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Analysis of marker substitutions in A/chicken/Astrakhan/2171-1/2020 H5N8 isolate of avian influenza virus recovered in the Astrakhan Oblast

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SUMMARY

At the end of 2020, a large-scale bird death was registered at one of the poultry farms in the Astrakhan region, the cause of which was avian influenza. Data on detection of the marker substitutions in viral proteins of avian influenza virus A/chicken/Astrakhan/2171-1/2020 isolate are presented in the paper. Type A H5N8 avian influenza virus was identified with complex PCR-based methods in the submitted samples. Hemagglutinin gene fragment sequencing identified REKRRKR/GLF, highly pathogenic avian influenza virus isolate-characteristic amino acid sequence of the hemagglutinin cleavage site. Phylogenetic analysis of nucleotide sequences of hemagglutinin gene segment (848–1105 bp ORF) allowed A/chicken/Astrakhan/2171-1/2020 H5N8 isolate to be classified to highly pathogenic avian influenza virus genetic clade 2.3.4.4. Comparative analysis of genome segments using available databases showed that A/chicken/Astrakhan/2171-1/2020 H5N8 virus related to A/H5 avian influenza virus isolates detected in the Russian Federation in 2016–2020. Analysis of the studied virus isolate hemagglutinin amino acid identified AIV-characteristic $G_{225}QRG_{228}$ amino acids in the receptor-binding domain of the protein enabling high-affinity binding to avian epithelial cell SA*a*-2,3-gal receptors. Single mutations, 70G in NEP protein and 13P in PB1 protein, out of the list of the reported influenza virus mutations affecting successful influenza virus replication in mammals were identified. No mutations affecting virus sensitivity to anti-viral medicines, rimantadin, amantadine, oseltamivir and zanamivir, were detected. The following mutations recognized as pathogenicity determinants in mice were found: 42S in the NS1 protein and 30D protein 215A in M1 protein.

Keywords: Avian influenza, H5N8, genetic analysis, amino acid substitutions.

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Анализ маркерных замен изолята вируса гриппа A/chicken/Astrakhan/2171-1/2020 H5N8, выделенного на территории Астраханской области

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РЕЗЮМЕ

В конце 2020 г. на одной из птицефабрик в Астраханской области была зарегистрирована массовая гибель птиц, причиной которой стал грипп птиц. В работе представлены данные по выявлению маркерных замен вирусных белков изолята вируса гриппа птиц A/chicken/Astrakhan/2171-1/2020. В результате комплекса исследований с использованием полимеразной цепной реакции в полученных пробах был идентифицирован вирус гриппа птиц типа А подтипа H5N8. Согласно результатам секвенирования участка гена гемагглютинина установлена аминокислотная последовательность сайта расщепления гемагглютинина REKRRKR/GLF, характерная для изолятов высокопатогенного гриппа птиц. Филогенетический анализ нуклеотидных последовательностей участка гена гемагглютинина (848–1105 н. п. открытой рамки считывания) позволил установить принадлежность изолята A/chicken/Astrakhan/2171-1/2020 H5N8 к генетической кладе 2.3.4.4 высокопатогенного вируса гриппа птиц. В результате сравнительного анализа геномных сегментов с использованием доступных баз данных установлено родство вируса A/chicken/Astrakhan/2171-1/2020 H5N8 с изолятами вируса гриппа A/H5, выявленными на территории Российской Федерации в 2016–2020 гг. Анализ аминокислотной последовательности вирусного гемагглютинина анализированного изолята выявил в рецептор-связывающем центре белка аминокислоты $G_{225}QRG_{228}$, характерные для вируса гриппа птиц и обеспечивающие повышенный аффинитет к рецепторам SA*α*-2,3-gal эпителиальных клеток птиц. Из числа описанных мутаций вируса гриппа, влияющих на успешную репродукцию его в организме млекопитающих, были выявлены единичные мутации 70G в белке NEP и 13P в белке PB1. Мутаций, влияющих на чувствительность вируса к противовирусным препаратам: римантадину, амантадину, осельтамивиру и занамивиру – не обнаружено. Выявлены мутации 42S в белке NS1 и 30D, 215A в белке M1, признанные детерминантами патогенности для мышей.

