DOI: 10.29326/2304-196X-2023-12-2-147-153

Molecular identification of Newcastle disease virus isolated on the poultry farm of the Moscow Oblast in summer of 2022

A. A. Treshchalina[1], A. A. Rtishchev[2], E. Yu. Shustova[1], A. V. Belyakova[1], A. S. Gambaryan[1], E. Yu. Boravleva[1]

[1] FSASI "Chumakov Federal Scientific Center for Research and Development of Immune-and-Biological Products of Russian Academy of Science" (Institute of Poliomyelitis) (FSASI "Chumakov FSC R&D IBP RAS" (Institute of Poliomyelitis), Moscow, Russia

[2] FSBSI "I. Mechnikov Research Institute of Vaccines and Sera" (I. Mechnikov NIIVS), Moscow, Russia

SUMMARY

In August 2022, a sudden death in backyard chickens was reported in the Moscow Oblast (urban district Chernogolovka, settlement Starki). As a result, within just a few days 45 chickens on this farm died or fell ill with the following signs – gray mucus discharge from nostrils and beak, coughing, gasping and rales. On day 1–3 after the onset of symptoms, the chicken died. The Newcastle disease virus, which is a representative of the *Paramyxoviruses* family, was isolated from the dead poultry. We determined the nucleotide sequences of fragments in F gene (encodes the fusion surface protein) and in NP gene (encodes the nucleocapsid protein). The motif of $_{109}$SGGRRQKRFIG$_{119}$ proteolysis site, typical for the velogenic pathotype, was determined for the F gene, and a phylogenetic analysis was carried out to demonstrate that the isolate belonged to Subgenotype VII, Class II of the subfamily *Avulavirinae*. The Basic Local Alignment Search Tool revealed that they are most genetically related with isolates from Iran. It was found that the average death time of developing chicken embryos, infected with a minimum infectious dose, was 52 hours, which is typical for the velogenic pathotype. The virus caused 100% death in six-week-old chickens after oral infection and 100% death in all contact chickens, including those kept in cages at a distance, which proves the high level of pathogenicity and contagiousness of the recovered isolate and its ability to transmit both via fecal-oral and aerosols–borne routes. No death cases were reported in mice after intranasal infection with high doses.

Keywords: Newcastle disease virus, pathogenicity, molecular genetic analysis, contagiousness

Acknowledgements: The study was funded by the FSASI "Chumakov Federal Scientific Center for Research and Development of Immune-and-Biological Products of Russian Academy of Science" (Institute of Poliomyelitis).

For citation: Treshchalina A. A., Rtishchev A. A., Shustova E. Yu., Belyakova A. V., Gambaryan A. S., Boravleva E. Yu. Molecular identification of Newcastle disease virus isolated on the poultry farm of the Moscow Oblast in summer of 2022. *Veterinary Science Today*. 2023; 12 (2): 147–153. DOI: 10.29326/2304-196X-2023-12-2-147-153.

Conflict of interest: The authors declare no conflict of interest.

For correspondence: Anastasiya A. Treshchalina, Junior Researcher, Laboratory for Molecular Biology of Viruses, FSASI "Chumakov FSC R&D IBP RAS" (Institute of Poliomyelitis), 108819, Russia, Moscow, poselenie Moskovskii, poselok Institute of Poliomyelitis, domovladenie 8, korp. 1, *e-mail: treshchalinaA@gmail.com*.

УДК 619:616:98:578.831.11

Молекулярная идентификация вируса ньюкаслской болезни, выделенного в домашнем птицехозяйстве Подмосковья летом 2022 года

А. А. Трещалина[1], А. А. Ртищев[2], Е. Ю. Шустова[1], А. В. Белякова[1], А. С. Гамбарян[1], Е. Ю. Боравлева[1]

[1] ФГАНУ «Федеральный научный центр исследований и разработки иммунобиологических препаратов им. М. П. Чумакова РАН» (Институт полиомиелита) (ФГАНУ «ФНЦИРИП им. М. П. Чумакова РАН» (Институт полиомиелита), г. Москва, Россия

[2] ФГБНУ «Научно-исследовательский институт вакцин и сывороток им. И. И. Мечникова» (ФГБНУ НИИВС им. И. И. Мечникова), г. Москва, Россия

РЕЗЮМЕ

В августе 2022 г. в Московской области (городской округ Черноголовка, деревня Старки) на личном подворье внезапно начался падеж кур, в результате которого в течение нескольких дней погибли или заболели все 45 голов этого хозяйства со следующими признаками: выделение серой слизи из ноздрей и клюва, резкие кашляющие звуки. Через 1–3 дня после появления симптомов птица погибала. Из тканей, полученных от павших кур, был выделен вирус ньюкаслской болезни, являющийся представителем обширного семейства парамиксовирусов. Определены нуклеотидные последовательности фрагментов гена F, кодирующего поверхностный белок слияния, и гена NP, кодирующего белок нуклеокапсида. Для гена F определен мотив сайта протеолиза $_{109}$SGGRRQKRFIG$_{119}$, типичный для велогенного патотипа, также проведен филогенетический анализ, по результатам которого установлена принадлежность изолята к субгенотипу VII класса II подсемейства *Avulavirinae*. С помощью Basic Local Alignment Search Tool выявлено наиболее генетически

