UDC 616.98:578.834COVID-19-036.22:616-051

WHOLE-GENOME SEQUENCING OF SOME UKRAINIAN ISOLATES OF SARS-COV-2 VIRUS AND ANALYSIS OF ITS GENETIC VARIABILITY

S. A. Nychyk [1], M. S. Mandygra [2], M. V. Bezymennyi [1], N. V. Hudz [1], A. V. Molozhanova [1], *O. A. Tarasov [1]

[1] Institute of Veterinary Medicine the National Academy of Agrarian Sciences of Ukraine
30, Donetska Str, Kyiv, Ukraine, 03154
[2] National Academy of Agrarian Sciences of Ukraine
9, Mykhaila Omelyanovycha-Pavlenka Str., Kyiv, Ukraine, 01010

*E-mail: snychyk@gmail.com, mandygra@gmail.com, nomax@ukr.net, gudznataly@gmail.com, vetereneri@ukr.net, * ast97@ukr.net*

ORCID: https://orcid.org/0000-0002-4789-4334, https://orcid.org/0009-0002-3944-7195, https://orcid.org/0000-0001-8042-0061, https://orcid.org/0000-0002-2175-1431, https://orcid.org/0009-0002-8166-8979, https://orcid.org/0000-0003-1481-5529

Received September 05, 2023 / Received October 10, 2023 / Accepted November 18, 2023

Aim. The aim was to gain experience of the animal health services to detect and properly identify SARS-CoV-2 virus with whole-genome sequencing method and its genetic variability in Ukraine in relation to possible future spread of the virus in animals. **Methods.** Sixteen SARS-CoV-2 positive samples, not sequenced before, were provided by the Center for Public Health of the Ministry of Health of Ukraine. These samples were obtained from hospitalized patients from early October to mid-November of 2021. The viral RNA was isolated from nasopharyngeal swab samples of SARS-CoV-2 qPCR positive (Ct 21-28) patients (males and females) with moderate and severe symptoms who were being hospitalized. The samples were fully anonymized. The Ion Torrent S5 instrument (Oxford Nanopore, the USA) was used to sequence the mentioned SARS-CoV-2 isolates, originating from Ukraine. TorrentSuite 5.16.1 was used for data processing and analysis. Nextclade 2.3.0 was used for phylogenetic analysis to locate the 6 sequenced samples on the global phylogenetic tree. It was determined phylogenetic relations between tested 6 sequences and 495 verified sequences of high quality, reported in Ukraine and deposited in the GISAID EpiCoV™ database, (https://gisaid.org/) for the period of January 2020 – December 2022. In the comparison of sequences obtained, the sequence of SARS-CoV-2 virus isolate Wuhan-Hu-1 (GenBank NC_045512.2) was used as a reference sequence, according to which the sequences were aligned. All studies were carried out in the laboratory of the Research Training Center for Animal Disease Diagnostics at the Institute of Veterinary Medicine of the National Academy of Sciences of Ukraine. **Results.** Among the 16 isolates tested, all were confirmed to contain SARS-CoV-2 RNA, of which only six isolates were sequenced with sufficient quality and could be classified, five of them as Delta variants (two belong to lineage AY.126 (B.1.617.2.33), two to AY.122 (B.1.617.2.122), and one to AY.4.2.3 (B.1.617.2.4.2)), and one isolate as an Omicron variant (BA.1.18). Important mutations detected in our isolates were a S:N501Y substitution and S:H69 deletion in the gene of the virus envelope spike protein. Among the examined isolates, the Omicron variant (BA.1.18) was found to exhibit greater genetic variability, with over 60 mutations compared to previous variants. In our investigation, we identified mutations in the sequenced Delta variants too, ranging from 35 mutations in AY.122 (B.1.617.2.122) to 41 mutations in AY.126 (B.1.617.2.33) in the genome compared to the reference Wuhan-Hu-1 (MN908947) variant. Important mutations found regarding infectivity were 1) for the Delta variants: T478K, L452R mutations in the RBD region, and 2) for the Omicron variant: S371L, G339D, S375F, S373P, K417N, N440K, S477N, G446S, E484A, T478K, Q493R, Q498R, G496S, N501Y, and Y505H mutations in the RBD region. **Conclusions**. The whole-genome sequencing of 6 isolates of SARS-CoV-2 virus was performed, and three sublines of the Delta variant were found: AY.126 (B.1.617.2.33), AY.122 (B.1.617.2.122), AY.4.2.3 (B.1.617.2.4.2) and one subline for the Omicron variant (BA.1.18), all of which were deposited in the international database GISAID as EPI_SET_230516yp. The data obtained in this study add

to the existing ones delivered by the Ministry of Health in Ukraine and can be used in laboratories, (including veterinary ones), detecting the SARS-CoV-2 virus in risk animal populations, in order to prevent the spread of the disease to humans and animals, as well as to detect possible mutational changes in the pathogen genome that may affect infectivity and pathogenicity.

Key words: SARS-CoV-2, Covid-19, whole genome sequencing, mutations, phylogenetics.

DOI: https://doi.org/10.15407/agrisp10.03.003

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a strain of coronavirus that causes COVID-19, a serious and often lethal respiratory disease in man, leading to a pandemic in 2019. This virus is a positive-sense, single-stranded enveloped ribonucleic acid (RNA) virus, related to *Sarbecovirus* subgenus of *Betacoronavirus genus (*Rambaut A et al, 2020; Rabi FA et al, 2020, Ahmed R et al, 2021; Ferretti L et al, 2020, Gorbalenya AE et al, 2020).

Epidemics caused by coronaviruses were observed in the past (from early 2000's), among others caused by SARS-CoV and the respiratory syndrome coronavirus (MERS-CoV) after 2012. In the context of zoonotic viral diseases, it is important to mention the dynamics of Middle East Respiratory Syndrome Coronavirus (MERS-CoV), which poses a significant public health concern. MERS-CoV is known for its zoonotic transmission, with dromedary camels serving as a crucial reservoir. Understanding the transmission dynamics of MERS-CoV, including its potential to persist in the environment, was essential for devising effective prevention and control strategies for SARS-CoV-2, hich appeared late 2019. Researchers developed experience in the detection and identification of these viruses (Yan Y Chang & Wang L, 2020; Lam T T-Y et al, 2020; Elaswad A et al, 2020; Lu R et al, 2020; Dunowska, 2023).

The pandemic outbreak of coronavirus (named coronavirus 2019 or COVID-19) was caused by a new coronavirus, namely SARS-CoV-2 virus, first diagnosed in humans in December 2019, in China (Wuhan, Hubei province). On January 30, 2020, the WHO announced that the outbreak of the diseases, caused by SARS-CoV-2 coronavirus, was an emergency situation in the field of public health care (Zhang, Y-Z, Holmes EC, 2020).

In Ukraine, COVID-19 was first diagnosed in March 2020 in Chernivtsi, and on March 13, the first lethal case, caused by SARS-CoV-2, was registered (Resolution of Cabinet of Ministry of Ukraine No. 211 dated March 11, 2020). As of early December 2023, according to the official statistics data for Ukraine, there were about 4,380,047 registered and confirmed cases of Covid-19, including 101,887 lethal ones Ukraine Ministry of Health, 'Update on the spread and prevention of COVID-19 coronavirus infection' (Kyiv, Ukraine, 2022).

