Original Study Article
https://doi.org/10.36233/0372-9311-395

The adaptive potential of North American subtype H7N2 avian influenza viruses to mammals

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Abstract

Introduction. H7 subtype avian influenza viruses causing severe epizootics among birds are phylogenetically different in the Eastern and Western hemispheres. Numerous human infections caused by these viruses in the Eastern hemisphere indicate that H7 viruses can overcome the interspecies barrier and pose a potential threat of a new pandemic.The H7N2 viruses with deletion of amino acids 221–228 (H3 numbering) in hemagglutinin (HA) had been circulating among poultry in the Western Hemisphere during 1996–2006, and had once again been detected in 2016 in an animal shelter, where they caused cat diseases.

The objective of this study is to elucidate the mechanism of adaptation to mammals of North American H7N2 influenza viruses with deletion in HA.

Materials and methods. The A/chicken/New Jersey/294598-12/2004 (H7N2) virus was adapted to mice by the lung passages. Complete genomes of original and mouse-adapted viruses were analyzed. The receptor specificity and thermostability of viruses, HA activation pH and virulence for mice were determined.

Results. The non-pathogenic H7N2 avian influenza virus became pathogenic after 10 passages in mice. Amino acid substitutions occurred in five viral proteins: one in PB2 (*E627K*), NA (*K127N*), NEP (*E14Q*), four in HA and six in NS1. Mutations in HA slightly changed receptor specificity but increased the pH of HA activation by 0.4 units. The NS1 protein undergone the greatest changes in the positions (*N73T*, *S114G*, *K118R*, *G171A*, *F214L* and *G224R*), where amino acid polymorphisms were observed in the original virus, but only minor amino acid variants have been preserved in the mouse adapted variant.

Conclusion. The results show that H7N2 viruses have the potential to adapt to mammals. The increase in virulence is most likely due to the adaptive *E627K* mutation in PB2 and possibly in HA.

Keywords: *H7N2 influenza virus; adaptation to mammals; adaptive mutations*

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 D.I. Ivanovsky Institute of Virology of the National Research Centre for Epidemiology and Microbiology named after honorary academician N.F. Gamaleya (protocol No. 12, 28 June, 2021).

Acknowledgement. The authors thank the staff of the Genome Analysis Laboratory of the N.F. Gamaleya Federal State Budgetary Institution of the Russian Ministry of Health O.L. Voronina, E.I. Aksenova, M.S. Kunda and N.N. Ryzhova for the complete genome sequencing of viruses on the Illumina platform.

Funding source. This study was not supported by any external sources of funding.

Conflict of interest. The authors declare no apparent or potential conflicts of interest related to the publication of this article.

For citation: Lyashko A.V., Rudneva I.A., Shcherbinin D.N., Lomakina N.F., Treshchalina A.A., Kupriyanova I.M., Gambaryan A.S., Timofeeva E.B., Shilov A.A., Sadykova G.K., Prilipov A.G., Timofeev B.I., Shmarov M.M., Ryazanova E.L., Timofeeva T.A. The adaptive potential of North American subtype H7N2 avian influenza viruses to mammals. *Journal of microbiology, epidemiology and immunobiology.* 2023;100(6):442–453.
DOI: https://doi.org/10.36233/0372-9311-395
EDN: https://www.elibrary.ru/yphkuy

Оригинальная статья
https://doi.org/10.36233/0372-9311-395

Потенциал адаптации к млекопитающим вирусов гриппа птиц H7N2 североамериканской линии

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Аннотация

Введение. Вирусы гриппа птиц подтипа H7 вызывают тяжёлые эпизоотии среди птиц и филогенетически различаются в Восточном и Западном полушарии. Многочисленные случаи заражения людей этими вирусами в Восточном полушарии указывают на то, что вирусы H7 могут преодолевать межвидовой барьер и представляют потенциальную угрозу новой пандемии. Вирусы H7N2 с делецией аминокислот 221–228 (в нумерации H3) в гемагглютинине (HA) циркулировали среди домашней птицы в Западном полушарии в 1996–2006 гг. и вновь были обнаружены в 2016 г. в приюте для животных, где вызвали заболевание кошек.

Цель работы — выяснить механизм адаптации к млекопитающим вирусов гриппа североамериканской линии H7N2 с делецией в HA.

Материалы и методы. Вирус A/chicken/New Jersey/294598-12/2004 (H7N2) был адаптирован к мышам пассированием через лёгкие. Проведён анализ полных геномов исходного и адаптированного к мышам вариантов вируса. Определены специфичность вирусов к аналогам клеточных рецепторов, термостабильность HA, значение pH активации HA, вирулентность для мышей.

Результаты. Непатогенный птичий вирус гриппа H7N2 после 10 пассажей на мышах стал патогенным. В 5 вирусных белках произошли аминокислотные замены: по 1 в PB2 (*E627K*), NA (*K127N*), NEP (*E14Q*), 4 в HA, 6 в NS1. Мутации в HA слабо повлияли на рецепторную специфичность, но способствовали возрастанию значения pH активации HA на 0,4 единицы. Наибольшим изменениям подвергся белок NS1 в позициях *N73T*, *S114G*, *K118R*, *G171A*, *F214L* и *G224R*, где в исходном варианте наблюдался полиморфизм, а в адаптированном варианте сохранились только минорные альтернативные аминокислоты.

Заключение. Из результатов следует, что вирусы H7N2 обладают потенциалом адаптации к млекопитающим. Возрастание вирулентности, скорее всего, обусловлено адаптационной мутацией *E627K* в PB2 и, возможно, мутациями в HA.

***Ключевые слова*:** *вирус гриппа подтипа H7N2, адаптация к млекопитающим, адаптационные мутации*

Этическое утверждение. Авторы подтверждают соблюдение институциональных и национальных стандартов по использованию лабораторных животных в соответствии с «Consensus Author Guidelines for Animal Use» (IAVES, 23.07.2010). Протокол исследования одобрен Комитетом по этике Национального исследовательского центра эпидемиологии и микробиологии имени почётного академика Н.Ф. Гамалеи (протокол № 12 от 28.06.2021).