Ключевые слова: Грипп птиц, H5N8, генетический анализ, аминокислотные замены.

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INTRODUCTION

Avian influenza virus (AIV) is a dangerous highly contagious causative agent of respiratory illnesses in birds. Mortality in birds infected with H5 and H7 AI viruses reaches 100%.

Since 1996, highly pathogenic avian influenza (HPAI) caused by A/H5N1 AI virus has become epidemic in the South-Eastern Asian countries. The disease outbreaks occurred in 2005–2007 caused significant losses to the poultry industry of the Russian Federation. H5N8 AI virus was detected in a migratory duck in the Republic of Sakha in 2014. Then, H5N8 AI viruses have repeatedly caused outbreaks in poultry and wild bird populations in Russia as well as in Asian, African and European countries up to the present time. Epidemic HPAI situation in the Russian Federation aggravated in late 2016. In 2016–2017 H5N8 HPAI outbreaks were reported in poultry in the Rostov, Astrakhan, Samara, Moscow, Nizhny Novgorod Oblasts, Krasnodar Krai, Republic of Tatarstan, Mariy El, Kalmykia as well as in the Udmurt and Chechen Republics. The disease outbreaks caused great economic losses to Russian commercial establishments [1]. In 2018 H5N8 AI virus was detected in poultry in the Kursk, Oryol, Voronezh, Kostroma, Smolensk, Saratov, Samara, Ulyanovsk, Penza, Nizhny Novgorod, Rostov Oblasts, Udmurt Republic, Republic of Mariy-El, Chuvash Republic and Republic of Tatarstan [2].

In 2020, H5N8 AI virus widely spread across the European and Middle East countries as well as Russian Federation and Kazakhstan territories. Moreover, H5N5 AI virus was detected in the Omsk and Rostov Oblasts. In late 2020, H5N8 AI virus was detected in humans contacting to the diseased poultry on the poultry farm located in the Astrakhan Oblast (https://www.interfax.ru/russia/752017).

Human AI cases require further investigations including whole-genome sequencing followed by analysis of deduced amino acid sequence A/chicken/Astrakhan/2171-1/2020 for detection of possible markers of tropism and virulence for mammals.

MATERIALS AND METHODS

RNA extraction. RNA was extracted with RIBO-sorb kit (FBIS "Central Research Institute of Epidemiology" of the RF Federal Service for Customers' Rights Protection and Human Well-Being Surveillance, Russia; cat. No. K2-1-Et-100) according to the manufacturer's instructions.

Real time reverse transcription-polymerase chain reaction (rt RT-PCR). One-step rt RT-PCR was carried out with OneStep RT-PCR Kit (Qiagen, Netherlands; cat. No. 210212) using 25 мМ magnesium chloride solution (Promega, USA; supplied with the kit, cat. No. M8296) and a set of primers for M gene and HA, NA genes of H5N8 AIV. Reaction mix (25 µl) was prepared. The reaction mix contained 1× buffer for RT-PCR, 1.25 мМ

$MgCl_2$, 0.4 мМ dNTP, 0.4 pmol/µl of forward primer and 0.4 pmol/µl of reverse primer, 0.3 pmol/µl of fluorescent probe, 1 µl of reverse transcriptase and polymerase mix (Qiagen, Netherlands; cat. No. 210212), 5 µl of total RNA solution. Reverse transcription was performed at 50 °C for 30 min. The following temperature/time conditions were used for amplification: 95 °C – 10 min. (polymerase activation), then 40 runs, each consisting of three steps (95 °C – 10 sec., 55 °C – 35 sec., 72 °C – 10 sec.).

