250	20
Total	27,100	979	103

[2] URL: https://www.ncbi.nlm.nih.gov/nucleotide/
[3] URL: https://www.gisaid.org/

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Table 2. Marker amino acid substitutions associated with HPAI adaptation to reproduction in mammalian CC

Protein	Amino acid's position number and HPAI isolates containing mutations	Phenotypic manifestation of the mutation
PB1	*622G* — all studied viruses	Increased polymerase activity [39]
	678S — all studied viruses, except *678N* — A/turkey/Rostov-on-Don/332-XX/2021, *678G* — A/dabchick/Tyva/767-58/2021	*678N* — increased polymerase activity [19]
PB2	*89V*, *309D, 339K*, *477G* — all studied viruses, *495V/I/A, 676T/I/M/A*	Aggregate mutations: *89V, 309D, 339K, 477G, 495V, 676T* — increased polymerase activity and replication in mammalian CC [15]
	292I/T, *588A* — all studied viruses, except *292V* — H5N8 2018–2020 (except chickens from Novosibirsk in 2020), A/chicken/Kostroma/1761-1 (H5N8), A/chicken/Tomsk/1797-7/20 (H5N8), A/duck/KChR/1590-14/20 (H5N8), A/crow/Khabarovsk/2712-1/2022 (H5N1), A/dabchick/Tyva/767-58/2021 (H5), *588V,* A/common gull/Saratov/1676/2018 (H5N6)	*292V, 588V* — increased polymerase activity and replication in mammalian CC, increased virulence in mice [40]
	389R, 598T — all studied viruses	*389R, 598T* — increased polymerase activity and replication in mammalian CC at low temperatures [41]
	482K — all studied viruses, except 482R, A/chicken/Kostroma/304-XX/2020 (H5N8), A/chicken/Kostroma/ 1761-1 (H5N8), A/crow/Khabarovsk/776-56/22 (H5N1), A/duck/Magadan/2272-8/2022 (H5N1), A/goose/Magadan/2272-5/22 (H5N1), A/poultry/Magadan/1560-1/2022 (H5N1)	*482R* — increased polymerase activity in mammalian CC [42]
PA	*37A, 100V* — all studied viruses, except *37S,* A/turkey/Stavropol/165-5/2022	*37A, 100V* — increased polymerase activity and replication in mammalian CC, increased virulence in mice [18]
	97T — all studied viruses, except *97I,* A/Chicken/Ryazan/1093-1/2022 (H5N1), A/Poultry/Samara/1659-1/2022 (H5N1), A/Poultry/Samara/1643-1/2022 (H5N1), A/Chicken/Kursk/1281-1/2022 (H5N1), A/Goose/Saratov/1965-1/2022 (H5N1), A/Goose/Belgorod/1498-1/2022 (H5N1), A/Duck/Ivanovo/1462-3/2022 (H5N1), A/Duck/Belgorod/1482-10/2022 (H5N1), A/Chicken/Orel/1484-5/2022 (H5N1), A/Chicken/Kaluga/1424-2/2022 (H5N1), A/Chicken/Rostov/1724-2/2022 (H5N1)	*97I* — increased polymerase activity and replication in mammalian CC, increased virulence in mice [43]
	127V, 44V, *241C*, *343A, 573I* — all studied viruses, except: *127A* — all H5N8/2018 viruses *343A, 347D* — all studied viruses, except: *343T* — A/Crow/Khabarovsk/2712-1/2022 (H5N1), *343S* — H5N5 and H5N8 viruses, circulating in 2020–2021	*127A, 44I, 241Y, 343T, 573V* — increased replication in mammalian ECs, increased virulence in mice [16] *343S, 347E* — increased replication in mammalian CC, increased virulence in mice [44]
	142K, 147I, *171I*, *182M* — all studied viruses; *142R,* A/common gull/Saratov/1676/2018 (H5N6), *182L,* A/waterfowl/Russia/1526-4/2021 (H5N5), A/shelduck/Kalmykia/1814-1/2021 (H5N5)	*142R, 147V, 171V, 182L* — increased polymerase activity and replication in mammalian CC [45]
	224S/A — all studied viruses, *383D* — all studied viruses	*224P, 383D* — increased polymerase activity and replication in mammalian CC [17]
NP	*41I* — all studied viruses, except: *41V* — A/common teal/Chelyabinsk/1379-1/2021 (H5N1)	*41V* — increased polymerase activity in mammalian CC at low temperature [46]
NS1	*3P/S, 41K, 74D* — all studied viruses, except: *41R* — A/chicken/Tomsk/1797-7/20 (H5N8)	*3S, 41K, 74N* — enhanced replication in mammalian CC and pathogenicity to mice [47]
	55E, 66E, 138F — all studied viruses, except: *66K* — viruses H5N8 2020–2021 *138L* A/goose/Omsk/3003/2020 (H5N8) A/goose/Omsk/3008/2020 (H5N8)	*55E, 66E, 138F* — enhanced replication in mammalian CC, decreased response to interferon [48]

acid substitutions of HPAI proteins that promote HPAI reproduction in mammals.