близкое родство с изолятами из Ирана. Установлено, что среднее время смерти развивающихся куриных эмбрионов при заражении минимальной инфекционной дозой составило 52 ч, что характерно для велогенного патотипа. Вирус вызывал 100%-ю гибель цыплят шестинедельного возраста при оральном заражении, а также 100%-й падеж всего контактного молодняка, включая содержащихся в клетках на отдалении, что доказывает высокий уровень патогенности и контагиозности выделенного изолята и его способность распространяться не только фекально-оральным, но и аэрозольным путем. Гибель мышей при интраназальном заражении высокими дозами не наблюдалась.

Ключевые слова: вирус ньюкаслской болезни, патогенность, молекулярно-генетический анализ, контагиозность

Благодарность: Исследование профинансировано ФГАНУ «Федеральный научный центр исследований и разработки иммунобиологический препаратов им. М. П. Чумакова РАН» (Институт полиомиелита).

Для цитирования: Трещалина А. А., Ртищев А. А., Шустова Е. Ю., Белякова А. В., Гамбарян А. С., Боравлева Е. Ю. Молекулярная идентификация вируса ньюкаслской болезни, выделенного в домашнем птицехозяйстве Подмосковья летом 2022 года. *Ветеринария сегодня*. 2023; 12 (2): 147–153. DOI: 10.29326/2304-196X-2023-12-2-147-153.

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

Для корреспонденции: Трещалина Анастасия Андреевна, младший научный сотрудник лаборатории молекулярной биологии вирусов ФГАНУ «ФНЦИРИП им. М. П. Чумакова РАН» (Институт полиомиелита), 108819, Россия, г. Москва, поселение Московский, поселок Института полиомиелита, домовладение 8, корп. 1, *e-mail: treshchalinaA@gmail.com*.

INTRODUCTION

Newcastle disease virus (NDV), or *Avian orthoavulavirus* 1 (AOAV-1), belongs to extensive *Paramixoviridae* family, *Avulavirinae* subfamily, its representatives cause avian diseases of various severity [1]. NDV affects more than 240 wild and domestic avian species and is an enveloped negative-sense single-stranded RNA virus. The genome is not segmented and is about 15,000 bases long, divided by conservative non-coding regions into six genes in 3'-NP-P-M-F-HN-L-5' sequence encoding eight proteins [2, 3].

One of the key factors that influence NDV pathogenicity is the number of basic acids in the F protein cleavage site [4]. Low pathogenic virus strains have sequence $_{109}$SGGGR(K)QGRLIG$_{119}$ in the proteolytic site and can be cleaved only by extracellular trypsin-like proteases. The motif of highly pathogenic NDV strains has several basic amino acids (lysine or arginine) and phenylalanine at amino acid position 117: $_{109}$SGGRRQ(K/R)RF(V/I)G$_{119}$. The protein of such a virus is able to transform into an active form with the help of furin-like proteases present in all body cells. This virus causes a generalized infection [5].

Phylogenetically, NDV is divided into 2 classes, Class I includes only one genotype and is represented mainly by apatogenic strains isolated from wild and domesticated birds [6]. Class II is divided into 21 genotypes and includes strains of different virulence affecting a wide range of hosts [7]. The class representatives are spread worldwide and have regularly induced epizooties.

NDV poses a serious threat to poultry. The mortality rate in the infected chickens can reach 100%. According to the pathogenicity for chickens, NDV can be lentogenic (low pathogenic), mesogenic (causing moderate disease in adult chickens and death in young birds) and velogenic (highly pathogenic for chickens of all ages) [3].

The virus may spread due to trade in poultry and poultry products, as well as during movement of workers from the infected poultry farms and during transportation [8]. Migration of wild birds is another way of the virus spread [9, 10]. Although most apatogenic NDV isolates recovered from wild birds do not pose a serious threat to chickens, a mutation accumulation increases the virus pathogenicity [11].

The aim of this research is to conduct a molecular and phylogenetic analysis of the Newcastle disease virus isolated from the chicken pathological material taken in one of the backyards located in the Moscow Region, to determine its pathotype; to study its pathogenicity and contagiousness.

MATERIALS AND METHODS

Reagents. MycoKill AB (PAA Laboratories GmbH, Austria); mini-kit for RNA virus isolation QIAamp Viral RNA Mini Kit (QIAGEN, Germany); set of reagents for reverse transcription MMLV RT kit, random decanucleotide primer, set of reagents Tersus Plus PCR kit, nuclease-free water, DNA markers and TAE buffer (Eurogen, Russia); ribonuclease inhibitor (Syntol, Russia); set reagents for sequencing ABI PRISM® BigDye™ Terminator v3.1 (ThermoFisher Scientific Inc., USA).