Reverse transcription-polymerase chain reaction (RT-PCR). Conventional RT-PCR was performed in one-step with OneStep RT-PCR Kit (Qiagen, Netherlands; cat. No. 210212) using 25 мМ magnesium chloride solution (Promega, USA; supplied with the kit, cat. No. M8296) and a set of primers for HA gene of H5 subtype AIV. Reaction mix (25 µl) was prepared. The reaction mix contained 1× buffer for RT-PCR, 1.25 мМ $MgCl_2$, 0.4 мМ dNTP, 0.4 pmol/µl of forward primer and 0.4 pmol/µl of reverse primer, 1 µl of reverse transcriptase and polymerase mix (Qiagen, Netherlands; cat. No. 210212), 5 µl of total RNA solution. Reverse transcription was performed at 50 °C for 30 min. Amplification was performed under the following temperature/time conditions: 95 °C – 10 min. (polymerase activation), then 40 runs, each consisting of three steps (95 °C – 30 sec., 58 °C – 60 sec., 68 °C – 120 sec.) and final elongation – for 7 min.

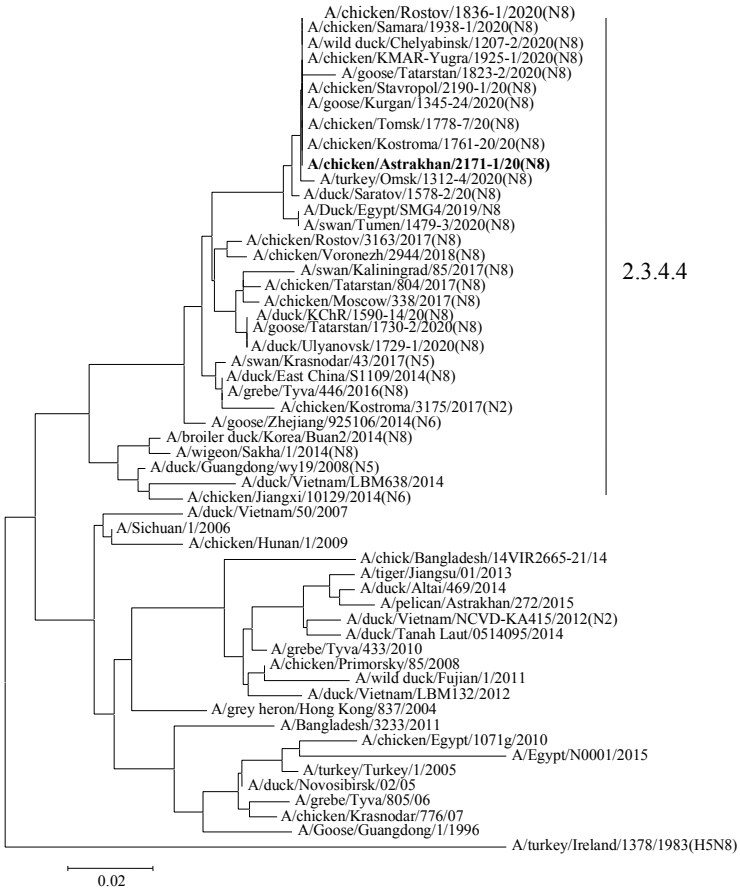

Fig. 1. Phylogenetic tree constructed using HA gene fragment sequence (848–1105 bp) of H5 HPAI virus isolates and strains

Рис. 1. Филогенетическое древо, построенное с помощью последовательностей фрагмента гена НА (848–1105 н. п.) изолятов и штаммов вируса ВПГП подтипа Н5

Sequencing. HA gene fragment nucleotide sequences were determined with automated ABI Prism 3100 sequencer using BigDye Terminator Cycle Sequencing kits (Applied Biosystems, USA) according to the manufacturer's instructions. Whole-genome sequencing was performed with MySeq analyzer (Illumina, USA) according to the manufacturer's instructions. Double stranded DNA synthesis was carried out with cDNA Synthesis System (Roche, Switzerland) according to the manufacturer's instructions. DNA libraries were prepared with commercial XT kit and Nextera XT Index Kit (Illumina, USA).

Nucleotide sequences. Nucleotide sequences of H5 subtype AIV isolates and strains published in the GenBank database, NCBI electronic source (www.ncbi.nlm.nih.gov/nucleotide), and the EpiFlu database (https://www.gisaid.org) were used.