Благодарность. Авторы благодарят сотрудников лаборатории анализа геномов НИЦЭМ им. Н.Ф. Гамалеи О.Л. Воронину, Е.И. Аксенову, М.С. Кунда, Н.Н. Рыжову за полногеномное секвенирование вирусов на платформе «Illumina».

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.

Для цитирования: Ляшко А.В., Руднева И.А., Щербинин Д.Н., Ломакина Н.Ф., Трещалина А.А., Куприянова И.М., Гамбарян А.С., Тимофеева Е.Б., Шилов А.А., Садыкова Г.К., Прилипов А.Г., Тимофеев Б.И., Шмаров М.М., Рязанова Е.Л., Тимофеева Т.А. Потенциал адаптации к млекопитающим вирусов гриппа птиц H7N2 североамериканской линии. *Журнал микробиологии, эпидемиологии и иммунобиологии.* 2023;100(6):442–453.
DOI: https://doi.org/10.36233/0372-9311-395
EDN: https://www.elibrary.ru/yphkuy

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Introduction

Subtype H7 influenza A viruses are associated with severe epizootics of avian influenza in poultry, predominantly of the order *Galliformes*. H7 influenza viruses are divided into low-pathogenic (non-pathogenic) and highly pathogenic viruses based on their virulence properties [1]. Low-pathogenic viruses circulate asymptomatically among domestic and wild aquatic and semi-aquatic birds [2, 3]. Highly pathogenic viruses are mainly isolated from domestic birds, in which they cause disease with characteristic signs and are fatal [4]. During epizootics in affected regions, highly pathogenic H7 viruses have also been detected in various species of synanthropic and wild birds [5, 6].

Cases of human infection with H7 virus were recorded in the twentieth century in individuals that were in close contact with infected birds. In 2003, the first mass infection was observed in 82 poultry farm workers during the epizootic of highly pathogenic influenza subtype H7N7 in the Netherlands. The infection manifested as conjunctivitis and/or mild influenza-like illness, and in 1 case the disease was fatal [7].

Between 2013 and 2017, 5 waves of H7N9 influenza epidemics affected China. Both low- and high-pathogenic avian influenza viruses caused the epidemics. During the entire period, 1,568 sick people were registered, of whom 616 died. The source of human infection were live poultry markets and backyard poultry farms, where low-pathogenic H7 viruses had been asymptomatically present since 2013 [3, 6]. In 2016, a highly pathogenic H7 virus emerged in southern China (Guangdong Province) that differed dramatically in properties and clinical symptoms from those previously present and evolved from a precursor of one of the numerous lineages of the low-pathogenic H7 virus that had been circulating in China since 2013 [6, 8-10].

Fatal severe pulmonary illnesses in humans have raised the concerns of the World Health Organization (WHO) about the potential threat of a new H7 subtype virus pandemic. A program to counteract a potential pandemic was developed[1], which drove scientists from different countries to study the features of avian influenza viruses of subtype H7.

Phylogenetically, all H7 subtype viruses are divided into Western Hemisphere viruses, Eastern Hemisphere viruses and the equine influenza virus lineage. Among the viruses of the Eastern Hemisphere, the Australian virus lineage is the one that stands out. The remaining isolates belong to the well-studied Eurasian lineage and form numerous branches on the evolutionary tree reflecting their geographical distribution [11]. H7 viruses of the Western Hemisphere are divided into three large clusters and several clades, based on HA sequence analysis [12]. The virus researched in the present study belongs to clade II-2 of the North American lineage.

Low-pathogenic H7 viruses cause asymptomatic infection in wild waterfowl. Their introduction into domestic bird populations may remain undetected for a long time if the virus does not cause clinical symptoms [2, 4, 10]. Circulation among domestic birds (part of the order *Galliformes*) promotes the accumulation of mutations that lead to changes in virulence and the formation of highly pathogenic viruses [4, 8, 10, 13].

During the years 1959–2019, 27 independent events of origin of highly pathogenic H7 influenza viruses from a low-pathogenic precursor were observed in different parts of the world [13].

The leading role in the evolution and pathogenicity of H7 influenza viruses is played by the surface protein hemagglutinin (HA), which contains the main antigenic determinants of the virus. At the stage of virus entry into the cell, HA allows binding to cellular receptors [14] and mediates the fusion of the viral and cellular membranes [15]. The determinant of pathogenicity of H7 viruses is the structure of the proteolytic cleavage site of the HA precursor: in nonpathogenic strains there is a single amino acid residue of arginine (R), while in highly pathogenic strains there is an insertion of several basic amino acids [1, 13].

The host specificity of influenza viruses is determined by their ability to recognize receptors characteristic of a particular host species. Avian receptors are sialoglycosides with the alpha2-3 binding type, and human (possibly other mammalian) receptors are sialoglycosides with the alpha2-6 binding type. The structure of the HA receptor-binding pocket of all influenza viruses is formed by three constituent elements (in H3 numbering): loop-130 (a.a. 134–138), loop-190 (a.a. 188–194) and loop-220 (a.a. 221–228). Mutations in these HA regions resulted in virus adaptation to humans and the formation of pandemic strains of H1N1 (*E190D/G225D*) and H2N2/H3N2 (*Q226L* and *G228S*) subtypes [14, 16].