Analysis of the predicted amino acid sequences of the polymerase complex proteins revealed single marker substitutions capable of enhancing the work of the virus replicative complex in mammalian cells, which were fixed in the avian influenza virus population. For example, only 1 substitution at position *622G* became fixed in the PB1 protein. Two other mutations (*678N* and *105S*) had a sporadic distribution.

Mutations *389R* and *598T* were fixed in the PB2 protein by natural selection. These mutations among avian influenza viruses have been recorded previously, but have now become dominant. The widespread distribution in 2018-2020 of the *292V* mutation and the appearance of single *482R* mutations in the population were noted. Analysis of the set of "adaptation mutations" *89V*, *309D*, *339K*, *477G*, *495V*, *676T* indicates the consolidation by natural selection of this set of amino acid substitutions. Experimental studies have shown that the set of these substitutions can compensate for the absence of lysine at position 627 of the PB2 protein for successful replication of HPAI in mammalian cells [15].

Analysis of the predicted amino acid sequence of the *PA* gene showed that the *383D* mutation was fixed in the population. A wide distribution of mutations *37A*, *61I*, *63V*, *100V*, *343S*, *383D* and single cases of mutations *224P*, *343T*, *142R* were detected. Although the largest number of "adaptation mutations" was found in the nucleotide sequences of the *PA* gene, this does not appear to be critical because they are randomly distributed among the viruses. Furthermore, a number of studies have shown the necessity of a synergistic effect for the phenotypic manifestation in mammals of "adaptation mutations" in the *PA* gene [16–18].

Analysis of marker amino acid substitutions associated with the virulent properties of HPAI showed that the 42S mutation in the NS1 protein is fixed in the HPAI population (**Table 3**). This substitution is a marker of virulent properties for mice and can counteract the induction of interferon in the host cell, as well as prevent activation of the NF-κB pathway during the immune response [19]. In addition, the amino acid substitutions *30D* and *215A* in the M1 protein, recognized as determinants of pathogenicity for mice, were detected in all isolates [20].

During the study of pathogenicity factors, the *NS1* gene encoding the corresponding protein with an additional 5 amino acid insertions at positions 80–84 was found to be fixed in the HPAI population. Experimental studies have shown that hybrid viruses with this insertion can induce a hyperimmune response in the organism, which is known as the cytokine storm [21]. The analysis showed that among influenza viruses, this mutation began to take hold after 2017.

Discussion

As a result of this study, it has been established that evolutionary selection has fixed a number of amino acid substitutions that contribute to the successful reproduction of HPAI in mammals. At the same time, questions remain about the mechanism of functioning of the viral receptor that allows the virus to make cross-species transmission. According to previous studies, the amino acid motif $G_{225}QRG_{228}$ is characteristic of influenza viruses isolated from birds and has high affinity for SAα-2,3-Gal group receptors [14, 22]. However, interpretation of the affinity properties of viral hemagglutinin to SAα-2,3-Gal group receptors or to SAα-2,6-Gal group receptors based on the primary amino acid sequence is difficult. It has been found that changes in the tropic

Table 3. Marker amino acid substitutions associated with increased HPAI virulence

Protein	Amino acid's position number and HPAI isolates containing mutations	Phenotypic manifestation of mutations
PB1	*105S* — A/chicken/Penza/300/2018	Increased virulence in mice [43]
PB1-F2	*66N* — all studied viruses, except: *66S* — HPAI isolates 2019-2022 subtypes H5N5, H5N1	*66S* — virulence and enhanced immune response in mice [34, 54]
NP	*319N* — all studied viruses, except: *319K* A/Crow/Khabarovsk/776-56/22 (H5N1) A/Duck/Magadan/2272-8/2022 (H5N1) A/Goose/Magadan/2272-5/22 (H5N1) A/Poultry/Magadan/1560-1/2022 (H5N1) A/Chicken/Ryazan/1093-1/2022 (H5N1)	*319K* — disruption of intranuclear transport in mammalian cells [23]
M1	*30D, 215A* — all studied viruses *43M* — all studied viruses	*30D, 215A* — increased virulence in mice Increased virulence in mice [49]
NS1	*42S* — all studied viruses	Increased virulence and decreased antiviral response in mice [50]
	92D — all studied viruses, except: 92E, A/common gull/Saratov/1676/2018 (H5N6)	*92D* — increased virulence in pigs and mice [51]
	103F/Y, 106M — all studied viruses	*103F, 106M* — increased virulence in mice [52, 53]

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properties of influenza viruses are possible as a result of both single mutations and a whole set of mutations in the amino acid sequence. In our unpublished studies and literature data comparing the predicted amino acid sequence of the *HA* gene of viruses isolated from birds and mammals, no amino acid substitutions were identified that were unique to AIV or unique to viruses isolated from mammals. Apparently, the hemagglutinin of influenza virus of genetic clade 2.3.4.4 as a result of single mutations is able to change its affinity properties with respect to sialic acid residues and, while retaining functionality, can overcome the cross-species barrier by combining with different types of viral neuraminidase.