The pandemic character of coronavirus spreading and the aggressive behavior of its causal agent made the detection of new mutations in its genome a priority (Zi-Wei Ye et al, 2020; Phun T, 2020; MacLean OA et al, 2021, Ridgway et al, 2022). A further field of investigation became the possible recombination of the virus, either of natural or laboratory origin, that can impact pathogenicity and virus contagion (Rabi FA et al, 2020; Zhou P et al, 2020; Lam T T-Y et al, 2020; Liu P et al, 2020; Sia S F et al, 2020; Ridgway et al, 2022).

Phylogenetic analysis demonstrated that the coronavirus of pangolin (pangolin-CoV-2020), along with the group of coronaviruses, circulating in populations of different bat species are genetically related to SARS-CoV-2. For example, the nucleotide sequence identities among the S protein genes between the Bat-CoV-RaTG13 and SARS-CoV-2 were 93.15 %, and between pangolin-CoV-2020 and SARS-CoV-2, 84.52, but the assumption that SARS-CoV-2 originated directly from pangolin-CoV-2020 was not confirmed (Liu P et al, 2020). Seroconversion was observed in racoon dogs, mink and cattle, while for pigs, antibodies were detected only in three out of six references. Transmission of SARS-CoV-2 was observed for mink and raccoon dogs, but no transmission has been observed for cattle and pigs so far (Dunowska, 2023; Nielsen et al, 2023). Moreover, the possibility of spontaneous infecting of domestic dogs with SARS-CoV-2 coronavirus from humans was demonstrated (Sit et al, 2020). The genome sequencing showed that the so-called feline SARS-CoV-2 belongs to the phylogenetic cluster A2a along with most recently isolated samples of human

SARS-CoV-2 (Michelitsch A et al, 2020; Garigliany M et al, 2020; Ruiz-Arrondo I et al, 2021).

According to Doliff and Martens (2022) cats appear to play only a limited role in the spread of SARS-CoV-2. Although cats are indeed are susceptible to the virus and reverse zoonotic transmission from humans to cats is occurring regularly, there is up till now no evidence that SARS-CoV-2 circulates among cats.

Recently, Lan et al (2020) determined that the structure of spike (S)-protein, which binds the receptor of human angiotensin-converting enzyme 2 (ACE2), has 20 key amino acids for the interaction with the receptor-binding site. The studies have already shown that some animals are potentially susceptible to SARS-CoV-2 because they have the same receptor ACE2, which is widespread in different mammal species, such as Syrian hamster (*Mesocricetus auratus*), ferret (*Mustela furo*), mink (*Neogale vison*), cattle (*Bos taurus*) and raccoon dog (*Nyctereutes procyonoides*) (Abdel-Moneim and Abdelwhab 2020; Li F, 2013; Nielsen SS, 2023). Mutations in the locus of the receptor binding domain (RBD) impact the efficiency ACE2, and thus, the susceptibility to SARS-CoV-2 (Jia et al, 2005; Wan Y et al, 2020; Zi-Wei Ye et al, 2020; Fenollar F et al, 2021, Ridgway, 2022; Cherian S et al, 2021; Hoffmann M et al, 2020).

The species of animals that have sequences K31, Y41, N90, and K353 are likely to be susceptible to the SARS-CoV-2 infection, while other species that do not have the mentioned fragments will surely be either less susceptible or resistant to the infection (Devaux et al, 2021).

In addition to currently unknown and susceptible animal hosts of SARS-CoV-2, new mutations in SARS-CoV-2 may become fixed in the virus which may make animal species with low sensitivity to SARS-CoV-2 more susceptible to infection (Gu H et al, 2020; Kumar A et al, 2021 and 2023; McAloose, D et al, 2020; Naqvi A et al, 2020; Dunowska, 2023).

A rapid spreading mutation, N501T in the RBD of S-protein was frequently observed in minks (*Neogale vison*) at mink farms (Elaswad et al, 2020; Domanska-Blicharz K et al, 2021) and in experimentally infected ferrets (*Mustela furo*) (Richard et al, 2020). This mutation N501T was found in almost all SARS-CoV-2 sequences, obtained from biological material of minks in the USA (99 %). The studies of Cai et al (Cai HY, Cai A, 2021) demonstrated that mutation N501T was first found in humans and only two months later – in minks. These authors assumed mutations and eventually new virus variants may first occur in humans, later penetrating and getting fixed in the population of susceptible animals.

Progressive virus mutations provoked the rise of new lineages such as Delta, Epsilon and Omicron (Davies NG et al, 2021; Damas J et al, 2020; Kumar et al, 2023; Markov et al, 2023). This requires testing as many samples of SARS-CoV-2 positive samples for timely detection of possible new variants, lines or mutations of the virus. In Ukraine the Ministry of Health has determined that the Delta and omicron variants have largely replaced the others in recent days (Yakovleva et al, 2022). These authors also mapped lineages and mutations occurring in Ukraine using a large database of Ukrainian whole genome data collected over the past years of the pandemic up to 2021.

The aim of the study. The aim was to gain experience of the animal health services to detect and properly identify SARS-CoV-2 virus and its mutations in Ukraine in relation to possible future spread of the virus in animals.

MATERIALS AND METHODS

Sixteen SARS-CoV-2 positive samples, not sequenced before, were provided by the Center for Public Health of the Ministry of Health of Ukraine. These samples were obtained from hospitalized patients from early October to mid-November of 2021. The viral RNA was isolated from nasopharyngeal swab samples of SARS-CoV-2 qPCR positive (Ct 21–28) patients (males and females) with moderate and severe symptoms who were being hospitalized. The samples were fully anonymized. All samples were transported in a cryobox with dry ice (–70 °C), and after the delivery to the laboratory prior to the tests kept at –70 °C without any protectant added.

Combined DNA/RNA isolation from the samples of biological material was conducted using IndiSpin Pathogen kit (Indical Bioscience) according to the manufacturer's recommendations, using 200 ul of sample. Efficacy of this kit was verified in our laboratory, using an internal control RNA sample provided by Seegene, South Korea as a part of 2019-nCoV Assay kit (Ref RV10248X, LOT RV9120H23) (synthetic specific fragments of viral RNA to be used as positive control for RT-qPCR assay for SARS-CoV-2 detection).

The amount of RNA isolated was determined using Qubit RNA HS assays kits (Life Technologies, Carlsbad, California, USA) on a Qubit 3.0 Fluorometer (Life Technologies, Carlsbad, California, USA) according to manufacturer's manual. The presence of SARS-CoV-2 in the purified RNA samples for the downstream steps was estimated by fluorescence detection of RdRp and N genes using the RT-qPCR kit Allplex SARS-CoV-2 Assay (Seegene, South Korea), Ref RV10248X, LOT RV9120H23 according to manufacturer's protocol on a plate-type amplifier Bio-Rad^{tm} 1000 with module CFX96^{tm} using the built-in software to evaluate the result.