For interspecies host transmission, influenza viruses must have the ability to recognize the receptors of the new host and adapt to use host cellular factors that the virus requires for successful reproduction. Mutations that facilitate adaptation to a new host primarily affect the HA and neuraminidase (NA) surface proteins, polymerase complex proteins, and the nonstructural NS1 protein. The NS1 protein inhibits host antiviral defense signaling and suppresses the expression of its proteins. NS1 performs multiple functions through interactions with proteins, RNA and other host cell factors, which differ between hosts. Differences in the primary structure of NS1 in specialized viruses of different host species reflect the high plasticity of this protein,

[1] The PIP Framework's Partnership Contribution (PC) High-Level Implementation Plan II 2018–2023 (HLIP-II). URL: https://www.who.int/publications/i/item/pip-pc-preparedness-high-level-implementation-plan-ii-2018-2023

which plays an important role in overcoming the interspecies barrier and adapting the virus to a new host [17, 18]. A virus adapted to new conditions must not only multiply in a new organism, but also be transmitted from one individual to another, i.e. it must be highly contagious. So far, no proven cases of human-to-human transmission of H7Nx viruses have been recorded[2], infection occurred through close contact with the source of infection, mainly with infected poultry.

In 1996, low-pathogenic H7 viruses with deletion of 8 amino acids (p. 221–228 in the H3 numbering) in the HA were detected in live poultry markets in the northeastern United States – they lacked loop-220 in the receptor-binding site region [2]. A North American virus lineage with the deletion (clade II-2) circulated in the northeastern United States from 1996 to 2006 in poultry [12]. The viruses had a HA cleavage site of a low virulence phenotype [2] and had a dual receptor specificity, i.e., along with high affinity for avian-type receptors (alpha2-3), they showed moderate binding to human-specific receptors (alpha2-6) [11, 16, 19]. Two arginine residues, R220 and R229, which are adjacent due to the deletion in positions 221–228 (in H3 numbering), play an essential role in preserving the tertiary structure of HA and providing dual receptor specificity, as shown by a study of the North American A/New York/107/2004 (H7N2) virus (clade II-2) isolated from humans [16].

The capability of H7N2 viruses to recognize human-type receptors indicates an increased potential to overcome the interspecies barrier and adapt to humans or other mammalian species.

During the circulation of H7N2 subtype viruses (1994–2006), two laboratory-confirmed human infection cases with these viruses were reported in the northeastern region of the United States. More recently, from December 2016 to February 2017, an outbreak of respiratory infection among domestic cats was observed in animal shelters in New York City. The cause of the illness in about 500 cats turned out to be a low-pathogenic H7N2 influenza virus with deletion in HA. An influenza-like infection was observed in a veterinarian who had close contact with an infected cat. Sequencing of 6 isolates from cats and 1 isolate from human showed that all 8 segments of the feline viruses were phylogenetically similar to low-pathogenic chicken viruses circulating in New York in the late 1990s and early 2000s. The viruses from cats and human were nearly identical and lacked mammalian adaptation mutations [20, 21].

The possibility of crossing the interspecies barrier by H7N2 viruses with deletion of loop-220 in HA prompted us to investigate the mechanism of adaptation of these viruses to mammals on the example of one of the representatives of this lineage — virus A/chicken/New Jersey/294598-12/2004.

The objective of this study was to elucidate the mechanism of adaptation to mammals of North American H7N2 influenza viruses with deletion in HA.

Materials and Methods

The A/chicken/NJ/294508-12/2004 (H7N2) virus (GenBank EU743253-EU743260) maintained in chicken embryos (CE) was provided by Dr A.I. Klimov, CDC USA (CDC-2004708596). Cloning of the virus by limiting dilutions in CE resulted in the A/chicken/New Jersey/294598-12/2004 (ch/NJ) variant, which was used in the present study as the original virus.

Adaptation of the virus to propagation in the lungs of white outbred mice was performed as described previously [25]. 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). Animal studies were approved by the Ethics Committee of the N.F. Gamaleya National Research Center for Epidemiology and Microbiology (Protocol No. 12 of 28.06.2021).

The hemagglutination reaction (HGA) was performed according to the generally accepted method [1]. The 50% embryonic infectious dose (EID_{50}/ml) of influenza viruses was determined as described in [25], each virus dilution was used for inoculation of 5 CEs. The mean EID_{50}/ml for each virus was calculated using the method developed by L.J. Reed and H. Muench [22].

The 50% lethal dose (LD_{50}) of influenza virus was determined in BALB/c mice according to [25], 6 mice per group were used for each virus dilution. The value of LD_{50}/ml was calculated using Kerber's method modified by Ashmarin [23].

Sequencing on the MiSeq platform (Illumina) was performed by O.L. Voronina, E.I. Aksenova, M.S. Kunda, and N.N. Ryzhova (GenBank, MN400380-MN400395) of the Laboratory of Genome Analysis of the N.F. Gamaleya NRCEM. Libraries were prepared according to [24], sequencing was performed on the MiSeq platform (Illumina) using the 600-cycle MiSeq Reagent Kit v3 cartridge. The *de novo* and reference A/chicken/NJ/294508-12/2004(H7N2) (EU743253-EU743260, GenBank) genomes were assembled using the CLC Genomics Workbench 10.0 program.

Sequencing by the Sanger method was performed as described previously in [25]. The National Center for Biotechnology Information[3] and Influenza Research Database[4] (IRD) were used for structural and functional analysis of nucleotide and amino acid sequences.

[2] Avian influenza A (H7N9) virus outbreak. URL: https://www.who.int/emergencies/situations/avian-influenza-a-(h7n9)-virus-outbreak

[3] URL: https://www.ncbi.nlm.nih.gov

[4] URL: https://www.bv-brc.org

DOI: https://doi.org/10.36233/0372-9311-395

The thermostability of influenza virus hemagglutinin was determined as described previously in [25]. The inactivation temperature was considered to be the temperature at which, after 40 min of virus incubation, the HA titer decreased by $6\log_2$.

The pH value of HA activation was determined by the erythrocyte hemolysis method [25], which is based on the ability of HA to agglutinate erythrocytes at neutral pH values and cause their hemolysis at low values due to a conformational change in HA.