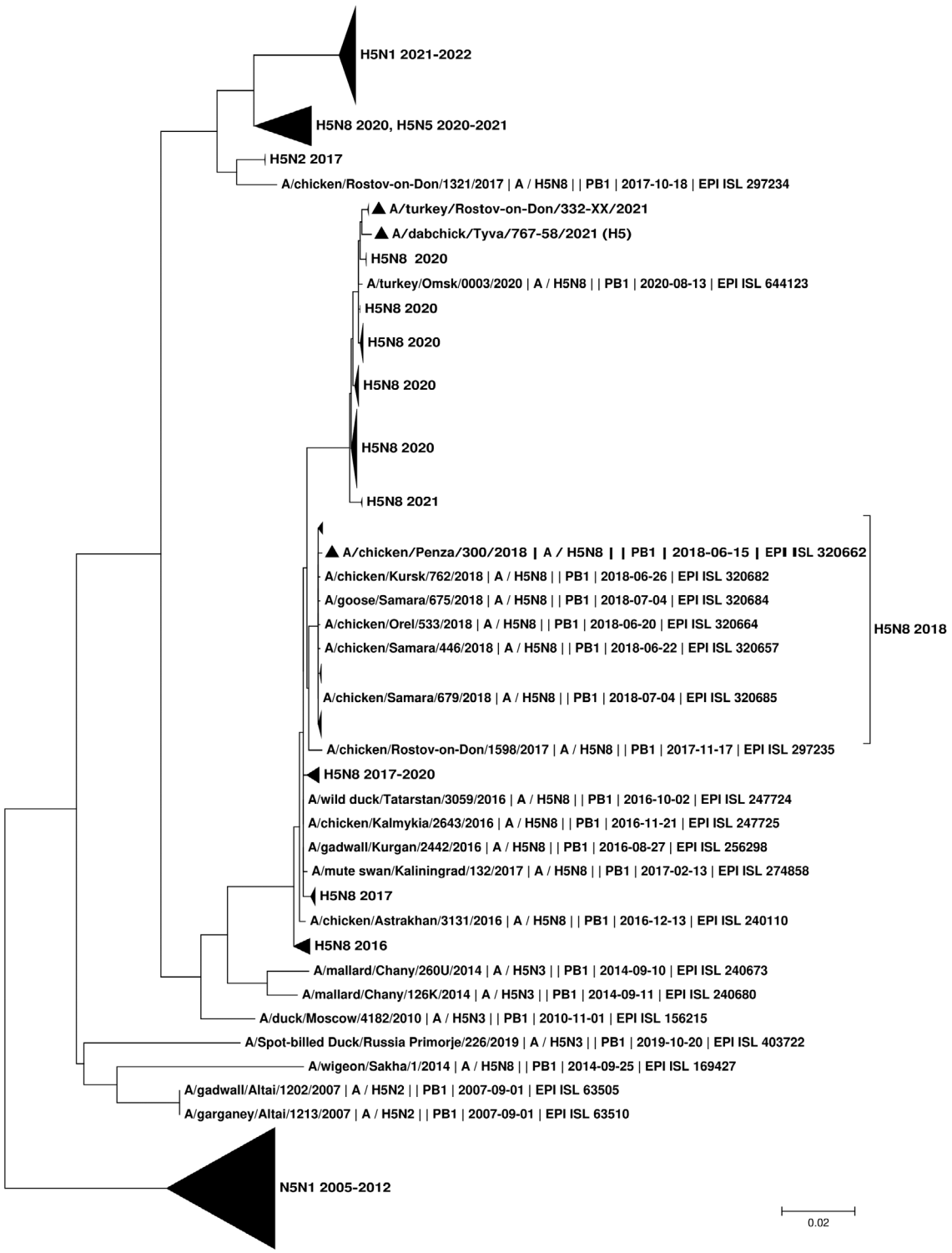
A *678N* mutation was detected in the PB1 protein of HPAI isolated in industrial farming in Rostov region — A/turkey/Rostov-on-Don/332-XX/2021; A/dabchick/Tyva/767-58/2021 virus with *678G* mutation was detected in the territory of the Republic of Tyva. According to experimental data, the combination of amino acids 13P and 678N causes a sharp increase in the polymerase activity of HPAI during replication in mammalian cells [19]. In the A/chicken/Penza/300/2018 isolate, a *105S* mutation was detected that increases the manifestation of virulent properties against mice. To verify the origin of these mutations, phylogenetic analysis was performed, which showed that the A/turkey/Rostov-on-Don/332-08/2021, A/dabchick/Tyva/767-58/2021 and A/chicken/Penza/300/2018 viruses are in groups that include viruses that spread in Russia between 2018 and 2022 and did not have similar mutations (**Figure**). Phylogenetic analysis confirms that the occurrence of mutations *105S*, *678N*, *678G* in the PB1 protein among HPAI detected in Russia is the result of antigenic drift rather than antigenic shifting.