Virus isolation. Lung and kidney samples from dead chickens were used to isolate the virus. Tissue fragments were rubbed with fine glass powder; a fourfold phosphate buffered saline (PBS) solution was added containing 0.4 mg/mL gentamicin, 0.1 mg/mL kanamycin, 0.01 mg/mL nystatin and 2% MycoKill AB solution. The suspension was centrifuged for 10 min at 4,000 rpm, and 0.2 mL of the supernatant was inoculated through allantoic cavity into 10-day-old developing chicken embryos (DCE). Incubation lasted 72 hours at 37 °C; embryo death was controlled twice a day. Then, virus-containing allantoic fluid (VAF) was collected and tested in the hemagglutination test (HA test) using a 1% suspension of chicken erythrocytes according to the generally accepted method [12]. The amount of virus was expressed in hemagglutinating

units. The 50% infectious dose (EID_{50}) was determined by titration in developing chicken embryos.

RNA extraction, cDNA synthesis and sequencing. The viral RNA was extracted from virus-containing allantoic fluids using QIAamp Viral RNA Mini Kit (QIAGEN, Germany) according to the manufacturer's instructions. The reverse transcription was carried out using a set of reagents MMLV RT kit (Eurogen, Russia) in presence of a random decanucleotide primer. The polymerase chain reaction (PCR) was carried out in a volume of 25 µL using a set of reagents Tersus Plus PCR Kit (Eurogen, Russia): sterile water for PCR – 17.5 µL; 10× Tersus Plus buffer – 2.5 µL; 50× dNTP mixture – 0.5 µL; direct primer fFapmv2 (10 µmol) – 1 µL; reverse primer rFapmv2 (10 µmol) – 1 µL, cDNA – 2 µL; 50× Tersus polymerase – 0.5 µL. Oligonucleotides used in the work: fFapmv2 (ATGGGCTCCAGACCTTCTAC); rFapmv2 (CTGCCACTGCTAGTTGCGATAATCC); fNPapmv (GGTATTCTGTCTTCGGATTG); rNPapmv (TCATCCGATATAAACGCAT). The PCR products were analysed by 2% agarose gel electrophoresis in a tris-acetate buffer. PCR fragments of about 500 bp were cut out for clean-up from the gel using the Qiagen MinElute Gel Extraction Kit (QIAGEN, Germany) according to the manufacturer's instructions. The nucleotide sequences of PCR fragments were determined using ABI PRISM® BigDye™ Terminator v3.1 reagent kit (ThermoFisher Scientific, USA), followed by PCR product analysis on the ABI PRISM 3130 sequencer (ThermoFisher Scientific, USA). The resulting chromatograms were analyzed using SnapGene Viewer program[1].

Phylogenetic analysis. All the sequences were downloaded from the GenBank database[2]. The sequences were processed using BioEdit 7.2[3] and MEGA X[4] [13, 14]. BEAST v1.10.4 software was used to perform a Bayesian Markov Chain Monte Carlo-based phylogenetic analysis (MCMC) of the nucleotide sequences (n = 150) using the General Time Reversible (GTR) model [15]. The iTOL v6[5] online service was used to visualize and annotate the tree. Genotype was identified using phylogenetic topology.

Determination of the virus pathotype. The virus pathotype was determined by the mean death time (MDT) method. 0.2 mL of 10-fold dilutions of fresh VAF on PBS (from 10^{-3} to 10^{-7}) were inoculated into the allantoic cavities of 9-day developing chicken embryos. After that, they were incubated for five days at 37 °C, checked 2 times a day to control embryo death. MDT was calculated as the mean death time of an embryo infected with a minimum lethal dose. If MDT is up to 60 hours, the virus pathotype is defined as velogenic, from 60–90 – as mesogenic, more than 90 – as lentogenic.

Analysis of pathogenicity and contagiousness of the virus for chickens. 6-week-old Leghorn chickens were infected orally, with the virus added to the drinking bowl. Virus-free fresh allantoic fluid was added into the drinking bowl for the control and contact groups. Each group included 5 chickens; the groups were initially put into separate cages. The chickens were daily examined for 10 days after infection.

Analysis of the virus pathogenicity for mice. BALB/c mice weighing 10–12 g were infected intranasally (under light ether anesthesia) with 50 µL containing doses from 10^3 to 10^6 EID_{50}/mouse. A group of 5–6 mice was infected with each viral dose. The control group received virus-free allantoic fluid diluted with saline solution. Survival rate and mice weight were recorded within 15 days after infection.

Ethical status. Animal experiments were conducted in accordance with the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, March 18, 1986)"[6]. The Ethics Committee of the FSASI "Chumakov FSC R&D IBP RAS" (Institute of Poliomyelitis), Resolution No. 4 of December 2, 2014, approved the experiment design. All measures were taken to reduce the suffering of animals.

RESULTS AND DISCUSSION

In late August 2022, mass death was reported in chickens in the Moscow region (urban district Chernogolovka, settlement Starki). Pathological material from the dead birds was sent for laboratory tests to the (FSASI "Chumakov FSC R&D IBP RAS" (Institute of Poliomyelitis). Lung and kidney tissue suspensions were inoculated into 10-day DCE through allantoic cavities. Newcastle disease virus was detected in all HA-positive VAF samples tested in RT-PCR. The isolate was named as NDV/chicken/Moscow/6081/2022.