The cDNA was obtained using a SuperScript^{tm} VILO^{tm} cDNA synthesis kit (Invitrogen, Carlsbad, CA, the USA) according to the manufacturer's protocol and amplicons was produced using the Ion AmpliSeq SARS-CoV-2 panel and a protocol published by ThermoFisher. The samples selected for sequencing had the cDNA concentration of 12–15ng/µl) measured using a Qubit 3.0 fluorometer (ThermoFisher Scientific, USA) with Qubit™ dsDNA HS Assay Kit (ThermoFisher Scientific, USA).

All libraries were prepared using the Ion One Touch^{tm} instrument (ThermoFisher Scientific, USA), and subjected to templating runs. The library quality was checked and confirmed by standard agarose gel electrophoresis.

Sequencing of SARS-CoV-2 virus was conducted by NGS using the Ion GeneStudio™ S5 System and Ion 520™ Chip Kit with 6 barcodes per chip (ThermoFisher Scientific, USA).

Reads were basecalled using TorrentSuite 5.16.1 software, (ThermoFisher Scientific, the USA). The following plugins were used to conduct the bioinformational analysis: SARS_CoV_2_coverageAnalysis 5.16.0.4, SARS_CoV_2_variantCaller 5.16.0.5, COVID19AnnotateSnpEff 5.16.0.5, IRMAreport 1.3.0.2 and generate Consensus 5.16.0.10.

To conduct the phylogenetic analysis, we used Nextclade 2.3.0 (Aksamentov I et al, 2021) to locate the 6 sequenced samples on the global phylogenetic tree. We also determined phylogenetic relations between our 6 sequences and 495 verified sequences of high quality, reported in Ukraine and deposited in the GISAID EpiCoV™ database, (https://gisaid.org/).

The bioinformational analysis was conducted using Augur v.15.0.2, which is a component of Nextstrain open-source toolkit (Hadfield et al, 2018). The phylogenetic tree was visualized by the web-realization of Auspice 2.37.3 (Hadfield et al, 2018). The sequence of SARSCoV2 virus isolate Wuhan-Hu-1 (GenBank NC_045512.2) was used as a reference sequence.

Table 1. Characteristics of the sequenced SARS-CoV-2 isolates

Sample ID/GISAID Accession	Collection date	Mapped Reads (NB: not too many reads)	% Target base coverage at 20x genome sequencing	Mean Depth	% Uniformity
hCoV-19/Ukraine/IVM-12/2021 EPI_ISL_13199869	September 15, 2021	247466	99.72	1295	99.07
hCoV-19/Ukraine/CGZ-7/2021 EPI_ISL_13199872	October 4, 2021	1457798	99.96	9604	97.66
hCoV-19/Ukraine/IVM-592/2021 EPI_ISL_13199580	October 23, 2021	268556	98.40	716	95.7
hCoV-19/Ukraine/CGZ-15/2021 EPI_ISL_13200025	November 8, 2021	313298	98.09	475	92.72
hCoV-19/Ukraine/CGZ-17/2021 EPI_ISL_13477111	November 8, 2021	2252933	99.90	13822	92.44
hCoV-19/Ukraine/IVM-an1/2022 EPI_ISL_13199861	January 27, 2022	2987475	99.90	20120	97.52

All studies were carried out in the laboratory of the Research Training Center for Animal Disease Diagnostics at the Institute of Veterinary Medicine of the National Academy of Sciences of Ukraine, BSL-2+ biosafety level, ISO/IEC 17025: 2019 accredited by National Accreditation Agency of Ukraine.

RESULTS

The first stage of the studies involved the confirmation of the state and concentration of the target virus RNA and QC check and the presence of SARS-CoV-2 virus using the RT-qPCR test kit. The best quality samples had an RNA concentration 12–15 ng/which was used to prepare the libraries for sequencing.

From the 16 samples we obtained, six sequences with sufficient quality were deposited in the database https://gisaid.org, inventory number EPI_SET_230516yp (https://doi.org/10.55876/gis8.230516yp). Other samples either had a very low RNA concentration or were not suitable for further sequencing due to considerable degradation.

General description of six samples is presented in **Table 1.**

Table 2. The mutations found in the genome of the investigated SARS-CoV-2 isolates

Nextstrain clade/ Pango lineage	Aminoacid substitutions and deletions
hCoV-19/Ukraine/IVM-12/2021 EPI_ISL_13199869	
21J (Delta)/AY.126 (B.1.617.2.33),	M:I82T, N:D63G, N:R203M, N:G215C, N:D377Y, ORF1a:A206V, ORF1a:V561F, ORF1a:A1306S, ORF1a:S1510F, ORF1a:I1714V, ORF1a:P2046L, ORF1a:P2287S, ORF1a:V2930L, ORF1a:T3255I, ORF1a:T3646A, ORF1b:P314L, ORF1b:M592I, ORF1b:G662S, ORF1b:P1000L, ORF1b:A1918V, ORF1b:K2557R, ORF3a:S26L, ORF3a:G100C, ORF3a:G172V, ORF7a:V82A, ORF7a:T120I, ORF7b:T40I, ORF8:D119-, ORF8:F120-, ORF9b:T60A, S:T19R, S:G142D, S:R158G, S:L452R, S:T478K, S:D614G, S:P681R, S:S689I, S:D950N, S:E156G, S:F157-
hCoV-19/Ukraine/CGZ-7/2021 EPI_ISL_13199872	
21J (Delta)/AY.122 (B.1.617.2.122)	M:I82T, N:D63G, N:R203M, N:G215C, N:D377Y, ORF1a:K261N, ORF1a:A498V, ORF1a:A1306S, ORF1a:P2046L, ORF1a:P2287S, ORF1a:V2930L, ORF1a:T3255I, ORF1a:T3646A, ORF1b:P314L, ORF1b:G662S, ORF1b:P1000L, ORF1b:M1719T, ORF1b:A1918V, ORF3a:S26L, ORF7a:V82A, ORF7a:R118G, ORF7a:T120I, ORF7a:F101-, ORF7a:L102- ORF7b:F16S, ORF7b:T40I, ORF8:D119-, ORF8:F120-, ORF9b:T60A, S:T19R, S:G142D, S:R158G, S:W258R, S:L452R, S:T478K, S:D614G, S:P681R, S:D950N, S:C1248F, S:E156G, S:F157-