Analysis of the occurrence of substitutions in the replicative proteins of HPAI (as exemplified by mutations *678N* and *105S* of the PB1 protein) showed that the occurrence of single mutations as a result of antigenic drift is extremely rare, even in conditions of frequent epizootics. The single and insignificant spread of mutations in the HPAI population that contribute to the development of the disease in mammals in case of cross-species transmission demonstrates the feasibility and effectiveness of the stepping-out strategy in the control of avian influenza as a disease with zooanthroponotic potential. The use of a stepping-out strategy, involving the total eradication of all infected animals, is recommended by the World Organization for Animal Health for a number of emergent diseases that have panzootic potential and can persist in wildlife populations. With proper surveillance of poultry populations using molecular biology techniques, HPAI can be detected in a timely manner and eradication of the outbreak can be carried out without allowing the possibility of cross-species transmission to mammals. The effectiveness of the stepping-out strategy is confirmed by the disappearance from the viral population of such mutations as *127A* in the PA protein and *292V* in the PB2 protein. These mutations were identified in viruses during the 2018 H5N8 subtype VHP epizootic. The *292V* mutation in the PB2 protein was registered in isolated cases after 2018, but timely eradication of infected stock prevented HPAI from entering the mammalian population. From the HPAI population, these mutations were eliminated by natural selection because they had a negative effect on virus reproduction in birds. Apparently, there is a dynamic equilibrium in HPAI populations, maintained by natural selection, due to which adaptation mutations to mammals disappear from the population. This can be seen in the scattering of such mutations in various genes among HPAI – they occur in different viruses with different frequencies, but no viruses have been found that contain in their genome the full range of adaptation mutations to mammals. In addition, new substitutions in positions, changes in which affect replication in mammalian cells, were identified in the viruses we studied (Table 3).

The effect of new mutations on the replication and virulence properties of HPAI in mammals has not been experimentally studied. Despite the lack of experimental data indicating an increase in virus replication from new substitutions, this is worrisome because early work on the evolution of HPAI showed that, after crossing the species barrier, it undergoes a phase that allows it to gradually acquire adaptive mutations without losing adaptation to the old host [23, 24]. Earlier studies on the transmissibility of HPAI performed by other researchers indicate the complex and often complex nature of changes in the HPAI genome during cross-species transmission and fixation of the virus in the population of a new species [25–28]. Studies carried out at the N.F. Gamaleya Research Center for Epidemiology and Microbiology also showed the possibility of adaptation and acquisition of pathogenic properties for laboratory mice during 7–10 cycles of experimental infection [29].

In our analysis, in addition to single substitutions, it was noted that viruses capable of translating the full-length PB1-F2 protein became established in the post-2020 HPAI population in Russia. Translation became possible due to the nucleotide mutation *A129C* (numbering from the beginning of the open reading frame of the *PB1* gene), which eliminated the stop codon. The PB1-F2 gene is located within the reading frame of the *PB1* gene and encodes a protein that influences the severity of the inflammatory process. At present, there is no unambiguous description of the effect of this protein on the virulence properties of the virus. It is reliably known that the phenotypic expression of the same form of the *PB1-F2* gene differs between birds and mammals. It has already been shown that expression of *PB1-F2* reduces virulence for birds [30-32]. The results of experimental infection revealed that while no virulence enhancement was observed in birds, infection of mice revealed a clear involvement

of the PB1-F2 protein of the H7N1 virus in the host inflammatory response, as previously shown for the H1N1 and H5N1 subtype HPAI strains [32]. The results of experimental infection in ferrets when infected with PB1-F2-expressing HPAI differed from the course of the infection process when the virus without PB1-F2 expression was used. Infection with a virus expressing PB1-F2 correlated with a significant dysregulation of leukocyte counts on days 3 and 7 post-infection; PB1-F2 expression was associated with both lymphopenia and increased neutrophil counts. Lymphopenia was transient in all ferrets, and leukocyte levels



Phylogenetic tree constructed by the NJ method based on the nucleotide sequences of the *PB1* gene fragment (1–2275 bp ORF) of H5 subtype HPAI.

Triangles indicate HPAI with adaptation mutations.

returned to baseline on day 19 post-infection [33]. All viruses with an active form of the PB1-F2 gene have a *66S* amino acid mutation. Several studies have shown that viruses with this mutation caused a more severe infection process in infected laboratory mice [34]. This mutation was present in HPAI isolated from minks in Spain in 2022. [5]. Furthermore, this mutation was one of those that distinguished the deadly virus A/Brevig Mission/18, also known as the "Spanish flu" that swept the world in the early twentieth century [34]. In addition to the direct effect of PB1-F2 protein on the course of the infectious process, data have been obtained that indicate the possibility of a more severe infectious process in case of mutual expression of full-length PB1-F2 and PA-X proteins [35]. The analysis showed that all the HPAI studied in this study are capable of expressing the full-size PA-X protein.