Molecular and phylogenetic analysis. While studying the isolate, 438 bp F gene fragment (which includes a site encoding the F protein proteolytic sites) was amplified and sequenced. The obtained nucleotide sequence was analyzed using the Basic Local Alignment Search Tool (BLAST)[7] and the analysis showed that NDV/chicken/Moscow/6081/2022 isolate is most closely related to the viruses recovered in Iran from chickens in 2011–2013 (similarity is 97.03–97.48% with the first five sequences). Several basic amino acid residues (arginine/lysine) with $_{109}$SGGRRQKRFIG$_{119}$ motif were detected in F protein proteolytic site of the studied virus, as demonstrated by the obtained sequence. This sequence is specific for virulent strains based on the criteria used by the World Organisation for Animal Health to assess the virulence of NDV isolates [12].

Additionally, nucleotide sequence of the NP gene encoding the nucleocapsid protein (697 bp) was obtained. This gene may also indirectly affect NDV virulence. Thus, nucleotides 546 and 555 are different for lentogenic and velogenic strains [16]. According to the sequencing results, positions 546 and 555 in NP gene of the recovered isolate correspond to the velogenic variant (two thymines (T) in positions 546 and 555). Both sequences were uploaded to the GenBank database under numbers OQ190211 and OQ190212.

For phylogenetic analysis, a sample was taken from representative sequences of each genotype of Class II (n = 125) [7]. The sample was combined with

[1] SnapGene Viewer. Available at: https://www.snapgene.com/snapgene-viewer.

[2] GenBank. Available at: https://www.ncbi.nlm.nih.gov/genbank.

[3] BioEdit. Available at: https://bioedit.software.informer.com.

[4] MEGA X. Available at: https://www.megasoftware.net.

[5] iTOL. Available at: https://itol.embl.de.

[6] The European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, March 18, 1986). Available at: rm.coe.int›168007a67b.

[7] BLAST. Available at: https://blast.ncbi.nlm.nih.gov/Blast.cgi.