Continuation of Table 2

Nextstrain clade/ Pango lineage	Aminoacid substitutions and deletions
hCoV-19/Ukraine/IVM-592/2021 EPI_ISL_13199580	
21J (Delta)/AY.4.2.3 (B.1.617.2.4.2)	M:A2S, M:I82T, N:D63G, N:R203M, N:G215C, N:D377Y, ORF1a:A1306S, ORF1a:P2046L, ORF1a:P2287S, ORF1a:A2529V, ORF1a:V2930L, ORF1a:T3255I, ORF1a:T3646A, ORF1b:P314L, ORF1b:G662S, ORF1b:P1000L, ORF1b:A1918V, ORF1b:V1961F, ORF3a:S26L, ORF7a:V82A, ORF7a:T120I, ORF7b:T40I, ORF8:D119-, ORF8:F120-, ORF9b:T60A, S:T19R, S:R158G, S:A222V, S:L452R, S:T478K, S:D614G, S:P681R, S:D950N, S:E156G, S:F157-
hCoV-19/Ukraine/CGZ-15/2021 EPI_ISL_13200025	
21J (Delta)/AY.126 (B.1.617.2.33)	M:I82T, N:D63G, N:R203M, N:G215C, N:D377Y, ORF1a:S216F, ORF1a:A1306S, ORF1a:P2046L, ORF1a:P2287S, ORF1a:D2506N, ORF1a:V2930L, ORF1a:T3255I, ORF1a:T3646A, ORF1b:I162M, ORF1b:P314L, ORF1b:T634K, ORF1b:G662S, ORF1b:P1000L, ORF1b:A1918V, ORF1b:K2557R, ORF3a:K16T, ORF3a:S26L, ORF7a:V82A, ORF7a:T120I, ORF7b:T40I, ORF8:D119-, ORF8:F120-, ORF9b:T60A, S:T19R, S:R158G, S:L452R, S:T478K, S:D614G, S:P681R, S:I850L, S:D950N, S:E156G, S:F157-
hCoV-19/Ukraine/CGZ-17/2021 EPI_ISL_13477111	
21J (Delta)/AY.122 (B.1.617.2.122)	M:I82T, N:D63G, N:R203M, N:G215C, N:D377Y, ORF1a:K261N, ORF1a:A1306S, ORF1a:P2046L, ORF1a:P2287S, ORF1a:V2930L, ORF1a:T3255I, ORF1a:T3646A, ORF1b:P314L, ORF1b:G662S, ORF1b:P1000L, ORF1b:A1918V, ORF3a:S26L, ORF3a:S40L, ORF7a:P45L, ORF7a:V82A, ORF7a:T120I, ORF7b:T40I, ORF8:D35Y, ORF8:D119-, ORF8:F120-, ORF9b:T60A, S:T19R, S:G142D, S:R158G, S:L452R, S:T478K, S:D614G, S:P681R, S:D950N, S:E156G, S:F157-

End of Table 2

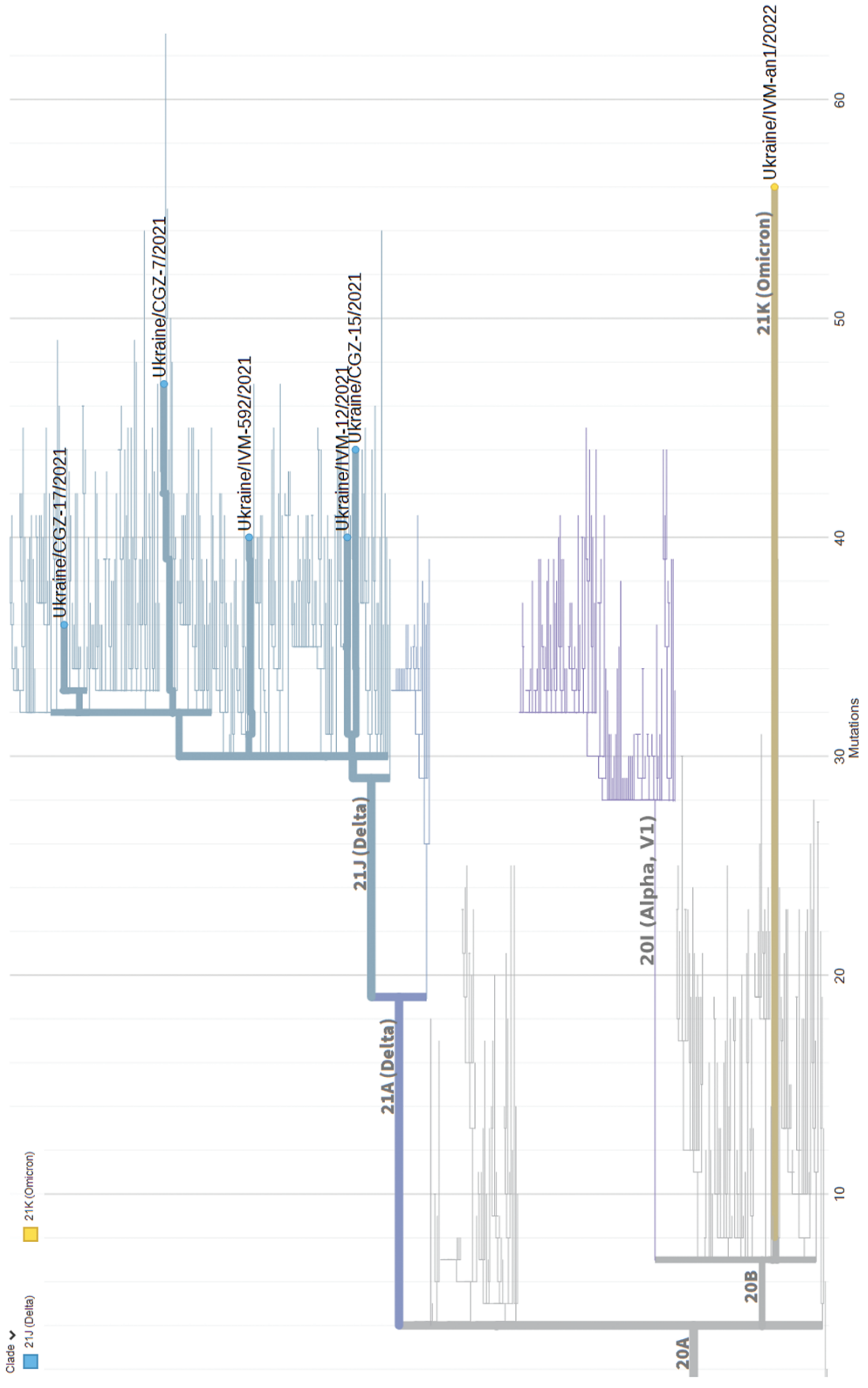
Nextstrain clade/ Pango lineage	Aminoacid substitutions and deletions
hCoV-19/Ukraine/IVM-an1/2022 EPI_ISL_13199861	
21K (Omicron)/ BA.1.18	E:T9I, M:D3G, M:Q19E, M:A63T, N:P13L, N:R203K, N:G204R, N:E31-, N:R32-, N:S33- ORF1a:K856R, ORF1a:T1822I, ORF1a:L2084I, ORF1a:A2710T, ORF1a:T3255I, ORF1a:P3395H, ORF1a:I3758V, ORF1a:S2083-, ORF1a:L3674-, ORF1a:S3675-, ORF1a:G3676- ORF1b:P314L, ORF1b:I1566V, ORF3a:D155Y, ORF8:I121-, ORF9b:P10S, ORF9b:E27-, ORF9b:N28-, ORF9b:A29-, S:A67V, S:Y145D, S:L212I, S:G339D, S:S371L, S:S373P, S:S375F, S:K417N, S:N440K, S:G446S, S:S477N, S:T478K, S:E484A, S:Q493R, S:G496S, S:Q498R, S:N501Y, S:Y505H, S:T547K, S:D614G, S:H655Y, S:N679K, S:P681H, S:N764K, S:D796Y, S:N856K, S:D936Y, S:Q954H, S:N969K, S:L981F, S:H69-, S:V70-, S:G142-, S:V143-, S:Y144-, S:N211-

The sequences of all the samples, collected and aligned according to the reference genome showed areas of uneven reading, the depth of coverage in which is less than 20 times. These areas were also observed in the envelope spike protein gene (G-gene), responsible for binding to the receptors of the target cell, the mutations in which are of special interest since they are related to the changes in pathogenicity.