The variability of the C-terminal sequence of the NS1 protein is worth special attention. Earlier studies have shown that single substitutions in the last 4 amino acids affect the possibility of effective cross-species transmission from pigs to mice, accompanied by the manifestation of pathogenic properties in relation to the new host [36]. Thus, H1N1 subtype HPAI that had an NS1 protein with the last 4 amino acids being PEQK and RSEV could not infect mice, whereas the same virus whose NS1 protein ended with the amino acid motif of GSEI and EPEV successfully induced the infectious process in mice, reaching a titer of 2300 BOU/g [37]. Among the HPAI detected in Russia during 2018-2022, the NS1 protein end motif had variations of GSEV, LPPK, FPPK, ESEV and ESEI. Such diversity of the NS1 protein end motif may ensure a wide distribution of HPAI among different bird species [38].

The data obtained by analyzing the presence of marker substitutions in HGP/H5 indicate an active evolutionary process currently taking place in the HPAI population. The presence of mammalian pathogenicity factors in the HPAI population may contribute to successful cross-species transmission of the virus by suppressing certain elements of immune defense. After the cross-species transmission, the virus may end up in the channel of accumulative variability, when in the process of natural selection, single mutations that enhance the phenotypic manifestation or functional properties of certain proteins and provide competitive advantages relative to other viruses are consolidated.

This scenario of cross-species transmission emphasizes the necessity to use the strategy of stepping-out and ban on vaccination against HPAI in industrial poultry production as a deterrent to HPAI as a zooanthroponotic pathogen. Timely and complete elimination of infected poultry stock allows avoiding cross-species transmission of HPAI to mammals via stray dogs, cats or small rodents. In case of uncontrolled vaccination against HPAI, there may be a latent circulation of HPAI among susceptible stock without clinical signs, which will result in an increase in genetic diversity of the HPAI population and active appearance of new mutations, among which may be useful for the virus, contributing to cross-species transmission.

Conclusion

As a result of these studies, it was found that among the H5 subtype HPAI detected in Russia in 2018–2022, viruses with an enzyme complex adapted to replication in avian cells predominated. Adaptation mutations to replication in mammalian cells are sporadic and chaotically distributed in the virus population. Viral hemagglutinin has affinity predominantly to avian cell receptors. The appearance and distribution of virulence factors for mammals in the HPAI population has been shown. The presence of such factors may contribute to successful cross-species transmission of the virus with subsequent adaptation of viral hemagglutinin to mammalian cell receptors as a result of antigenic drift and fixation of new mutations in the course of natural selection.

The results obtained indicate the effectiveness of the strategy of stepping-out and ban on vaccination against HPAI in industrial poultry farming as a deterrent factor for HPAI as a pathogen of zooanthroponotic disease. Timely and complete elimination of poultry stock infected with HPAI, which is capable of producing pathogenicity factors for mammals in the process of reproduction and possessing separate adaptive mutations in replicative proteins, allows to avoid or significantly reduce the probability of cross-species transmission of HPAI from birds to mammals via stray animals or small rodents.

СПИСОК ИСТОЧНИКОВ|REFERENCES

1. Марченко В.Ю., Гончарова Н.И., Tran T.N. и др. Обзор эпизоотологической ситуации по высокопатогенному вирусу гриппа птиц в России в 2019 г. *Проблемы особо опасных инфекций.* 2020;(2):31–7. Marchenko V.Yu., Goncharova N.I., Tran T.N., et al. Overview of the epizootiological situation on highly pathogenic avian influenza virus in Russia in 2019. *Problems of Particularly Dangerous Infections.* 2020;(2):31–7. DOI: https://doi.org/10.21055/0370-1069-2020-2-31-37 EDN: https://elibrary.ru/rnioio
2. Lewis N.S., Banyard A.C., Whittard E., et al. Emergence and spread of novel H5N8, H5N5 and H5N1 clade 2.3.4.4 highly pathogenic avian influenza in 2020. *Emerg. Microbes Infect.* 2021;10(1):148–51. DOI: https://doi.org/10.1080/22221751.2021.1872355
3. Виткова О.Н., Караулов А.К., Ирза В.Н. и др. Эпизоотическая ситуация по высокопатогенному гриппу птиц и болезни ньюкасла в Российской Федерации в 2016–2020 годах. *Эффективное животноводство.* 2021;(4):76–8. Vitkova O.N., Karaulov A.K., Irza V.N., et al. Epizootic situation of highly pathogenic avian influenza and Newcastle disease in the Russian Federation in 2016–2020. *Efficient Animal Husbandry.* 2021;(4):76–8. EDN: https://elibrary.ru/utlbye
4. Ирза В.Н., Волков М.С., Варкентин А.В. О текущей панзоотии высокопатогенного гриппа птиц. *Эффективное животноводство.* 2022;(5):85–6. Irza V.N., Volkov M.S.,