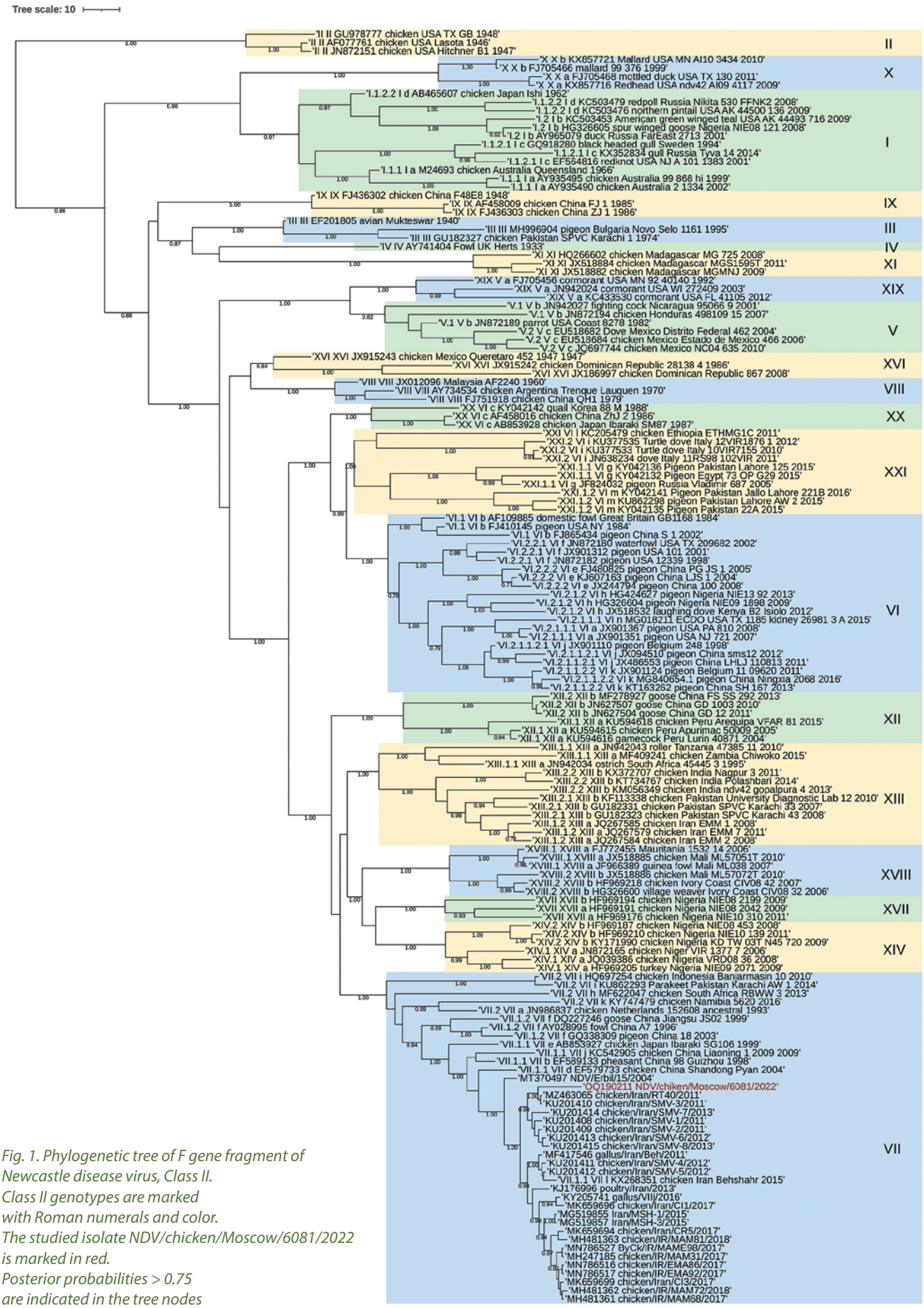

Fig. 1. Phylogenetic tree of F gene fragment of Newcastle disease virus, Class II. Class II genotypes are marked with Roman numerals and color. The studied isolate NDV/chicken/Moscow/6081/2022 is marked in red. Posterior probabilities > 0.75 are indicated in the tree nodes

the sequence of F gene fragment of the Moscow isolate and a sample from 24 most closely related viruses identified in BLAST. Phylogenetic analysis of the F gene fragment showed that NDV/chicken/Moscow/6081/2022 isolate belongs to Genotype VII, Class II (Fig. 1).

Genotype VII of Class II originated in Asia presumably in the 1980s and is now widespread in Eurasia and Africa; it is as well recorded in South America [17–19]. The virus was the cause of Newcastle disease Fourth Panzootic, which has been ongoing since the 1980s [9]. The genotype includes only velogenic NDV strains that cause high mortality in birds [20].

Determination of pathogenicity and contagiousness of NDV/chicken/Moscow/6081/2022 isolate for chickens. It was found that MDT for 9-day-old DCE is 52 hours, which corresponds to the velogenic type (up to 60 hours).

To study the isolate contagiousness, three groups of five 6-week-old chickens were formed. To infect the birds of Group 1, 10^8 EID_{50} of NDV/chicken/Moscow/6081/2022 virus were added to the drinking bowl. The next day, the infected chickens were put into a cage of Group 2. Cage No. 1 was removed and disinfected. The chickens from Group 3 were in Cage 3, located two meters away from Cages 1 and 2, so that to exclude contamination with feed particles and faeces, however, air and fine dust circulation between the cages was possible. The death dynamics in infected and contact birds is shown in Figure 2. All the infected chickens died by Day 5, contact birds from Group 2 died on Day 6, and in Group 3 chickens fell ill on Days 6 and 7, after that all the birds died by Day 10. NDV/chicken/Moscow/6081/2022 virus was detected in the organs of the dead birds using PCR.

Thus, contact chickens (Group 2) caught the disease from the infected ones almost immediately. The death of chickens from Group 3, who did not come into a direct contact with the sick ones, may be explained by the fact that at some point one of the chickens became infected through airborne droplets, thus causing the infection spread and rapid death of the entire group. The experiment results demonstrate a very high pathogenicity and contagiousness of NDV/chicken/Moscow/6081/2022 isolate and show that the birds are infected not only by fecal-oral route, but also through the air.

Pathogenicity of the virus for mice. Avulavirinae representatives, as a rule, are non-pathogenic to mammals. However, given the exceptionally high pathogenicity and contagiousness of NDV/chicken/Moscow/6081/2022 isolate for chickens, it was decided to make sure that it is safe for mammals.

The weight change dynamics in mice infected intranasally with NDV/chicken/Moscow/6081/2022 is shown in Figure 3. Groups 1, 2, 3 and 4 were infected at doses of 10^3, 10^4, 10^5 and 10^6 EID_{50}/mouse, respectively. The control group was given placebo (virus-free allantoic fluid, diluted with saline solution). Survival rate and mice weight were recorded within 12 days after infection. No mice died during the experiment. Figure 3 shows that groups infected with high viral doses slightly became slightly underweight on Days 2–5 after infection, but by Day 12 almost all the mice were healthy.

Thus, NDV/chicken/Moscow/6081/2022 isolate of Newcastle disease virus is practically not pathogenic for mice, despite its very high pathogenicity for chickens.