According to the PANGO nomenclature (Aine O'Toole et al, 2021), five isolates belonged to Delta variants (two of lineage AY.126 (B.1.617.2.33), two of AY.122 (B.1.617.2.122), and one AY.4.2.3 (B.1.617.2.4.2)) and one to the Omicron variant (BA.1.18) (Table 2). According to the data of the global database of the sequenced GISAID isolates, the virus of Delta variant was first detected in India on October 2020. One of our six isolates was related to Omicron variant (line BA.1.18), which was first detected in South Africa and Botswana in November 2021. This variant is characterized by multiple mutations in RGB region, more than observed in the Delta variant.

The presence of the following mutations related to infectivity was determined 1) for the Delta variants: T478K, L452R mutations in the RBD regions, and 2) for the Omicron variant: S371L, G339D, S375F, S373P, K417N, N440K, S477N, G446S, E484A, T478K, Q493R, Q498R, G496S, N501Y, and Y505H in the RBD (**Table 2**).

Among the samples, sequenced by us, the furthest one from the reference sequence in the phylogenetic tree is Omicron variant (BA.1.18) sequenced in this study over 60 mutations (**Figure**), with 32 specifically found in the spike protein (A67V, L212I, Y145D, G339D, S373P, S371L, K417N, S375F, N440K, G446S, S477N, E484A, T478K,Q493R, N856K, Q498R, G496S, N501Y, Y505H, T547K, Q954H, P681H, D614G, H655Y,N764K, N679K, N969K, and D796Y,), distinguishing it from the wild-type strain. In comparison, the Delta variant isolates display nine spike protein mutations and an additional thirteen in other regions. Among the Omicron isolate mutations, 26 are unique to this variant, while 10 and 6 are unique to Delta and the Beta strain, respectively. Noteworthy mutations in Omicron span various regions of the viral genome, including ORF1a, ORF1b, ORF9b, and structural proteins like envelope (E), membrane (M), and nucleocapsid (N). Omicron's spike protein mutations are particularly extensive, encompassing substitutions such as N501Y and deletions like H69/V70. Additionally, other mutations involve amino acid substitutions and deletions at specific genomic positions (Table 2).



The phylogenetic tree of a total of 501 samples of SARS-CoV-2 genome from GISAID database, selected within the period from December 2019 to January 2022 in Ukraine. Complete sequences of the 6 isolates sequenced by us are indicated in the phylogenetic tree. The horizontal axis shows the divergence level i.e. the number of mutations in the genome as compared to the initial strain Wuhan-Hu-1 (number in the GenBank database: MN908947)

DISCUSSION

The results, obtained in the study, are in agreement with the published data, according to which the five Delta isolates and the one Omicron variant we tested, were characterized by multiple mutations in S gene, which is accompanied by faster spreading and a considerable decrease in virus pathogenicity (Davies NG et al, 2021; Chatterjee S et al, 2023; Jacobs et al, 2023; Liang, 2023; Jung et al, 2022).

The method and equipment we used enables determination of mutations in the SARS-CoV-2 genome, including unknown ones (Elaswad A et al, 2020; Yuan S et al, 2020; Phun T, 2020; Kumar A et al, 2021; Fischhoff IR et al, 2021; MacLean OA et al, 2021).

The volume of sequence reading is crucial because it directly impacts coverage, accuracy, sensitivity, and reliability of sequencing data. Higher volume ensures better coverage, accuracy in variant detection, sensitivity to detect rare variants, and statistical confidence in results; it should be at least one million readings for SARS-CoV-2 genome, which is minimally sufficient to obtain a whole sequence of SARS-CoV-2 virus genome. We obtained more than one million readings per sample (Aine O'Toole et al., 2021). The Spike protein mutation H69/V70 which we detected in our Omicron isolate belonging to lineage BA.1.18 was reported before in lineage BA.1 by Zaman et al (2022) and Kandeel et al (2023).

The combination of high transmissibility and the presence of a considerable number of people who may be hosts of SARS-CoV-2 virus creates a threat of virus transmission into the populations of animals. Seroconversion was observed in racoon dogs, mink and cattle, while for pigs, antibodies were detected only in three out of six references. Transmission of SARS-CoV-2 was observed for mink and raccoon dogs, and no transmission was observed for cattle and pigs (Dunowska, 2023; EFSA, 2023). In Ukraine according to official statistics the six mink farms united by the Ukrainian Stock Breeders Association raise 1.1 million minks, which is about 95 % of the Ukrainian market. All products are exported. Since government agencies do not test mink farms for the presence of SARS-CoV-2, it is possible that coronavirus outbreaks can occur at any time. These assumptions are in agreement with previous publications (Devaux et al, 2021; EFSA, 2023).

According to Doliff and Martens (2022) cats appear to play only a limited role in the spread of SARS-CoV-2. Although cats are indeed are susceptible to the virus and reverse zoonotic transmission from humans to cats is occurring regularly, there is up till now no evidence that SARS-CoV-2 circulates among cats.

Some published data have proposed that the Omicron variant may have arisen from cross-species transmission, given its specific combination of mutations, particularly in the spike protein responsible for host cell entry (Sun et al, 2022; Burki, 2022; Kupferschmidt, 2021; Doliff & Martens, 2022; Carabelli et al, 2023). Therefore, it seems unlikely that this hypothesis will be conclusively confirmed in the future. Nevertheless, these concerns underscore the critical importance of conducting extensive surveillance of potentially vulnerable animal populations.

At present the scientific community uses several classification systems of SARS-CoV-2 variants: GISAID, Nextstrain and PANGO. PANGO differs from other systems as it uses dynamic nomenclature, focused on actively circulating lines of the virus and those, spreading to new areas (Rambaut et al, 2020). At the time of the study, the Omicron variant was just spreading, but at the time of publication of this article, the lines that were common in 2020–2022 (Alpha, Beta, Delta) have completely disappeared and been replaced by newer variants (currently, according to https://covid.cdc.gov/covid-data-tracker/, the Omicron JN.1 variant dominates). This process is likely to continue, so the relevance of whole-genome studies aimed at identifying new mutations and new variants will remain (Rambaut et al, 2021; Aine O'Toole, 2021). To facilitate communication beyond the scientific audience, the World Health Organization developed a system of marking the virus variants using the letters of the Greek alphabet. At present, this system is for the following virus variants B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma), B.1.617.2 (Delta), and BA.1.18 (Omicron) are grouped into Variants of Concern (VOC), while B.1.427/B.1.429 (Epsilon), P.2 (Zeta), B.1.525 (Eta), P.3 (Theta), B.1.526 (Iota), B.1.617.1 (Kappa) are mentioned as Variants of Interest (VOI)(https://www.who.int/publications/m/item/updated-working-definitions-and-primary-actions-for--sars-cov-2-variants).

To refine the future goal, the researchers at the veterinary medicine institutions of National Academy of Agrarian Sciences (NAAS) of Ukraine, aim to create and implement a comprehensive survey and monitoring program for SARS-CoV-2 in animal populations. This initiative involves collaborating with relevant veterinary institutions to sample and monitor specific animal species susceptible to the virus, mainly minks

and cats. The goal is to conduct regular surveillance to detect any potential transmission or mutation events, thereby contributing to the early detection and control of the virus in animal populations.