Analysis of the nucleotide sequences and corresponding amino acid sequences was carried out with BioEdit software, version 7.0.5.3. The sequences were aligned with ClustalW multiple sequence alignment software. Phylogenetic tree was constructed with NJ algorithm using MEGA package, version 6.06.

RESULTS AND DISCUSSION

Mass mortality of poultry was reported on a poultry farm located in the Astrakhan Oblast in December 2020. Results of the tests carried out by local veterinary laboratory indicated the avian influenza virus presence in the tested samples. The samples were sent to the Reference Laboratory for Viral Avian Diseases of the FGBI "ARRIAH" for confirmation of the test results and further virus typing. Type A H5N8 AI virus was identified in the submitted samples with a complex PCR-based tests. Virus hemagglutinin cleavage site, REKRRKR/GLF, was identified based on analysis of deduced amino acid sequence. The obtained results allow detected virus to be identified as highly pathogenic avian influenza virus.

The isolated A/chicken/Astrakhan/2171-1/2020 virus was classified to genetic clade 2.3.4.4 based on phylogenetic analysis of its HA gene nucleotide sequence (Fig. 1). Diagnostic fragment of the hemagglutinin gene sequence (848–1105 bp of open reading frame – ORF) was used for the phylogenetic analysis.

Whole-genome sequencing was performed to identify marker substitutions indicative of A/chicken/Astrakhan/2171-1/2020 virus adaptation to mammals. Comparative and phylogenetic analyses showed high similarity of A/chicken/Astrakhan/2171-1/2020 virus to the vast majority of H5N8 AI virus isolates recovered in the Russian Federation in 2020 and described earlier [3]. No genomic segment reassortment events between A/chicken/Astrakhan/2171-1/2020 virus and the virus isolates of other types or H5 genetic clades were found.

HA protein amino acid sequence of A/chicken/Astrakhan/2171-1/2020 H5N8 isolate was analyzed. $G_{225}QRG_{228}$ amino acids (according to H3 subtype numbering) were detected in viral protein receptor-binding domain (Fig. 2).

According to the earlier studies, a group of $G_{225}QRG_{228}$ amino acids is characteristic of the viruses isolated from birds and targets to SA*α*-2,3-gal receptors [4].

According to the literature data, virus hemagglutinin contains marker amino acids that are located in the receptor-binding domain and conservative for the virus isolates recovered from birds but distinct from the relevant amino acids in the virus isolates recovered from mammals [4, 5].

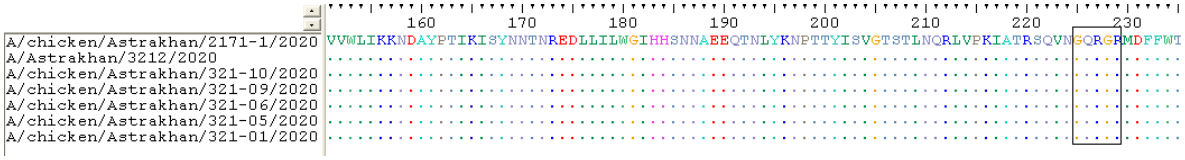

Fig. 2. Segment of deduced amino acid sequence of the virus HA receptor-binding domain

Рис. 2. Участок предсказанной аминокислотной последовательности рецептор-связывающего домена вирусного гемагглютинина

Table 1 shows HA protein amino acid residues related to receptor specificity characteristic of H5 AI viruses and human influenza virus.

For A/chicken/Astrakhan/2171-1/2020 isolate, AIV-characteristic amino acid residues are located in all above-mentioned positions (except for 159, 222, 227). Amino acid residues non-characteristic of human and avian influenza viruses are located in three positions.

H5 HPAI viruses can infect humans despite that their hemagglutinins interact predominantly with SA*α*-2,3-gal cell receptors. However, in cases of successful influenza virus replication in mammalian cells, the researchers identified mutations in other virus genes supposed to be markers of the influenza virus adaptation to mammals for its replication [6–11]. Table 2 shows amino acid residues of A/chicken/Astrakhan/2171-1/2020 isolate proteins responsible for successful influenza virus replication in birds or mammals. Amino acid substitutions facilitating virus replication in mammalian cells were identified only in NEP protein (70G substitution) and PB1 protein (13P substitution). In all other positions AIV-characteristic amino acid residues were identified.