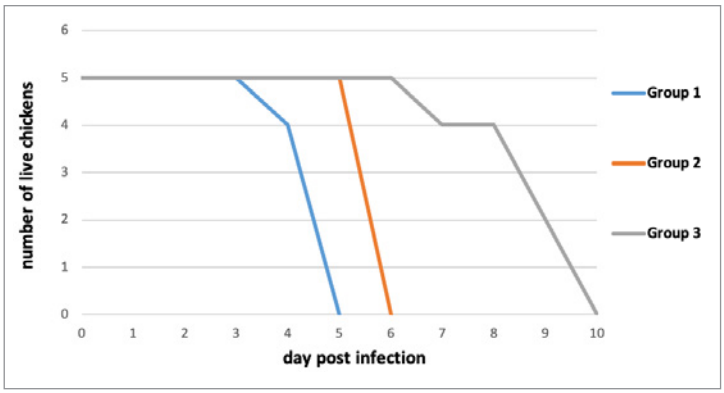

Fig. 2. Dynamics of death in infected and contact chickens

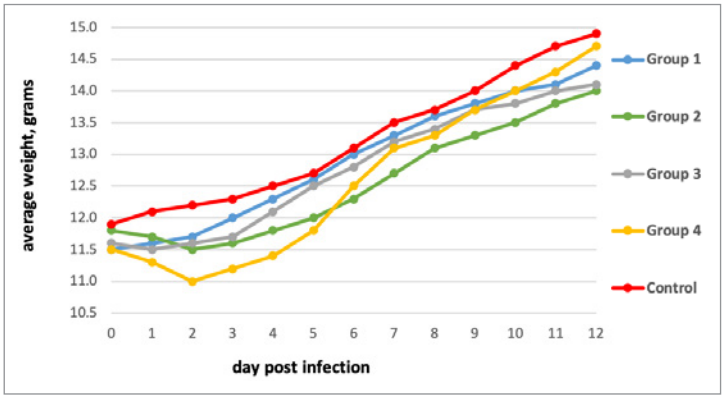

Fig. 3. Dynamics of the mouse weight changes upon infection with NDV/chicken/Moscow/6081/2022

CONCLUSION

In August 2022, all chickens died in one of the backyards of the Moscow region shortly after the first disease symptoms appeared. Newcastle disease virus was detected in the dead bird tissues. Laboratory tests showed high pathogenicity of the isolate for chickens and no pathogenicity for mice.

Molecular analysis together with the MDT test helped to check several factors indicating how NDV/chicken/Moscow/6081/2022 isolate is related to velogenic NDV: presence of a polybasic proteolytic site in F fusion protein, presence of two amino acids in NP nucleocapsid protein, which is typical for highly pathogenic NDV, MDT up to 60 hours.

The phylogenetically isolated virus belongs to Genotype VII, Class II. This genotype belongs to the "late" ones (emerged after the 1960s) and includes viruses of the velogenic pathotype only. Most current outbreaks in chickens in Asia and the Middle East are associated with this particular virus [21]. Its widespread is partly explained by the fact that the strains related to this genotype are able to spread in poultry vaccinated with popular commercial vaccines [20].

In the Russian Federation, NDV poses a potential economic threat to the poultry industry. As the Rosselkhoznadzor data show, more than 25 outbreaks of Newcastle disease have been registered in various regions of the country since 2019[8]. Serological data show an increase

[8] Rosselkhoznadzor. Available at: https://fsvps.gov.ru/ru. (in Russ.)

in the number of immune wild birds and non-vaccinated domestic ducks after 2017 [22–25]. To control the virus spread, it is required to continue monitoring, as well as to timely vaccinate poultry in backyards [26, 27].

REFERENCES

1. Rima B., Balkema-Buschmann A., Dundon W. G., Duprex P., Easton A., Fouchier R., et al. ICTV virus taxonomy profile: *Paramyxoviridae*. *J. Gen. Virol*. 2019; 100 (12): 1593–1594. DOI: 10.1099/jgv.0.001328.

2. The Negative Sense Single Stranded RNA Viruses. *In: Virus Taxonomy. Ed. by C. M. Fauquet, M. A. Mayo, J. Maniloff, U. Desselberger, L. A. Ball*. Academic Press; 2005; 607–738. DOI: 10.1016/B978-0-12-249951-7.50014-6.

3. Alexander D. J. Newcastle disease and other avian paramyxoviruses. *Rev. Sci. Tech*. 2000; 19 (2): 443–462. DOI: 10.20506/rst.19.2.1231.

4. Ganar K., Das M., Sinha S., Kumar S. Newcastle disease virus: current status and our understanding. *Virus Res*. 2014; 184: 71–81. DOI: 10.1016/j.virusres.2014.02.016.

5. Glickman R. L., Syddall R. J., Iorio R. M., Sheehan J. P., Bratt M. A. Quantitative basic residue requirements in the cleavage-activation site of the fusion glycoprotein as a determinant of virulence for Newcastle disease virus. *J. Virol*. 1988; 62 (1): 354–356. DOI: 10.1128/JVI.62.1.354-356.1988.

6. Czeglédi A., Ujvári D., Somogyi E., Wehmann E., Werner O., Lomniczi B. Third genome size category of avian paramyxovirus serotype 1 (Newcastle disease virus) and evolutionary implications. *Virus Res*. 2006; 120 (1–2): 36–48. DOI: 10.1016/j.virusres.2005.11.009.