CONCLUSIONS

The whole-genome sequencing of 6 isolates of SARS-CoV-2 virus was performed, and three sublines of the variant Delta were found: AY.126 (B.1.617.2.33), AY.122 (B.1.617.2.122), AY.4.2.3 (B.1.617.2.4.2) and one Omicron (BA.1.18), which were deposited in the international database GISAID as EPI_SET_230516yp. The data obtained in this study add to the existing ones delivered by the Ministry of Health in Ukraine and can be used in laboratories, (including veterinary laboratories), detecting the SARS-CoV-2 virus in risk animal populations to prevent the spread of the disease to humans and animals, as well as to detect possible mutational changes in the pathogen genome that may affect infectivity and pathogenicity of the virus.

Adherence to ethical principles. Only viral RNA was used in the study without any connection to any personal data; the methodological approaches complied with the requirements of IEC/ISO 17025 standard.

Conflict of interests. The authors declare the absence of any conflicts of interests.

Financing. The study was conducted with the financial support of the State Fund for Research of Ukraine, grant No. 2020.01/0121.

Повногеномне секвенування вірусу SARS-COV-2 та вивчення генетичної варіабільності

С. А. Ничик [1], М. С. Мандигра [2], М. В. Безименний [1], Н. В. Гудзь [1], А. В. Моложанова [1], *О. А. Тарасов [1]

[1] Інститут ветеринарної медицини Національної академії аграрних наук України, вул. Донецька, 30, м. Київ, Україна, 03154
[2] Національна академія аграрних наук України, вул. Михайла Омеляновича-Павленка, 9, м. Київ, Україна, 01010

E-mail: snychyk@gmail.com, mandygra@gmail.com, nomax@ukr.net, gudznataly@gmail.com, vetereneri@ukr.net, *tarasovaleksandr@gmail.com

orcid: https://orcid.org/0000-0002-4789-4334, https://orcid.org/0009-0002-3944-7195, https://orcid.org/0000-0001-8042-0061, https://orcid.org/0000-0002-2175-1431, https://orcid.org/0009-0002-8166-8979, https://orcid.org/0000-0003-1481-552988

Мета. Метою роботи було вивчення можливості установ ветеринарної медицини щодо виявлення та правильної ідентифікації вірусу SARS-CoV-2 методом повногеномного секвенування та його генетичної варіабільності в Україні у зв'язку з можливим поширенням вірусу серед тварин у майбутньому. **Методи.** Шістнадцять позитивних зразків SARS-CoV-2, які раніше не були секвеновані, були надані Центром громадського здоров'я Міністерства охорони здоров'я України. Ці зразки були отримані від госпіталізованих пацієнтів з початку жовтня до середини листопада 2021 року. Вірусна РНК була виділена зі зразків мазків з носоглотки пацієнтів (чоловіків і жінок) з позитивними результатами ПЛР (Ct 21-28), які перебували на стаціонарному лікуванні, з помірними та тяжкими симптомами SARS-CoV-2. Зразки були повністю анонімними. Для секвенування зазначених ізолятів SARS-CoV-2, що походять з України, використовували прилад Ion Torrent S5 (Oxford Nanopore, США). Для обробки та аналізу даних використовували програму TorrentSuite 5.16.1. Для філогенетичного аналізу використано програму Nextclade 2.3.0 та визначено місцезнаходження 6 секвенованих зразків на глобальному філогенетичному дереві. Визначено філогенетичні зв'язки між протестованими 6-ма послідовностями та 495 верифікованими високоякісними послідовностями, зареєстрованими в Україні та депонованими в базі даних GISAID EpiCoV™ (https://gisaid.org/) за період з січня 2020 р. по грудень 2022 р. При порівнянні отриманих послідовностей використовували послідовність ізоляту вірусу SARS-CoV-2 Wuhan-Hu-1 (GenBank NC_045512.2) як референтну послідовність, у відповідності до якої проводили вирівнювання отриманих послідовностей геному вірусу. Всі дослідження проводили в лабораторії Науково-навчального центру з проблем діагностики хвороб тварин Інституту ветеринарної медицини НААН. **Результати.** З 16 досліджених ізолятів, підтверджених як такі, що містять РНК вірусу SARS-CoV-2, лише шість ізолятів було секвеновано із достатньою кількістю прочитань, п'ять з них віднесено до дельта-варіантів (два належать до лінії AY.126 (B.1.617.2.33), два – до AY.122 (B.1.617.2.122) та один – до AY.4.2.3 (B.1.617.2.4.2)), а один ізолят – до омікрон-варіанту (BA.1.18). Важливими мутаціями, виявленими в досліджених ізолятах, були заміна S:N501Y та делеція S:H69 в гені білка шипа оболонки вірусу. Серед досліджених ізолятів було виявлено, що варіант Omicron (BA.1.18) демонструє більшу генетичну мінливість, з більш ніж 60 мутаціями порівняно з попередніми варіантами. У нашому дослідженні ми ідентифікували мутації і в секвенованих варіантах Delta, починаючи від 35 мутацій в AY.122 (B.1.617.2.122) до 41 мутації в AY.126 (B.1.617.2.33) в геномі відностно референтного варіанту Wuhan-Hu-1 (MN908947). Важливими мутаціями, що впливають на інфекційність, були: 1) для дельта-варіантів: T478K, L452R мутації в регіоні RBD, і 2) для варіанту Omicron: S371L, G339D, S375F, S373P, K417N, N440K,

S477N, G446S, E484A, T478K, Q493R, Q498R, G496S, N501Y та Y505H мутації в регіоні RBD. **Висновки.** Проведено повногеномне секвенування 6 ізолятів вірусу SARS-CoV-2 та виявлено три сублінії дельта-варіанту: AY.126 (B.1.617.2.33), AY.122 (B.1.617.2.122), AY.4.2.3 (B.1.617.2.4.2) та одну сублінію для варіанту Omicron (BA.1.18), які були депоновані в міжнародній базі даних GISAID як EPI_SET_230516ур. Дані, отримані в цьому дослідженні, доповнюють існуючі дані Міністерства охорони здоров'я України і можуть бути використані в лабораторіях (у тому числі ветеринарних), що можуть проводити генетичні дослідження щодо виявлення вірусу SARS-CoV-2 в популяціях тварин з групи ризику, з метою запобігання поширенню хвороби серед людей і тварин, а також для виявлення можливих мутаційних змін у геномі збудника, які можуть впливати на інфекційність і патогенність.

Ключові слова: SARS-CoV-2, Covid-19, повногеномне секвенування, мутації, філогенетика.