Varkentin A.V. About the current epizootic of highly pathogenic avian influenza. *Efficient Animal Husbandry.* 2022;(5):85–6. EDN: https://elibrary.ru/rcsitl
5. Agüero M., Monne I., Sanchez A., et al. Highly pathogenic avian influenza A(H5N1) virus infection in farmed minks, Spain, October 2022. *Euro Surveill.* 2023;28(3):2300001. DOI: https://doi.org/10.2807/1560-7917.ES.2023.28.3.2300001
6. Honglei S., Fangtao L., Qingzhi L., et al. Mink is a highly susceptible host species to circulating human and avian influenza viruses. *Emerg. Microbes Infect.* 2021;10(1):472–80. DOI: https://doi.org/10.1080/22221751.2021.1899058
7. Bordes L., Vreman S., Heutink R., et al. Highly pathogenic avian influenza H5N1 virus infections in wild red foxes (*Vulpes vulpes*) show neurotropism and adaptive virus mutations. *Microbiol. Spectr.* 2023;11(1):e0286722. DOI: https://doi.org/10.1128/spectrum.02867-22
8. Floyd T., Banyard A.C., Lean F.Z., et al. Encephalitis and death in wild mammals at a rehabilitation center after infection with highly pathogenic avian influenza A (H5N8) virus, United Kingdom. *Emerg. Infect. Dis.* 2021;27(11):2856–63. DOI: https://doi.org/10.3201/eid2711.211225
9. Postel A., King J., Kaiser F.K., et al. Infections with highly pathogenic avian influenza A virus (HPAIV) H5N8 in harbor seals at the German North Sea coast. *Emerg. Microbes Infect.* 2022;11(1):725–9. DOI: https://doi.org/10.1080/22221751.2022.2043726
10. Rodriguez Z., Picasso-Risso C., O'Connor A., Ruegg P.L. Hot topic: epidemiological and clinical aspects of highly pathogenic avian influenza H5N1 in dairy cattle. *JDS Commun.* 2024;5(Suppl. 1):S8–12. DOI: https://doi.org/10.3168/jdsc.2024-0650
11. Butt S.L., Nooruzzaman M., Covaleda L.M., Diel D.G. Hot topic: influenza A H5N1 virus exhibits a broad host range, including dairy cows. *JDS Commun.* 2024;5(Suppl. 1):S13–9. DOI: https://doi.org/10.3168/jdsc.2024-0638
12. Burrough E.R., Magstadt D.R., Petersen B., et al. Highly pathogenic avian influenza A(H5N1) clade 2.3.4.4b virus infection in domestic dairy cattle and cats, united states, 2024. *Emerg. Infect. Dis.* 2024;30(7):1335–43. DOI: https://doi.org/10.3201/eid3007.240508
13. Uyeki T.M., Milton S., Abdul Hamid C., et al. Highly pathogenic avian influenza A(H5N1) virus infection in a dairy farm worker. *N. Engl. J. Med.* 2024;390(21):2028–9. DOI: https://doi.org/10.1056/NEJMc2405371
14. Gabriel G., Abram M., Keiner B., et al. Structure and receptor specificity of the hemagglutinin from an H5N1 influenza virus. *Science*. 2006;312(5772):404–10. DOI: https://doi.org/10.1126/science.1124513
15. Li J., Ishaq M., Prudence M., et al. Single mutation at the amino acid position 627 of PB2 that leads to increased virulence of an H5N1 avian influenza virus during adaptation in mice can be compensated by multiple mutations at other sites of PB2. *Virus Res.* 2009;144(1-2):123–9. DOI: https://doi.org/10.1016/j.virusres.2009.04.008
16. Yamaji R., Yamada S., Le M.Q., et al. Mammalian adaptive mutations of the PA protein of highly pathogenic avian H5N1 influenza virus. *J. Virol.* 2015;89(8):4117–25. DOI: https://doi.org/10.1128/JVI.03532-14
17. Song J., Xu J., Shi J., et al. Synergistic effect of S224P and N383D substitutions in the PA of H5N1 avian influenza virus contributes to mammalian adaptation. *Sci. Rep.* 2015;5:10510. DOI: https://doi.org/10.1038/srep10510
18. Hu M., Yuan S., Ye Z.W., et al. PA_N substitutions A37S, A37S/I61T and A37S/V63I attenuate the replication of H7N7 influenza A virus by impairing the polymerase and endonuclease activities. *J. Gen. Virol.* 2017;98(3):364–73. DOI: https://doi.org/10.1099/jgv.0.000717