7. Dimitrov K. M., Abolnik C., Afonso C. L., Albina E., Bahl J., Berg M., et al. Updated unified phylogenetic classification system and revised nomenclature for Newcastle disease virus. *Infect. Genet. Evol*. 2019; 74:103917. DOI: 10.1016/j.meegid.2019.103917.

8. Beard C. W., Hanson R. P. Newcastle disease. *In: Diseases of Poultry. Ed. by M. S. Hofstad, H. J. Barnes, B. W. Calnek, W. M. Reid, H. W. Yoder, et al. 8th ed.* Ames: Iowa State University Press; 1984; 452–470.

9. Miller P. J., Haddas R., Simanov L., Lublin A., Rehmani S. F., Wajid A., et al. Identification of new sub-genotypes of virulent Newcastle disease virus with potential panzootic features. *Infect. Genet. Evol*. 2015; 29: 216–229. DOI: 10.1016/j.meegid.2014.10.032.

10. Karamendin K., Kydyrmanov A. Cormorants as a potentially important reservoir and carrier of Newcastle disease virus on the Asian continent. *Front. Vet. Sci*. 2021; 8:648091. DOI: 10.3389/fvets.2021.648091.

11. Shengqing Y., Kishida N., Ito H., Kida H., Otsuki K., Kawaoka Y., Ito T. Generation of velogenic Newcastle disease viruses from a nonpathogenic waterfowl isolate by passaging in chickens. *Virology*. 2002; 301 (2): 206–211. DOI: 10.1006/viro.2002.1539.

12. Newcastle disease (infection with Newcastle disease virus). *In: WOAH. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals.* 2021; Chapter 3.3.14. Available at: https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/3.03.14_NEWCASTLE_DIS.pdf.

13. Staden R. The Staden sequence analysis package. *Mol. Biotechnol*. 1996; 5 (3): 233–241. DOI: 10.1007/BF02900361.

14. Kumar S., Stecher G., Li M., Knyaz C., Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol*. 2018; 35 (6): 1547–1549. DOI: 10.1093/molbev/msy096.

15. Drummond A. J., Rambaut A., Shapiro B., Pybus O. G. Bayesian coalescent inference of past population dynamics from molecular sequences. *Mol. Biol. Evol*. 2005; 22 (5): 1185–1192. DOI: 10.1093/molbev/msi103.

16. Tan S. W., Ideris A., Omar A. R., Yusoff K., Hair-Bejo M. Detection and differentiation of velogenic and lentogenic Newcastle disease viruses using SYBR Green I real-time PCR with nucleocapsid gene-specific primers. *J. Virol. Methods*. 2009; 160 (1–2): 149–156. DOI: 10.1016/j.jviromet.2009.05.006.

17. Eid A. A. M., Hussein A., Hassanin O., Elbakrey R. M., Daines R., Sadeyen J. R., et al. Newcastle disease genotype VII prevalence in poultry and wild birds in Egypt. *Viruses*. 2022; 14 (10):2244. DOI: 10.3390/v14102244.

18. Xue C., Xu X., Yin R., Qian J., Sun Y., Wang C., et al. Identification and pathotypical analysis of a novel VIk sub-genotype Newcastle disease virus obtained from pigeon in China. *Virus Res*. 2017; 238: 1–7. DOI: 10.1016/j.virusres.2017.05.011.

19. Perozo F., Marcano R., Afonso C. L. Biological and phylogenetic characterization of a genotype VII Newcastle disease virus from Venezuela: efficacy of field vaccination. *J. Clin. Microbiol*. 2012; 50 (4): 1204–1208. DOI: 10.1128/JCM.06506-11.

20. Pandarangga P., McAllister M. M., Peaston A. E., Ngai Y. T., Cahyono M. I., Hemmatzadeh F. Performance comparison of homologous and heterologous Newcastle disease virus in vaccines and antibody tests. *Res. Vet. Sci*. 2022; 149: 82–89. DOI: 10.1016/j.rvsc.2022.06.014.

21. Miller P. J., Decanini E. L., Afonso C. L. Newcastle disease: evolution of genotypes and the related diagnostic challenges. *Infect. Genet. Evol*. 2010; 10 (1): 26–35. DOI: 10.1016/j.meegid.2009.09.012.

22. Gogoi P., Ganar K., Kumar S. Avian paramyxovirus: a brief review. *Transbound. Emerg. Dis*. 2017; 64 (1): 53–67. DOI: 10.1111/tbed.12355.

23. Volkova M. A., Chvala Ir. A., Yaroslavtseva P. S., Sosipatorova V. Yu., Chvala I. A. Serological monitoring of Newcastle disease in Russia 2017. *Veterinary Science Today*. 2018; (4): 26–30. DOI: 10.29326/2304-196X-2018-4-27-26-30.

24. Volkova M. A., Chvala Ir. A., Osipova O. S., Kulagina M. A., Andreychuk D. B., Chvala I. A. Serological monitoring of avian influenza and Newcastle disease in the Russian Federation in 2019. *Veterinary Science Today*. 2020; (2): 76–82. DOI: 10.29326/2304-196X-2020-2-33-76-82.