The affinity of viruses to cell receptor analogues was determined by interaction with sialooligosaccharides conjugated with biotin-labelled high-molecular-weight polyacrylamide [14]. The sialooligosaccharides used were Neu5Acα2-3Galβ1-4GlcNAcβ (3'SLN) and Neu5Acα2-6Galβ1-4GlcNAcβ (6'SLN). Results were expressed as dissociation constant calculated in sialic acid nanomoles (K_{diss}, nM SA).

Statistical processing of the data was performed using the parametric Student's t-test (activation pH and thermostability of HA), non-parametric Friedman's criterion (ANOVA) and Mann-Whitney (virus titer) at a critical significance level of $p \leq 0.05$. MS Office Excel 2016 and Statistica 8.0 programs were used to perform the corresponding calculations. The obtained results are presented as the arithmetic mean (activation pH and thermostability of HA) or geometric mean (virus titer) with standard deviation.

Results

Adaptation to mice

Adaptation of avian influenza A/chicken/NJ/294598-12/2004 (ch/NJ) virus to reproduction in the lungs of mice was carried out by serial passages through the lungs. After 10 passages, a variant was obtained that caused death of mice with extensive hemorrhagic lung lesions. The obtained variant was cloned once by the method of limiting dilutions in chicken embryos to obtain a homogeneous viral population, named A/chicken/NJ/294598-12MA/2004 (MA/NJ) and deposited in the State Virus Collection (division of the Ivanovsky Institute of Virology of the N.F. Gamaleya NRCEM) under the number 2890.

Phenotypic properties of viruses

The original cloned ch/NJ variant accumulated in the EC at a titer of 9.6 $lgEID_{50}$, while the MA/NJ variant adapted to mice had a similar titer of 9.7 $lgEID_{50}$.

The original ch/NJ and mouse-adapted MA/NJ variants were examined for pathogenicity to mice, receptor specificity, virus thermostability and HA activation pH value (**Table 1**).

The original virus had an avirulent phenotype for mice: mice remained alive after intranasal infection with extremely high virus doses of 8.6 and 9.6 $lgEID_{50}$ per mouse. In contrast, the adapted MA/NJ variant at lower doses of 4.7 $lgEID_{50}$ per mouse caused death of 100% of the animals with characteristic changes in the lungs.

The increased virulence of MA/NJ was accompanied by an increase in the pH value of HA activation by 0.4 units compared to the original ch/NJ virus (Table 1).

When receptor specificity was determined, both virus variants bound to fetuin, which is characteristic of all influenza viruses. Their affinity for the avian receptor analogue 3'SLN was slightly higher than for the human receptor analogue 6'SLN; the adapted MA/NJ variant had a greater decrease in affinity for the human receptor than the original ch/NJ virus.

Sequencing

Sequencing on the Illumina platform yielded ambiguous results for some positions in segments 4 and 8, so additional sequencing was performed using the Sanger method. The results of sequencing by different methods coincided (**Table 2**). The original ch/NJ virus was found to be a heterogeneous population with polymorphisms in segments 4 and 8. Sequential passages in mouse lungs yielded a homogeneous virus population with fixation of amino acids that were present as minor amino acid variants in the original virus. The exception was position 125/133 in HA, where heterogeneity (F < L) was observed in the MA/NJ variant, which was absent in the original virus. In the MA/NJ variant, amino acid substitutions were detected in 5 proteins: 1 each in PB2, NA, NEP, 4 in HA, and 6 in NS1.

Table 1. Phenotypic properties of the original (ch/NJ) and mouse-adapted (MA/NJ) variants of the virus A/chicken/NJ/294598-12/2004 (H7N2)

Virus	Virulence*	Thermostability, ºC	HA activation pH values#	Affinity for cell receptor analogues, K_{diss}, nm SA**		
				Fet-HRP	3'SLN	6'SLN#
ch/NJ	> 9,6 (avirulent)	62,3 ± 0,2	5,2 ± 0,1	200 ± 100	100 ± 50	200 ± 50
MA/NJ	4,0 ± 0,2	62,3 ± 0,2	5,6 ± 0,1	200 ± 100	100 ± 50	500 ± 50

Note. The average values of three independent experiments are presented. # $p \leq 0.05$ (Student's criterion).
* Virulence for mice is represented as $\log_{10}$ of EID_{50} in one unit of LD_{50}. A lower value corresponds to a higher pathogenicity.
** The results of titration with peroxidase-labeled fetuin conjugate (Fet-HRP) and biotinylated polymers 3'SLN (Neu5Acα2-3Galβ1-4GlcNAcβ) and 6'SLN (6'SLN — Neu5Acα2-6Galβ1-4GlcNAcβ) are presented as a dissociation constant expressed in nanomoles of sialic acid. A higher value corresponds to a lower affinity for cellular receptor analogues.

Table 2. Amino acid differences in the original (ch/NJ) and mouse-adapted (MA/NJ) variants of the A/chicken/New Jersey/294598-12/2004 (H7N2) virus

Protein	Amino acid position	ch/NJ[2]	MA/NJ
PB2	627	E	K
HA[1]	125/133	F	**L** > F
HA	156/164	**D** > N	N
HA	198/207	G	E
HA	328/330	K	T
NA	127	K	N
NS1	73	**N** > T	T
NS1	114	**S** > G	G
NS1	118	**K** > R	R
NS1	171	**G** > A	A
NS1	214	**F** > L	L
NS1	224	R = G	R
NEP	14	**E** > Q	Q

Note. [1]The numbering of HA corresponds to H3/H7. Numbering is given for the mature HA/H3 protein of strain A/Aichi/2/68, and for HA/H7 according to the sequence ACF25499 (GenBank).
[2] The ratio of alternative amino acids. The predominant amino acid is in bold font.