19. Gabriel G., Dauber B., Wolff T., et al. The viral polymerase mediates adaptation of an avian influenza virus to a mammalian host. *Proc. Natl Acad. Sci. USA.* 2005;102(51):18590–5. DOI: https://doi.org/10.1073/pnas.0507415102
20. Fan S., Deng G., Song J., et al. Two amino acid residues in the matrix protein M1 contribute to the virulence difference of H5N1 avian influenza viruses in mice. *Virology*. 2009;384(1):28–32. DOI: https://doi.org/10.1016/j.virol.2008.11.044
21. Chen S., Miao X., Huangfu D., et al. H5N1 avian influenza virus without 80–84 amino acid deletion at the NS1 protein hijacks the innate immune system of dendritic cells for an enhanced mammalian pathogenicity. *Transbound. Emerg. Dis.* 2021;68(4):2401–13. DOI: https://doi.org/10.1111/tbed.13904
22. Matrosovich M.N., Gambaryan A.S., Teneberg S., et al. Avian influenza A viruses differ from human viruses by recognition of sialyloligosaccharides and gangliosides and by a higher conservation of the HA receptor-binding site. *Virology*. 1997;233(1):224–34. DOI: https://doi.org/10.1006/viro.1997.8580
23. Gabriel G., Herwig A., Klenk H.D. Interaction of polymerase subunit PB2 and NP with importin alpha1 is a determinant of host range of influenza A virus. *PLoS Pathog.* 2008;4(2):e11. DOI: https://doi.org/10.1371/journal.ppat.0040011
24. Gabriel G., Abram M., Keiner B., et al. Differential polymerase activity in avian and mammalian cells determines host range of influenza virus. *J. Virol.* 2007;81(17):9601–4. DOI: https://doi.org/10.1128/JVI.00666-07
25. Imai M., Watanabe T., Hatta M., et al. Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets. *Nature*. 2012;486(7403):420–8. DOI: https://doi.org/10.1038/nature10831
26. Russell C.A., Fonville J.M., Brown A.E., et al. The potential for respiratory droplet-transmissible A/H5N1 influenza virus to evolve in a mammalian host. *Science*. 2012;336(6088):1541–7. DOI: https://doi.org/10.1126/science.1222526
27. Herfst S., Schrauwen E.J., Linster M., et al. Airborne transmission of influenza A/H5N1 virus between ferrets. *Science*. 2012;336(6088):1534–41. DOI: https://doi.org/10.1126/science.1213362
28. Herfst S., Imai M., Kawaoka Y., Fouchier R.A.M. Avian influenza virus transmission to mammals. *Curr. Top. Microbiol. Immunol.* 2014;385:137–55. DOI: https://doi.org/10.1007/82_2014_387
29. Timofeeva T.A., Rudneva I.A., Lomakina N.F., et al. Mutations in the genome of avian influenza viruses of the H1 and H5 subtypes responsible for adaptation to mammals. *Microbiol. Indep. Res. J.* 2021;8(1):80–91. DOI: https://doi.org/10.18527/2500-2236-2021-8-1-50-61
30. Leymarie O., Embury-Hyatt C., Chevalier C., et al. PB1-F2 attenuates virulence of highly pathogenic avian H5N1 influenza virus in chickens. *PLoS One.* 2014;9(6):e100679. DOI: https://doi.org/10.1371/journal.pone.0100679
31. James J., Howard W., Iqbal M., et al. Influenza A virus PB1-F2 protein prolongs viral shedding in chickens lengthening the transmission window. *J. Gen. Virol*. 2016;97(10):2516–27. DOI: https://doi.org/10.1099/jgv.0.000584
32. Mettier J., Marc D., Sedano L., et al. Study of the host specificity of PB1-F2-associated virulence. *Virulence*. 2021;12(1):1647–60. DOI: https://doi.org/10.1080/21505594.2021.1933848
33. Hai R., Schmolke M., Varga Z.T., et al. PB1-F2 expression by the 2009 pandemic H1N1 influenza virus has minimal impact on virulence in animal models. *J. Virol.* 2010;84(9):4442–50. DOI: https://doi.org/10.1128/JVI.02717-09
34. Conenello G.M., Zamarin D., Perrone L.A., et al. A single mutation in the PB1-F2 of H5N1 (HK/97) and 1918 influenza A viruses contributes to increased virulence. *PLoS Pathog.* 2007;3(10):1414–21. DOI: https://doi.org/10.1371/journal.ppat.0030141
35. Ma J., Li S., Li K., et al. Effects of the PA-X and PB1-F2 proteins on the virulence of the 2009 pandemic H1N1 influenza