25. Kulagina M. A., Volkova M. A., Chvala Ir. A., Osipova O. S., Yaroslavtseva P. S., Andreychuk D. B., Chvala I. A. Serological monitoring of avian influenza and Newcastle disease in the Russian Federation in 2020. *Veterinary Science Today*. 2022; 11 (2): 142–148. DOI: 10.29326/2304-196X-2022-11-2-142-148.

26. Frolov S. V., Moroz N. V., Chvala I. A., Irza V. N. Effectiveness of vaccines produced by the Federal State-Financed Institution "ARRIAH" against topical genotype VII Newcastle disease viruses. *Veterinary Science Today*. 2021; (1): 44–51. DOI: 10.29326/2304-196X-2021-1-36-44-51.

27. Instructions on control measures against Newcastle disease (pseudo-poultry plague): approved by the Chief Veterinary Department of the USSR MoA on 09 June 1976

(with amendments and additions dated back to August 28, 1978). Available at: http://vetorel.ru/website/img/docs/newak.pdf. (in Russ.)

Received 28.02.2023
Revised 29.03.2023
Accepted 11.04.2023

INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Anastasiya A. Treshchalina, Junior Researcher, Laboratory of Molecular Biology of Viruses, FSASI "Chumakov FSC R&D IBP RAS" (Institute of Poliomyelitis), Moscow, Russia; *https://orcid.org/0000-0003-3801-2413, e-mail: treshchalinaA@gmail.com.*

Artem A. Rtishchev, Researcher, Laboratory of RNA-containing Viruses, I. Mechnikov NIIVS, Moscow, Russia; *https://orcid.org/0000-0002-4212-5093, e-mail: rtishchevartyom@gmail.com*.

Elena Yu. Shustova, Researcher, Laboratory of Molecular Biology of Viruses, FSASI "Chumakov FSC R&D IBP RAS" (Institute of Poliomyelitis), Moscow, Russia; *https://orcid.org/0000-0003-1314-0152, e-mail: shustova_eu@chumakovs.su.*

Alla V. Belyakova, Candidate of Science (Biology), Scientific Secretary, FSASI "Chumakov FSC R&D IBP RAS" (Institute of Poliomyelitis), Moscow, Russia; *https://orcid.org/0000-0003-4363-6394, e-mail: belyakova_av@chumakovs.su.*

Aleksandra S. Gambaryan, Doctor of Science (Biology), Leading Researcher, Laboratory of Molecular Biology of Viruses, FSASI "Chumakov FSC R&D IBP RAS" (Institute of Poliomyelitis), Moscow, Russia; *https://orcid.org/0000-0002-1892-0548, e-mail: al.gambaryan@gmail.com.*

Elizaveta Yu. Boravleva, Candidate of Science (Biology), Senior Researcher, Laboratory of Molecular Biology of Viruses, FSASI "Chumakov FSC R&D IBP RAS" (Institute of Poliomyelitis), Moscow, Russia; *https://orcid.org/0000-0001-8491-4640, e-mail: elisavetbor@gmail.com.*

Трещалина Анастасия Андреевна, младший научный сотрудник лаборатории молекулярной биологии вирусов ФГАНУ «ФНЦИРИП им. М. П. Чумакова РАН» (Институт полиомиелита), г. Москва, Россия; *https://orcid.org/0000-0003-3801-2413, e-mail: treshchalinaA@gmail.com.*

Ртищев Артем Андреевич, научный сотрудник лаборатории РНК-содержащих вирусов ФГБНУ НИИВС им. И. И. Мечникова, г. Москва, Россия; *https://orcid.org/0000-0002-4212-5093, e-mail: rtishchevartyom@gmail.com.*

Шустова Елена Юрьевна, научный сотрудник лаборатории молекулярной биологии вирусов ФГАНУ «ФНЦИРИП им. М. П. Чумакова РАН» (Институт полиомиелита), г. Москва, Россия; *https://orcid.org/0000-0003-1314-0152, e-mail: shustova_eu@chumakovs.su.*

Белякова Алла Владимировна, кандидат биологических наук, ученый секретарь ФГАНУ «ФНЦИРИП им. М. П. Чумакова РАН» (Институт полиомиелита), г. Москва, Россия; *https://orcid.org/0000-0003-4363-6394, e-mail: belyakova_av@chumakovs.su.*

Гамбарян Александра Сергеевна, доктор биологических наук, ведущий научный сотрудник лаборатории молекулярной биологии вирусов ФГАНУ «ФНЦИРИП им. М. П. Чумакова РАН» (Институт полиомиелита), г. Москва, Россия; *https://orcid.org/0000-0002-1892-0548, e-mail: al.gambaryan@gmail.com.*

Боравлева Елизавета Юрьевна, кандидат биологических наук, старший научный сотрудник лаборатории молекулярной биологии вирусов ФГАНУ «ФНЦИРИП им. М. П. Чумакова РАН» (Институт полиомиелита), г. Москва, Россия; *https://orcid.org/0000-0001-8491-4640, e-mail: elisavetbor@gmail.com.*