Discussion

Analysis of amino acid substitutions in viral proteins

The *E627K* substitution in the **PB2** polymerase protein is considered to be a mammalian adaptation. It increases the polymerase activity of viral polymerase and increases the pathogenicity of the virus for mammals [26-28]. It can be assumed that the increase in virulence for mice of the MA/NJ variant is because of this mutation.

Neuraminidase of both variants has deletion of 16 amino acids (p. 56-71)[5] in the stem part of the molecule. The *K127N* (143) substitution (numbering according to QEL43992, GenBank) in the neuraminidase of subtype N2 is located in the head of the molecule protruding on the virion surface. It is part of experimentally determined epitopes that are polymorphic at this position according to IRD data. Amino acid 127K (143K) makes contact with amino acid 450F (466F) of the neighboring NA homotetramer chain. Replacement of amino acid 127K with an extended side chain with 127N with a short side chain could theoretically affect the contact of neighboring chains. For North American H7N2 viruses, this substitution is unique. However, in different years, 127N was present in 146 natural isolates of exclusively avian (mainly chicken) viruses of other subtypes – H9N2 and H6N2 in China, non-pathogenic H5N2 viruses in the USA and Mexico.

In **HA** belonging to clade II-2 of the North American virus with deletion, adaptation to mice resulted in 4 substitutions: F125/133L, D156/164N, G198/207E, K328/330T (Table 2, **Table 3**), which had little effect on the receptor specificity of the MA/NJ variant (Table 1) and did not alter the thermostability of HA.

In MA/NJ, the K328/330T mutation located in the HA cleavage site at position –2 was observed. The cleavage site structure of ch/NJ and MA/NJ viruses, EKPKK**K**R↓G and EKPK**T**R↓G, corresponds to the apathogenic phenotype. According to IRD data, both structures are found in H7 viruses from different avian species in North America.

The IRD database has 1107 complete HA sequences of H7 subtype viruses isolated in North America between 1996 and 2022, among them 230 that have deletion of loop 220 in the HA (Table 3). Viruses with loop-220 deletion are represented by isolates from 1996–2006 and 2016. The viruses were isolated from the environment, from chickens, some poultry species, wild duck, 2 strains were isolated from humans — one in 2003 and another in 2016, when 6 strains were isolated from cats. We analyzed the variability of amino acids at positions where substitutions were found in MA/NJ virus. Table 3 shows that only 2 substitutions are noteworthy in the mouse-adapted variant, the L-dominated F/**L** polymorphism at positions F125/133L and G198/207E, since the other two positions (156/164 and 328/330) represent one of the alternative amino acids present in natural isolates.

In the original virus, F is located at position 125/133, as in natural H7 viruses. In MA/NJ, an F/L polymorphism with a predominance of L is observed. The presence of L at this position is very rare. Among natural H7 viruses with this substitution, 13 isolates were found in different parts of the world, among which only 2 were found in the USA (A/chicken/New York/Sg-00307/1998, H7N2 and A/American green-winged teal/Illinois/10OS4014/2010, H7N3). This rare occurrence indicates low competitive fitness of HA with this mutation among avian influenza viruses.

Analysis of H7N2 viruses of the North American lineage isolated in 1999-2006 indicates that all these viruses have an N at position 156/164. The observed polymorphism in the original ch/NJ variant with a predominance of D at this position is most likely a special case.

The D156/164N mutation in the mouse-adapted virus replaces a negatively charged amino acid with a neutral one. The G198/207E substitution with a negative charge shift is also rare among the group of viruses studied. It is possible that the two charge-shifting mutations at positions D156/164N and G198/207E located on the HA surface may affect its conformation depending on the pH of the medium.

[5] In this subsection, the NA numbering of the reference strain is given in parentheses A/Tokyo/3/1967(H2N2), AAO46245, GenBank; PDB: 1INH.

DOI: https://doi.org/10.36233/0372-9311-395

Table 3. Mutations in HA/H7 of a mouse-adapted virus and alternative amino acids in these positions in natural isolates isolated in North America in 1996-2022

Position H3/H7	Amino acid, virus variant		The number of H7 viruses with the indicated amino acid		Functional domain	Reference
	ch/NJ	MA/NJ	viruses of different hosts, *n* = 1107	HA with 220 loop deletion, *n* = 230*		
125/133	F	F/L	L = 3, F = 1104	F = 230	Antigenic site	[9]
156/164	N/D	N	N = 1094, D = 4, K = 1, S = 8	N = 227, S = 3	Antigenic site	[9]
198/207	G	E	E = 5, G = 1102	E = 1, G = 229	Antigenic site	[9]
328/330	K	T	A = 8, K = 47, P = 211, T = 824	K = 46, P = 184	Cleavage site	[2]

Note. *The sampling did not include laboratory obtained strains, for example, escape mutants.

Changes in NS1 and NEP proteins

In the adaptation process of the chicken ch/NJ virus to the mammalian organism, the segment 8, in which a polymorphism at 9 positions in nucleotide sequence was initially observed, underwent the greatest changes. In the adapted variant, the nucleotides present in the original variant in a smaller proportion became fixed. Among these, 6 nucleotides resulted in the substitution of 6 amino acids in NS1 protein and 1 substitution in NEP (Table 2, **Table 4**).

It should be noted that in natural viruses, the NS gene is represented by two alleles: allele A is present in all mammalian influenza viruses and certain avian influenza viruses, while allele B is characteristic of avian influenza viruses only [29]. The studied A/chicken/New Jersey/294598-12/2004(H7N2) virus contains the B allele. This likely explains the high variability of the NS gene of the mouse-adapted MA/NJ variant.

The lineage of North American viruses of subtype H7 with deletion of loop-220 in HA, to which the ch/NJ virus we studied belongs, is represented by influenza viruses isolated from poultry. Among them, according to GenBank data, there is only one virus isolated from humans, A/New York/107/2003(H7N2), which is, in fact, a virus of avian origin. Comparison of the HA and NS1 protein sequences of this virus with the ch/NJ and MA/NJ variants studied by us revealed no matching mutations in HA, while NS1 contains three mutations, *N73T*, *G171A*, and *F214L*, identical to the mouse-adapted variant.