A virus in mice. *Front. Cell. Infect. Microbiol.* 2019;9:315. DOI: https://doi.org/10.3389/fcimb.2019.00315
36. Lloren K.K.S., Lee T., Kwon J.J., Song M.S. Molecular markers for interspecies transmission of avian influenza viruses in mammalian hosts. *Int. J. Mol. Sci.* 2017;18(12):2706. DOI: https://doi.org/10.3390/ijms18122706
37. Wang J., Qi X., Lu C. Mutations in the C-terminal tail of NS1 protein facilitate the replication of classical swine H1N1 influenza A virus in mice. *Folia Microbiol. (Praha)*. 2012;57(3):169–75. DOI: https://doi.org/10.1007/s12223-012-0110-0
38. Soubies S.M., Volmer C., Croville G., et al. Species-specific contribution of the four C-terminal amino acids of influenza A virus NS1 protein to virulence. *J. Virol.* 2010;84(13):6733–47. DOI: https://doi.org/10.1128/jvi.02427-09
39. Feng X., Wang Z., Shi J., et al. Glycine at position 622 in PB1 contributes to the virulence of H5N1 avian influenza virus in mice. *J. Virol.* 2015;90(4):1872–9. DOI: https://doi.org/10.1128/JVI.02387-15
40. Xiao C., Ma W., Sun N., et al. PB2-588V promotes the mammalian adaptation of H10N8, H7N9 and H9N2 avian influenza viruses. *Sci. Rep.* 2016;6:19474. DOI: https://doi.org/10.1038/srep19474
41. Hu M., Yuan S., Zhang K., et al. PB2 substitutions V598T/I increase the virulence of H7N9 influenza A virus in mammals. *Virology*. 2017;501:92–101. DOI: https://doi.org/10.1016/j.virol.2016.11.008
42. Yamayoshi S., Kiso M., Yasuhara A., et al. Enhanced replication of highly pathogenic influenza A (H7N9) virus in humans. *Emerg. Infect. Dis.* 2018;24(4):746–50. DOI: https://doi.org/10.3201/eid2404.171509
43. Taft A.S., Ozawa M., Fitch A., et al. Identification of mammalian-adapting mutations in the polymerase complex of an avian H5N1 influenza virus. *Nat. Commun.* 2015;6:7491. DOI: https://doi.org/10.1038/ncomms8491
44. Zhong G., Le M.Q., Lopes T.J.S., et al. Mutations in the PA protein of avian H5N1 influenza viruses affect polymerase activity and mouse virulence. *J. Virol.* 2018;92(4):e01557-17. DOI: https://doi.org/10.1128/JVI.01557-17
45. Liang L., Jiang L., Li J., et al. Low polymerase activity attributed to PA drives the acquisition of the PB2 E627K mutation of H7N9 avian influenza virus in mammals. *mBio*. 2019;10(3):e01162-19. DOI: https://doi.org/10.1128/mBio.01162-19
46. Zhu W., Zou X., Zhou J., et al. Residues 41 V and/or 210D in the NP protein enhance polymerase activities and potential replication of novel influenza (H7N9) viruses at low temperature. *Virol. J.* 2015;12:71. DOI: https://doi.org/10.1186/s12985-015-0304-6
47. Kanrai P., Mostafa A., Madhugiri R., et al. Identification of specific residues in avian influenza A virus NS1 that enhance viral replication and pathogenicity in mammalian systems. *J. Gen. Virol.* 2016;97(9):2135–48. DOI: https://doi.org/10.1099/jgv.0.000542
48. Li J., Zhang K., Chen Q., et al. Three amino acid substitutions in the NS1 protein change the virus replication of H5N1 influenza virus in human cells. *Virology*. 2018;519:64–73. DOI: https://doi.org/10.1016/j.virol.2018.04.004
49. Nao N., Kajihara M., Manzoor R., et al. A single amino acid in the M1 protein responsible for the different pathogenic potentials of H5N1 highly pathogenic avian influenza virus strains. *PLoS One.* 2015;10(9):e0137989. DOI: https://doi.org/10.1371/journal.pone.0137989
50. Jiao P., Tian G., Li Y., et al. A single-amino-acid substitution in the NS1 protein changes the pathogenicity of H5N1 avian influenza viruses in mice. *J. Virol.* 2008;82(3):1146–54. DOI: https://doi.org/10.1128/JVI.01698-07
51. Lipatov A.S., Andreansky S., Webby R.J., et al. Pathogenesis of Hong Kong H5N1 influenza virus NS gene reassortants in mice: the role of cytokines and B- and T-cell responses. *J. Gen. Virol.* 2005;86(Pt. 4):1121–30. DOI: https://doi.org/10.1099/vir.0.80663-0
52. Ayllon J., Domingues P., Rajsbaum R., et al. A single amino acid substitution in the novel H7N9 influenza A virus NS1 protein increases CPSF30 binding and virulence. *J. Virol.* 2014;88(20):12146–51. DOI: https://doi.org/10.1128/JVI.01567-14
53. Spesock A., Malur M., Hossain M.J., et al. The virulence of 1997 H5N1 influenza viruses in the mouse model is increased by correcting a defect in their NS1 proteins. *J. Virol.* 2011;85(14):7048–58. DOI: https://doi.org/10.1128/JVI.00417-11
54. Schmolke M., Manicassamy B., Pena L., et al. Differential contribution of PB1-F2 to the virulence of highly pathogenic H5N1 influenza A virus in mammalian and avian species. *PLoS Pathog.* 2011;7(8):e1002186. DOI: https://doi.org/10.1371/journal.ppat.1002186

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