Additionally, deduced amino acid sequences of the virus proteins mediating the virus sensitivity to medicines were analyzed. Currently adamantanes (rimantadin and amantadine) are the medicines with known mechanism of action. These medicines are blockers of ion channels formed by type A influenza virus M2 protein. Influenza virus resistance to rimantadin and amantadine could be accounted for mutations in M2 protein (L26F, 27 V27A, 30 (A→V/P), 31 (S→N/R), 34 G34E) resulting in changes in ion channel configuration. The following amino acids were identified in M2 protein of A/chicken/Astrakhan/2171-1/2020 isolate: leucine (26L), isoleucine (27I), alanine (30A), serine (31S), glycine (34G) that indicates the virus sensitivity to the adamantanes [10].

Besides adamantanes, there are also virus neuraminidase inhibitors, such as the most common oseltamivir and zanamivir. The most oseltamivir-resistant viruses have histidine-to-tyrosine substitution in position 274 (H274Y) [11]. Marker for oseltamivir resistance was not detected in A/chicken/Astrakhan/2171-1/2020 during the analysis. However, some studies showed that resistance to neuraminidase inhibitors varied depending on NA subtype of influenza virus and different NA mutations could result in different resistance levels. Thus, four marker substitutions related to complete or partial resistance to oseltamivir and zanamivir were identified for N2 subtype viruses [12].

Finally, the analysis for markers of virulence for mammals was carried out. Analysis identified serine (S) amino acid in position 42 of NS1 protein. This substitution is a marker of virulence for mice and is able to antagonize the host cell interferon induction, as well as to prevent NF-*κ*B pathway activation during immune response [7]. Moreover, 30D, 215A amino acid substitutions in M1 protein recognized as determinants of pathogenicity for mice were found [13].

Thus, despite the absence of known marker amino acid substitutions enabling effective replication in the mammals in the studied virus, identification of markers of virulence for laboratory animals indicates the need for further investigations of the biological properties of H5N8 avian influenza viruses.

Table 1
HA protein amino acid residues related to receptor specificity of influenza virus (according to H3 type)

Таблица 1
Аминокислотные остатки белка гемагглютинина, определяющие рецепторную специфичность вируса гриппа (по подтипу H3)

Position No. (according to H3 subtype)	A/chicken/ Astrakhan/ 2171-1/2020	Avian influenza virus	Human influenza virus
136	S	S	T
153	W	W	–
158	N	N/D	N
159	D	N	S
183	H	H	–
190	E	E	D
194	L	L	I
221	S	S	P
222	Q	K	–
225	G	G/N	D
226	Q	Q	L/I
227	R	S	A
228	G	G	S

CONCLUSION

Analyses of A/chicken/Astrakhan/2171-1/2020 virus have showed that it is closely related genetically to H5N8 AIV isolates recovered in 2020 during avian influenza outbreaks occurred in the Russian Federation and belongs to genetic clade 2.3.4.4 of H5 subtype. No genomic segment reassortment between the analyzed virus and both influenza viruses of other types and H5N8

Table 2
Amino acid residues determining influenza virus host range

Таблица 2
Аминокислотные остатки, определяющие спектр хозяев вируса гриппа

Protein / position No.	A/chicken/Astrakhan/ 2171-1/2020	Avian influenza virus	Mammalian influenza virus
PB1			
13	P	L	P
99	H	H	Y
368	I	I	V
PB2			
44	A	A	S
81	T	T	M
199	A	A	S
271	T	T	A
588	A	A	I
613	V	V	I
627	E	E	K
661	A	A	T
674	A	A/S	T
701	D	D	N
702	K	K	R
PA			
28	P	P	L
55	D	D	N
65	S	S	L
100	V	V	A
356	K	K	R
382	E	E	D
400	S	Q/T/S	L
409	S	S	N
552	T	T	S
NP			
33	V	V	I
61	I	I	L
100	R	R	V
109	I	I	V
136	L	L	M
214	R	R	K
283	L	L	P
293	R	R	K
313	F	F	Y
375	D	D	G/E
M1			
137	T	T	A
M2			
16	E	E	G
20	S	S/N	N
28	I	I	I/V
55	L	L	F
78	Q	Q	K
NEP			
70	G	S	G