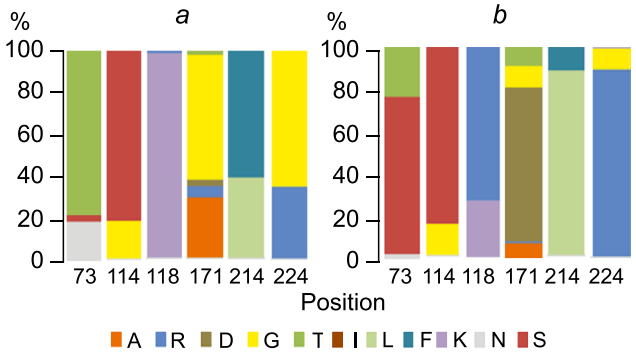
We analyzed NS1 protein variability among H7 influenza viruses of all NA (H7Nx) subtypes that were present in birds in North America from 1994–2022, as well as for H7N2 subtype viruses for 1994–2007, which included the circulating years of the virus lineage with deletion in the NA and its precursors (Table 4; **Figure**).

At positions 73, 114, 118, 171, 214 and 224, where MA/NJ variant substitutions occurred, there are no significant differences in the set of varying amino acids between these samples, only the percentage of specific amino acids differs (Figure). The observed difference in the distribution of amino acids in NS1 between two samples of avian influenza viruses of subtype H7, differing in subtype NA, may indicate the isolation of H7N2 viruses circulating in 1994–2007 and the existence of some relationship (linkage) between segments 4, 6, and 8 encoding HA, NA, and NS proteins in them.

Table 4. NS1 substitutions in the mouse-adapted MA/NJ variant, and their variability among North American avian H7 viruses

Mutation[1]	Amino acid variability		Domen	Location	Function[2]
	H7N2, 1994–2007 (*n* = 263)	H7Nx, 1994–2022 (*n* = 908)			
N73T	N = 47, S = 7, T = 138	A = 1, N = 23, F = 1, P = 1, S = 656, T = 226	RNA binding domain	1–73	Binding with several RNA species, including dsRNA. Interactions with host proteins RIG-I, PABPI, and importin-alpha
S114G	G = 44, P = 1, S = 123	G = 133, P = 1, S = 774	Effector domain	87–203	Mediated interaction with several host cellular proteins; nuclear-cytoplasmic transport of mature mRNAs; stabilization of the N-terminal domain (1-73) due to RNA interaction
K118R	R = 7, K = 161	R = 658, K = 250			
G171A	A = 60, R = 5, D = 7, G = 85, T = 11	A = 61, R = 5, N = 2, D = 648, G = 92, T = 100			
F214L	I = 1, L = 75, F = 91, X = 1	I = 1, L = 790, F = 116, X = 1	C-terminal domain	204–230	Contains signaling motifs for phosphorylation (CDK/ERK), Crk/CrkL SH3 binding, PDZ ligand and NoLS/NLS2
G224R	R = 77, G = 90	R = 807, G = 94, K = 5, S = 1			

Note. [1]Mutation in the mouse-adapted variant (original/adapted variant). [2]Based on data from [17, 18].



Positions in NS1 in which mutations occurred in the mouse-adapted MA/NJ virus variant and the variability of amino acids in them (ordinate) among avian influenza viruses subtype H7 isolated in different years in North America.

a — H7N2 subtype viruses isolated in 1994–2006;
b — H7Nx subtype viruses isolated in 1994–2022.

All NS1 positions that underwent substitutions in the MA/NJ variant show variability (Table 4). In both the original ch/NJ and MA/NJ variants, one of the alternative amino acids is present at these positions. The detected substitutions, according to IRD data, are part of experimentally identified short linear epitopes and are also located in structurally functional domains that make multiple contacts with host factors [17, 18].

In the NEP protein adapted to mice, one *E14Q* substitution occurred in the NES signaling region (positions 12-21, nuclear export signal), which binds to the cellular transport protein CRM1 and, with its participation, ensures the exit of viral nucleoprotein complexes from the nucleus [30]. The NES signal sequence is hydrophobic (leucine- or methionine-rich region), in which the 14M substitution does not significantly affect the structure of the signaling region. Nevertheless, in WSN virus, amino acid substitution *M14Y* at this position resulted in delayed export of viral ribonucleinoprotein complexes (vRNPs) from the nucleus, reduced growth properties of the virus and its attenuation in mice [30]. The structure of NES in the viruses we studied differs by only one position from the WSN virus: *M14E* in the original ch/NJ virus and *M14Q* in MA/NJ. In our case, the replacement of an amino acid with a charge change (*E14Q*) — acidic to neutral polar hydrophilic — could hypothetically influence the functioning of NES signaling.

Adaptive potential

There are only 8 H7N2 clade II-2 influenza viruses isolated from mammals for which the structure of the complete genome has been determined: these are 2 isolates from humans (A/NY/107/2003 and A/NY/108/2016) and 6 from cats [20, 21, 31]. The genome of the A/NY/108/2016 virus isolated from a human in 2016 was almost identical to the genome of the virus isolated from the cat with whom the infected person had a contact [20]. We compared the HA and NS proteins of these viruses, as well as the MA/NJ virus with their closely related chicken virus A/chicken/NJ/294508-12/2004 to find common differences in isolates from mammals. There are no overlapping substitutions in HA between MA/NJ and mammalian viruses relative to chicken virus. For the HA protein, the similarity (divergence) to chicken virus for MA/NJ was 99.5% (0.5%), 96.6% (3.5%) for human A/NY/107/2003 virus, and 94% (6.2%) for feline and human A/NY/108/2016 viruses.