REFERENCES

Abdel-Moneim AS, Abdelwhab EM (2020) Evidence for SARS-CoV-2 Infection of Animal Hosts. Pathogens, 9(7). https://doi.org/10.3390/pathogens9070529

Ahmed R et al (2021) Host range projection of SARS-CoV-2: South Asia perspective. Infection, Genetics and Evolution 87:104670. https://doi.org/10.1016/j.meegid.2020.104670

Aine O'Toole, Emily Scher, Anthony Underwood et al (2021) Assignment of epidemiological lineages in an emerging pandemic using the pangolin tool. Virus Evolution. https://doi.org/10.1093/ve/veab064

Aksamentov I, Roemer C, Hodcroft EB, & Neher RA (2021) Nextclade: clade assignment, mutation calling and quality control for viral genomes. J Open Source Software 6(67):3773. https://doi.org/10.21105/joss.03773

Burki T (2022) The origin of SARS-CoV-2 variants of concern. Lancet Infect Dis 22:174–175. https://doi.org/10.1016/S1473-3099(22)00015-9

Cai HY, Cai A (2021) SARS-CoV-2 spike protein gene variants with N501T and G142D mutation dominated infections in minks in the US. J Veter Diagnostic Investigation 33(5):939–942. https://doi.org/10.1177/1040638 7211023481

Carabelli AM, Peacock TP, Thorne LG et al (2023) COVID-19 SARS-CoV-2 variant biology: immune escape, transmission and fitness. Nat Rev Microbiol 21(3):162–177. https://doi.org/10.1038/s41579-022-00841-7

Chatterjee S et al (2023) A Detailed Overview of SARS-CoV-2 Omicron: Its Sub-Variants, Mutations and Pathophysiology, Clinical Characteristics, Immunological Landscape, Immune Escape, and Therapies. Viruses 15(1): 167. https://doi.org/10.3390/v15010167

Cherian S et al (2021) Convergent evolution of SARS-CoV-2 spike mutations, L452R, E484Q, and P681R, in the second wave of COVID-19 in Maharashtra, India. BioRxiv https://doi.org/10.1101/2021.04.22.440932

Damas J et al (2020) Broad host range of SARS-CoV-2 predicted by comparative and structural analysis of ACE2 in vertebrates. Proceedings of the National Academy of Sciences 117(36):22311–22322. https://doi.org/10.1073/pnas.2010146117

Davies NG, Abbott S, Barnard RC, Jarvis CI et al (2021) Estimated transmissibility and severity of the novel SARS-CoV-2 variant of concern 202012/01 in England. *MedRxiv. https://doi.org/10.1101/2020.12.24.20248822

Devaux CA et al (2021) Spread of Mink SARS-CoV-2 Variants in Humans: A Model of Sarbecovirus Interspecies Evolution. Front Microbiol 12. https://doi.org/ 10.3389/fmicb.2021.675528

Doliff R, Martens P. (2022) Cats and SARS-CoV-2: A Scoping Review. Animals (Basel) 12(11):1413. https://doi.org/10.3390/ani12111413

Domanska-Blicharz K et al (2021) Mink SARS-CoV-2 Infection in Poland – short communication. J Veter Res 65(1):1–5. https://doi.org/10.2478/jvetres-2021-0017

Dunowska M (2023) Cross-species transmission of coronaviruses with a focus on severe acute respiratory syndrome coronavirus 2 infection in animals: a review for the veterinary practitioner. N Z Vet J 71(4):159–171. https://doi.org/10.1080/00480169.2023.2191349.

Elaswad A et al (2020) Mutational spectra of SARS-CoV-2 isolated from animals. PeerJ 8:e10609. https://doi.org/10.7717/peerj.10609

Fenollar F et al (2021) Mink, SARS-CoV-2, and the Human-Animal Interface. Frontiers in Microbiology 12:663815. https://doi.org/10.3389/fmicb.2021.663815

Ferretti L et al (2020) Quantifying SARS-CoV-2 transmission suggests epidemic control with digital contact tracing. Science 368(6491):eabb6936. https://doi.org/10.1126/science.abb6936

Fischhoff IR et al (2021) Predicting the zoonotic capacity of mammal species for SARS-CoV-2 (preprint) https://doi.org/10.1101/2021.02.18.431844

Garigliany M et al (2020) SARS-CoV-2 Natural Transmission from Human to Cat. Emerging Infectious Diseases 26(12):3069–3071. https://doi.org/10.3201/eid2612.202223

Gorbalenya AE et al (2020) Coronaviridae Study Group of the International Committee on Taxonomy of Viruses. Nature Microbiology 5(4):536–544. https://doi.org/10.1038/s41564-020-0695-z

Gu H et al (2020) Adaptation of SARS-CoV-2 in BAB/c mice for testing vaccine efficacy. Science, 369(6511), 1603–1607. https://doi.org/10.1126/science.abc4730

Hadfield J et al (2018) Nextstrain: real-time tracking of pathogen evolution. Bioinformatics 34(23):4121–4123. https://doi.org/10.1093/bioinformatics/bty407

Hoffmann M et al (2020) SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clini-

cally Proven Protease Inhibitor. Cell 181(2). https://doi.org/10.1016/j.cell.2020.02.052

Jia HP et al (2005) ACE2 receptor expression and severe acute respiratory syndrome coronavirus infection depend on differentiation of human airway epithelia. J Virol 79(23):14614–14621. https://doi.org/10.1128/JVI.79.23.14614-14621.2005

Jacobs J et al (2023) COVID-19: Challenges of Viral Variants. Ann Rev Med.74:31–53. https://doi.org/10.1146/annurev-med-042921-020956

Jung et al (2022) Omicron: What Makes the Latest SARS-CoV-2 Variant of Concern So Concerning? J Virol 96. https://doi.org/10.1128/jvi.02077-21

Kandeel A et al (2023) Comparison of SARS-Cov-2 omicron variant with the previously identified SARS-Cov-2 variants in Egypt, 2020–2022: insight into SARS-Cov-2 genome evolution and its impact on epidemiology, clinical picture, disease severity, and mortality. BMC Infect Dis 23(1):542. https://doi.org/10.1186/s12879-023-08527-y

Kim YI et al (2020) Infection and Rapid Transmission of SARS-CoV-2 in Ferrets. Cell Host & Microbe 27(5): 704–709.e2. https://doi.org/10.1016/j.chom.2020.03.023

Kumar A et al (2021) Predicting susceptibility for SARS-CoV-2 infection in domestic and wildlife animals using ACE2 protein sequence homology. Zoo Biology 40(1): 79–85. https://doi.org/10.1101/2021.02.18.431844

Kumar R, Srivastava Y, Muthuramalingam P, et al (2023) Understanding Mutations in Human SARS-CoV-2 Spike Glycoprotein: A Systematic Review & Meta-Analysis. Viruses 15(4):856. https://doi.org/10.3390/v15040856

Kupferschmidt K (2021) Where did 'weird' Omicron come from? Science 374:1179. https://doi: 10.1126/science.acx9754

Lam TT-Y et al (2020) Identifying SARS-CoV-2-related coronaviruses in Malayan pangolins. Nature 583(7815): 282–285. https://doi.org/10.1038/s41586-020-2169-0

Lan J et al (2020) Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. Nature 581(7807):215–220. https://doi.org/10.1038/s41586-020-2180-5

Li F (2013) Receptor recognition and cross-species infections of SARS coronavirus. Antiviral Res 100(1):246–254. https://doi.org/10.1016/j.antiviral.2013.08.014

Li R, Pei S, Chen B et al (2020). Substantial undocumented infection facilitates the rapid dissemination of the novel coronavirus (SARS-CoV-2). Science 368(6490):489–493. https://doi.org/10.1126/science.abb3221

Liang F (2023) Quantitative Mutation Analysis of Genes and Proteins of Major SARS-CoV-2 Variants of Concern and Interest. Viruses 15:1193. https://doi.org/10.3390/v15051193