genetic clade 2.3.4.4 viruses isolated earlier in the Russian Federation was found with whole-genome sequencing. Analysis of virus molecular markers indicates A/chicken/Astrakhan/2171-1/2020 virus adaptation to birds and absence of mutations related to adaptation to mammals, including humans. No markers of the virus resistance to anti-viral medicines of adamantane class, oseltamivir and zanamivir, were identified.

REFERENCES

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

2. Volkova M. A., Chvala I. A., Yaroslavtseva P. S., Sosipatorova V. Yu., Osipova O. S., Chvala I. A. Serological monitoring for avian influenza in the Russian Federation in 2017–2018. *Veterinary Science Today*. 2019; 2 (29): 3–11. DOI: 10.29326/2304-196X-2019-2-29-3-7.

3. Lewis N. S., Banyard A. C., Whittard E., Karibayev T., Al Kafagi T., Chvala I., 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): 148–151. DOI: 10.1080/22221751.2021.1872355.

4. Stevens J., Blixt O., Tumpey T. M, Taubenberger J. K., Paulson J. C., Wilson I. A. Structure and receptor specificity of the hemagglutinin from an H5N1 influenza virus. *Science.* 2006; 312 (5772): 404–410. DOI: 10.1126/science.1124513.

5. Matrosovich M. N., Gambaryan A. S., Teneberg S., Piskarev V. E., Yamnikova S. S., Lvov D. K., 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): 224–234. DOI: 10.1006/viro.1997.8580.

6. Seo S. H., Hoffmann E., Webster R. G. The NS1 gene of H5N1 influenza viruses circumvents the host anti-viral cytokine responses. *Virus Res.* 2004; 103 (1–2): 107–113. DOI: 10.1016/j.virusres.2004.02.022.

7. Jiao P., Tian G., Li Y., Deng G., Jiang Y., Liu C., 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): 1146–1154. DOI: 10.1128/JVI.01698-07.

8. Gabriel G., Dauber B., Wolff T., Planz O., Klenk H. D., Stech J. The viral polymerase mediates adaptation of an avian influenza virus to a mammalian host. *Proc. Natl. Acad. Sci. USA.* 2005; 102 (51): 18590–18595. DOI: 10.1073/pnas.0507415102.

9. Dreier C., Resa-Infante P., Thiele S., Stanelle-Bertram S., Walendy-Gnirß K., Speiseder T., et al. Mutations in the H7 HA and PB1 genes of avian influenza a viruses increase viral pathogenicity and contact transmission in guinea pigs. *Emerg. Microbes Infect*. 2019; 8 (1): 1324–1336. DOI: 10.1080/22221751.2019.1663131.

10. Dong G., Pens C., Luo J., Wang C., Han L., Wu B., et al. Adamantane-resistant influenza a viruses in the world (1902–2013): Frequency and distribution of M2 gene mutations. *PLoS One*. 2015; 10 (3):e0119115. DOI: 10.1371/journal.pone.0119115.

11. McAuley J. L., Gilbertson B. P., Trifkovic S., Brown L. E., McKimm-Breschkin J. L. Influenza virus neuraminidase structure and functions. *Front. Microbiol.* 2019; 10:39. DOI: 10.3389/fmicb.2019.00039.

12. Aoki F. Y., Boivin G., Roberts N. Influenza virus susceptibility and resistance to oseltamivir. *Antivir. Ther.* 2007; 12 (4 Pt B): 603–616. PMID: 17944268.

13. Fan S., Deng G., Song J., Tian G., Suo Y., Jiang Y., 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: 10.1016/j.virol.2008.11.044.

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