For the NS1 protein, the similarity (divergence) to chicken virus for MA/NJ was 97.4% (2.7%), 97.8% (2.2%) for human NY/107/2003 virus, 92.6% (7.3%) for 5 feline viruses (WDL) from the same shelter, and 92.2% (7.8%) for cat virus A/feline/New York/16-040082-1/2016 and human contact virus (A/NY/108/2016). In NS1, among the substitutions relative to chicken ch/NJ virus, five (*N73T*, *S114G*, *G171A*, *F214L* and *G224R*) matched in mouse-adapted MA/NJ and feline viruses, of which three (*N73T*, *G171A*, *F214L*) were identical to substitutions in the human A/New York/107/2003(H7N2) virus.

Only one matching substitution, *E14Q*, is present in NEP. In feline viruses, aside from that substitution, there are 4 other differences from the chicken virus.

As for identical mutations in mammalian H7N2 viruses (in NS1 — *N73T*, *G171A*, *F214L*, in NEP — *E14Q*), the substitution occurred on the predominant or less common amino acid among alternative ones present in natural avian isolates. This does not allow them to be recognized as adaptations without further studies.

It is should be noted that H7N2 viruses isolated from humans and cats lacked the *E627K* mutation in PB2 [20, 21], which is considered to be adaptive to mammals [26–28]. According to different authors, the appearance of the *E627K* mutation during adaptation of avian influenza viruses to mice was accompanied by an increase in virulence of the virus [27, 28], as in the current case with MA/NJ. In addition to the mutation in PB2, the change in MA/NJ virulence could be affected by substitutions in HA, which contributed to an increase in the pH value of HA activation [10, 25].

Conclusion

Our studies on the adaptation of the low pathogenic H7N2 chicken influenza virus to mice, as well as the information that viruses of this lineage were able to cross the species barrier and cause an outbreak of respiratory infection in cats 10 years after their last detection in natural conditions [20, 21], indicate the presence of adaptation potential to mammals in H7N2 viruses of the North American lineage with a deletion of loop-220 in HA. Adaptation of these viruses to different mammalian species seems to have its own peculiarities, and it takes some time of circulation in a new host for the virus to acquire mutations capable of causing the clinical manifestation of infection. As the source of infection in

cats has not been identified, the question remains as to how H7N2 viruses have managed to remain undetected and out of sight of veterinary services for more than 10 years.