Liu P et al (2020) Are pangolins the intermediate host of the 2019 novel coronavirus (SARS-CoV-2)? PLoS Pathogens 16(5):e1008421. https://doi.org/10.1371/journal.ppat.1008421

Liu Y et al (2021) Functional and genetic analysis of viral receptor ACE2 orthologs reveals a broad potential host range of SARS-CoV-2. Proc Nat Acad Sci 18(12): e2025373118. https://doi.org/10.1073/pnas.2025373118

Lu R, Zhao X, Li J et al (2020) Genomic characterisation and epidemiology of the 2019 novel coronavirus: implications for virus origins and receptor binding. The Lancet 395(10224):565–574. https://doi.org/10.1016/s0140-6736(20)30251-8

MacLean OA et al (2021) Natural selection in the evolution of SARS-CoV-2 in bats created a generalist virus and highly capable human pathogen. PLoS Biology 19(3): e3001115. https://doi.org/10.1371/journal.pbio.3001115

Markov PV, Ghafari M, Beer M et al (2023) The evolution of SARS-CoV-2. Nat Rev Microbiol 21(6):361–379. https://doi.org/10.1038/s41579-023-00878-2

McAloose D et al (2020) From People to Panthera: Natural SARS-CoV-2 Infection in Tigers and Lions at the Bronx Zoo. mBio 11(5):e02220-20. https://doi.org/10.1128/mBio.02220-20

Michelitsch A et al (2020) Occurrence of Antibodies against SARS-CoV-2 in the Domestic Cat Population of Germany. Vaccines 8(4):772. https://doi.org/10.3390/vaccines8040772

Naqvi AAT, Fatima K, Mohammad T et al (2020) Insights into SARS-CoV-2 genome, structure, evolution, pathogenesis, and therapies: Structural genomics approach. Biochimica et Biophysica Acta (BBA) – Molecular Basis of Disease 1866(10):165878. https://doi.org/10.1016/j.bbadis.2020.165878

Nielsen SS et al (2023) SARS-CoV-2 in animals: susceptibility of animal species, risk for animal and public health, monitoring, prevention and control. EFSA J 21(2):e07822. https://doi.org/10.2903/j.efsa.2023.7822

Patterson EI et al (2020) Evidence of exposure to SARS-CoV-2 in cats and dogs from households in Italy. Nature Communications 11:6231. https://doi.org/10.1038/s41467-020-20097-0

Phun T (2020) Genetic diversity and evolution of SARS-CoV-2. Infection, Genet Evolut 81:104260. https://doi.org/10.1016/j.meegid.2020.104260

Rabalski L et al (2020) Detection and molecular characterization of SARS-CoV-2 in farmed mink (Neovison vison) in Poland (preprint). https://doi.org/10.1101/2020.12.24.422670.

Rabi FA et al (2020) SARS-CoV-2 and Coronavirus Disease 2019: What We Know So Far. Pathogens 9(3)231. https://doi.org/10.3390/pathogens9030231

Rambaut A et al (2020) A dynamic nomenclature proposal for SARS-CoV-2 lineages to assist genomic epidemiology. Nat Microbiol. https://doi.org/ 10.1038/s41564-020-0770-5

Richard M et al (2020) SARS-CoV-2 is transmitted via contact and via the air between ferrets. Nature Communications 11(1):3496. https://doi.org/10.1038/s41467-020-17367-2

Ridgway et al (2022) Understanding the Driving Forces That Trigger Mutations in SARS-CoV-2: Mutational Energetics and the Role of Arginine Blockers in COVID-19 Therapy, Viruses 14:1029. https://doi.org/10.3390/v14051029

Ruiz-Arrondo I et al (2021) Detection of SARS-CoV-2 in pets living with COVID-19 owners diagnosed during the COVID-19 lockdown in Spain: A case of an asymptomatic cat with SARS-CoV-2 in Europe. Transboundary and Emerging Diseases 68(2):973–976. https://doi.org/10.1111/tbed.13803

Sagulenko P, Puller V, Neher RA (2018) TreeTime: Maximum-likelihood phylodynamic analysis. Virus Evol 4(1). https://doi.org/10.1093/ve/vex042

Sia S F et al (2020) Pathogenesis and transmission of SARS-CoV-2 in golden hamsters. Nature 583(7818):834–838. https://doi.org/10.1038/s41586-020-2342-5

Sit THC, Brackman CJ, Ip SM et al (2020) Infection of dogs with SARS-CoV-2. Nature 586:776–778 https://doi.org/10.1038/s41586-020-2334-5

Sun Y, Lin W, Dong W, Xu J (2022) Origin and evolutionary analysis of the SARS-CoV-2 Omicron variant. J. Biosaf. Biosecurity 4:33–37. https://doi.org/10.1016/j.jobb.2021.12.001

Ukraine, M.O.H.O. (2022) Update on the spread and prevention of COVID-19 coronavirus infection https://www.kmu.gov.ua/en/news/operativna17-informaciya-proposhirennya-ta-profilaktiku-covid-19

Wan Y et al (2020) Receptor Recognition by the Novel Coronavirus from Wuhan: an Analysis Based on Decade-Long Structural Studies of SARS Coronavirus. J Virology 94(7). https://doi.org/10.1128/JVI.00127-20

Yakovleva et al (2022) Tracking SARS-COV-2 variants using Nanopore sequencing in Ukraine in 2021. Nat Sci Rep 12:15749. https://doi.org/10.1038/s41598-022-19414-y

Yan Y, Chang L, Wang L (2020) Laboratory testing of SARS-CoV, MERS-CoV, and SARS-CoV-2 (2019-nCoV): Current status, challenges, and countermeasures. Reviews in Medical Virology 30(3):e2106. https://doi.org/10.1002/rmv.2106

Ye Z-W et al (2020) Zoonotic origins of human coronaviruses. Inter J Biol Sci 16(10):1686–1697. https://doi.org/10.7150/ijbs.45472

Yuan S, Jiang S C, & Li Z L (2020) Analysis of Possible Intermediate Hosts of the New Coronavirus SARS-CoV-2. Front Veter Sci 7:379. https://doi.org/10.3389/fvets.2020.00379

Zaman K, Shete AM, Mishra SK, Kumar A et al (2022) Omicron BA.2 lineage predominance in severe acute respiratory syndrome coronavirus 2 positive cases during the third wave in North India. Front Med (Lausanne) 2(9):955930. https://doi.org/10.3389/fmed.2022.955930

Zhang Y-Z, Holmes EC (2020) A Genomic Perspective on the Origin and Emergence of SARS-CoV-2. Cell 181(2):223–227. https://doi.org/10.1016/j.cell.2020.03.035

Zhao J, Cui W, Tian BP (2020) The Potential Intermediate Hosts for SARS-CoV-2. Front Microbiol 11:580137. https://doi.org/10.3389/fmicb.2020.580137

Zhou P et al (2020) A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 579(7798):270–273. https://doi.org/ 10.1038/s41586-020-2012-7

Zi-Wei Ye et al (2020) Zoonotic origins of human coronaviruses. Inter J Biol Sci 16(10):1686–1697. https://doi.org/10.7150/ijbs.45472