СПИСОК ИСТОЧНИКОВ | REFERENCES

1. World Organization for Animal Health (WOAH). Avian influenza (including infection with high pathogenicity avian influenza viruses). Chapter 3.3.4. In: *OIE Terrestrial Manual.* Paris;2021.
2. Suarez D.L., Garcia M., Latimer J., et al. Phylogenetic analysis of H7 avian influenza viruses isolated from the live bird markets of the Northeast United States. *J. Virol.* 1999;73(5):3567–73. DOI: https://doi.org/10.1128/JVI.73.5.3567-3573.1999
3. Shi J., Deng G., Ma S., et al. Rapid evolution of H7N9 highly pathogenic viruses that emerged in China in 2017. *Cell Host Microbe*. 2018;24(4):558–68.e7. DOI: https://doi.org/10.1016/j.chom.2018.08.006
4. Capua I., Cattoli G., Terregino C., Marangon S. Avian influenza in Italy 1997–2006. In: Klenk H.D., Matrosovich M.N., Stech J., eds. *Avian Influenza. Monographs in Virology. Volume 27.* Basel;2008:59–70. DOI: https://doi.org/10.1159/000151608
5. Yao Y., Zhang T., Yang W., et al. Avian influenza A (H7N9) virus in a wild land bird in central China, late 2015. *Virol Sin.* 2018;33(1):96–9. DOI: https://doi.org/10.1007/s12250-018-0001-x
6. Li C., Chen H. H7N9 influenza virus in China. *Cold Spring Harb. Perspect. Med.* 2021;11(8):a038349. DOI: https://doi.org/10.1101/cshperspect.a038349
7. Fouchier R.A., Schneeberger P.M., Rozendaal F.W., et al. Avian influenza A virus (H7N7) associated with human conjunctivitis and a fatal case of acute respiratory distress syndrome. *Proc. Natl Acad. Sci. USA*. 2004;101(5):1356–61. DOI: https://doi.org/10.1073/pnas.0308352100
8. Lu J., Raghwani J., Pryce R., et al. Molecular evolution, diversity, and adaptation of influenza A(H7N9) viruses in China. *Emerg. Infect. Dis.* 2018;24(10):1795–805. DOI: https://doi.org/10.3201/eid2410.171063
9. Yang H., Carney P.J., Chang J.C., et al. Structural and molecular characterization of the hemagglutinin from the fifth-epidemic-wave A(H7N9) influenza viruses. *J. Virol.* 2018; 92(16):e00375-18. DOI: https://doi.org/10.1128/JVI.00375-18
10. Shi J., Deng G., Kong H., et al. H7N9 virulent mutants detected in chickens in China pose an increased threat to humans. *Cell Res*. 2017;27(12):1409–21. DOI: https://doi.org/10.1038/cr.2017.129
11. Gambaryan A.S., Matrosovich T.Y., Philipp J., et al. Receptor-binding Profiles of H7 subtype influenza viruses in different host species. *J. Virol*. 2012;86(8):4370–9. DOI: https://doi.org/10.1128/JVI.06959-11
12. Xu Y., Bailey E., Spackman E., et al. Limited antigenic diversity in contemporary H7 avian-origin influenza A viruses from North America. *Sci. Rep.* 2016;6:20688. DOI: https://doi.org/10.1038/srep20688
13. Lee D.H., Criado M.F., Swayne D.E. Pathobiological origins and evolutionary history of highly pathogenic avian influenza viruses. *Cold Spring Harb. Perspect. Med.* 2020;11(2):a038679. DOI: https://doi.org/10.1101/cshperspect.a038679
14. Matrosovich M., Tuzikov A., Bovin N., et al. Early alterations of the receptor-binding properties of H1, H2, and H3 avian influenza virus hemagglutinins after their introduction into mammals. *J. Virol*. 2000;74(18):8502–12. DOI: https://doi.org/10.1128/jvi.74.18.8502-8512.2000
15. Hamilton B.S., Whittaker G.R., Daniel S. Influenza virus-mediated membrane fusion: determinants of hemagglutinin fusogenic activity and experimental approaches for assessing virus fusion. *Viruses.* 2012;4(7):1144–68. DOI: https://doi.org/10.3390/v4071144
16. Yang H., Chen L.M., Carney P.J., et al. Structures of receptor complexes of a North American H7N2 influenza hemagglutinin with a loop deletion in the receptor binding site. *PLoS Pathog.* 2010;6(9):e1001081. DOI: https://doi.org/10.1371/journal.ppat.1001081
17. Васин А.В., Петрова-Бродская А.В., Плотникова М.А. и др. Эволюционная динамика структурных и функциональных доменов белка NS1 вирусов гриппа А человека. *Вопросы вирусологии*. 2017;62(6):246–58. Vasin A.V., Petrova-Brodskaya A.V., Plotnikova M.A., et al. Evolutionary dynamics of structural and functional domains of influenza A virus NS1 protein. *Problems of Virology.* 2017;62(6):246–58. DOI: https://doi.org/10.18821/0507-4088-2017-62-6-246-258 EDN: https://elibrary.ru/zuqetp
18. Evseev D., Magor K.E. Molecular evolution of the influenza A virus non-structural protein 1 in interspecies transmission and adaptation. *Front. Microbiol*. 2021;12:693204. DOI: https://doi.org/10.3389/fmicb.2021.693204
19. Gambaryan A.S., Tuzikov A.B., Pazynina G.V., et al. 6-sulfo sialyl Lewis X is the common receptor determinant recognized by H5, H6, H7 and H9 influenza viruses of terrestrial poultry. *Virol. J.* 2008;5:85. DOI: https://doi.org/10.1186/1743-422X-5-85
20. Marinova-Petkova A., Laplante J., Jang Y., et al. Avian influenza A(H7N2) virus in human exposed to sick cats, New York, USA, 2016. *Emerg. Infect. Dis*. 2017;23(12):2046–9. DOI: https://doi.org/10.3201/eid2312.170798
21. Hatta M., Zhong G., Gao Y., et al. Characterization of a feline influenza A(H7N2) virus. *Emerg. Infect. Dis*. 2018;24(1):75–86. DOI: https://doi.org/10.3201/eid2401.171240
22. Reed L.J., Muench H. A simple method of estimating fifty per cent endpoints. *Am. J. Epidemiol*. 1938;27(3):493–7.
23. Ашмарин И.П. Вычисление LD50 при малом числе подопытных животных. *Журнал микробиологии, эпидемиологии и иммунобиологии.* 1959;30(2):102–8. Ashmarin I.P. Calculation of LD50 with a small number of experimental animals. *Journal of Microbiology, Epidemiology and Immunobiology.* 1959;30(2):102–8.
24. Voronina O.L., Ryzhova N.N., Aksenova E.I., et al. Genetic features of highly pathogenic avian influenza viruses A(H5N8), isolated from the European part of the Russian Federation. *Infect. Genet. Evol.* 2018;63:144–50. DOI: https://doi.org/10.1016/j.meegid.2018.05.022
25. Тимофеева Т.А., Руднева И.А., Ломакина Н.Ф. и др. Мутации в геноме вируса гриппа птиц подтипов H1 и H5, ответственные за адаптацию к млекопитающим. *Независимые микробиологические исследования.* 2021;8(1):50–61. 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. *Microbiology Independent Research Journal.* 2021;8(1):50–61. DOI: https://doi.org/10.18527/2500-2236-2021-8-1-50-61 EDN: https://elibrary.ru/vsvzqt
26. Subbarao E.K., London W., Murphy B.R. A single amino acid in the PB2 gene of influenza A virus is a determinant of host range. *J. Virol.* 1993;67(4):1761–4. DOI: https://doi.org/10.1128/JVI.67.4.1761-1764.1993

27. Hatta M., Gao P., Halfmann P., Kawaoka Y. Molecular basis for high virulence of Hong Kong H5N1 influenza A viruses. *Science*. 2001;293(5536):1840–2.
DOI: https://doi.org/10.1126/science.1062882
28. Zhang H., Li X., Guo J., et al. The PB2 E627K mutation contributes to the high polymerase activity and enhanced replication of H7N9 influenza virus. *J. Gen. Virol*. 2014;95 (Pt. 4):779–86.
DOI: https://doi.org/10.1126/science.1062882
29. Suarez D.L., Perdue M.L. Multiple alignment comparison of the non-structural genes of influenza A viruses. *Virus Res.* 1998;54(1):59–69.
DOI: https://doi.org/10.1016/s0168-1702(98)00011-2
30. Iwatsuki-Horimoto K., Horimoto T., Fujii Y., Kawaoka Y. Generation of influenza A virus NS2 (NEP) mutants with an altered nuclear export signal sequence. *J. Virol*. 2004;78(18):10149–55.
DOI: https://doi.org/10.1128/JVI.78.18.10149-10155.2004
31. Ostrowsky B., Huang A., Terry W., et al. Low pathogenic avian influenza A (H7N2) virus infection in immunocompromised adult, New York, USA, 2003. *Emerg. Infect. Dis.* 2012; 18(7):1128–31.
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The article was submitted 28.09.2023;
accepted for publication 18.11.2023;
published 28.12.2023

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Статья поступила в редакцию 28.09.2023;
принята к публикации 18.11.2023;
опубликована 28.12.2023