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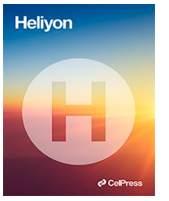

Research article

Genetic characteristics of SARS-CoV-2 virus variants observed upon three waves of the COVID-19 pandemic in Ukraine between February 2021–January 2022

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A R T I C L E I N F O

Keywords:
COVID-19
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VOC Alpha
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A B S T R A C T

The aim: of our study was to identify and characterize the SARS-CoV-2 variants in COVID-19 patients' samples collected from different regions of Ukraine to determine the relationship between SARS-CoV-2 phylogenetics and COVID-19 epidemiology.
Patients and methods: Samples were collected from COVID-19 patients during 2021 and the beginning of 2022 (401 patients). The SARS-CoV-2 genotyping was performed by parallel whole genome sequencing.
Results: The obtained SARS-CoV-2 genotypes showed that three waves of the COVID-19 pandemic in Ukraine were represented by three main variants of concern (VOC), named Alpha, Delta and Omicron; each VOC successfully replaced the earlier variant. The VOC Alpha strain was presented by one B.1.1.7 lineage, while VOC Delta showed a spectrum of 25 lineages that had different prevalence in 19 investigated regions of Ukraine. The VOC Omicron in the first half of the pandemic was represented by 13 lines that belonged to two different clades representing B.1 and B.2 Omicron strains. Each of the three epidemic waves (VOC Alpha, Delta, and Omicron) demonstrated their own course of disease, associated with genetic changes in the SARS-CoV-2 genome. The observed epidemiological features are associated with the genetic characteristics of the different VOCs, such as point mutations, deletions and insertions in the viral genome. A phylogenetic and transmission analysis showed the different mutation rates; there were multiple virus sources with a limited distribution between regions.
Conclusions: The evolution of SARS-CoV-2 virus and high levels of morbidity due to COVID-19 are still registered in the world. Observed multiple virus sourses with the limited distribution between regions indicates the high efficiency of the anti-epidemic policy pursued by the Ministry of Health of Ukraine to prevent the spread of the epidemic, despite the low level of vaccination of the Ukrainian population.

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1. Introduction

In the last one and half years (2021–2022) at least three epidemic COVID-19 waves were observed in each country in the world, caused by SARS-CoV-2 virus infection. The genomic material of this virus is encoded by a single-stranded positive-sense RNA. Actually, the SARS-CoV-2 RNA is longer than any other viral RNA [1]. Evolution of the SARS-CoV-2 virus by genetic alterations lead to appearance of new strains and lineages with different epidemiological and clinical characteristics [2–5], leading to the emergence of new epidemic waves of morbidity in the world. These waves differ in properties in different countries, as well as in variants of SARS-CoV-2. The WHO has classified the variants identified with fitness-enhancing mutations as variants of concern (VOC), variants of interest (VOI) or variants under monitoring (VUM) [6]. During the last two years, at least 6 emergent strains – the VOCs - SARS-CoV-2 strains were registered by WHO, from the VOC Alpha to Omicron [7–9]. Every VOC strain of SARS-CoV-2 has specific structural genomic alterations, acquired during active viral evolution [10]. VOCs showed increased transmissibility compared to the original virus and have the potential for increasing disease severity [11]. The biggest epidemic waves were caused by VOC Alpha (first registered in UK), Delta (first registered in India) and Omicron (first registered in South Africa) (https://cov-lineages.org./lineage_list.html) [12]. The VOC Alpha wave was registered from January to June of 2021, according to the Covariant [13], NEXTSTRAIN (https://nextstrain.org/ncov/gisaid/global/all-time) and GISAID databases (https://www.epicov.org/epi3/frontend#3a964b). The highest rate of Alpha strain detection was observed in April–May 2021. The number of such cases reached 42–43% of the total number of detected SARS-CoV-2 variants. It should be noted that clinical symptoms and the number of hospitalized patients and death cases were significantly higher, according to the WHO data in all countries, compared with the earlier COVID-19 wave observed in 2020 [14]. During this time, 8 of the Aplha variants were found (Q1-Q8), descended from the maternal B.1.1.7 strain.

Later on, the more aggressive and contagious Delta variant of SARS-CoV-2 appeared, causing the next world epidemic COVID-19 wave, in parallel with the other VOC strains (GISAID database, 2022). This wave was more prominent than the previous Alpha outbreak; transmissibility was higher by more than 60% over the VOC Alpha [15]. The Delta wave began in May–June of 2021, and the infection rate reached up to 97–98% from all detected variants in the world in September–November 2021. COVID-19 was diagnosed in millions of people, high mutagenicity was observed for the Delta strain. Due to that, 211 new variants (AY.x) were registered in different countries [12]. Combined with high human mobility, the Delta strain got caught quickly continent by continent, despite anti-epidemic measures.

The last of the study period and the most prominent epidemic wave was caused by the VOC Omicron (B.1.1.529). This highly contagious strain is still actively spreading around the world, infecting hundreds of thousands of people in different countries. In the Pangolin classification, more than 100 Omicron-related lineages were registered.

Of note, these SARS-CoV-2 variants have become dangerous not only for the elderly population, but also for all adults, young people and even children. That made it even more difficult to stop the spread of COVID-19 around the world [16]. Importantly, many SARS-CoV-2 lineages induced different clinical symptoms, partial vaccine escape and high transmissibility during virus evolution [17].

Considering all mentioned above, the aim of the present study was to identify and characterize the SARS-CoV-2 variants isolated from samples of COVID-19 patients that were collected in different regions of Ukraine. The relationship between SARS-CoV-2 phylogenetics and COVID-19 epidemiology has been investigated as well.

2. Materials and methods

2.1. Patient samples

Nasopharyngeal swabs from Ukrainian Covid-19 patients were collected in the period February 2021–January 2022 by the staff of the Center for Public Health of the Ministry of Health of Ukraine. Sampling was conducted in accordance with the Helsinki declaration and approved by the Ministry of Health of Ukraine in agreement between Public Health of the Ministry of Health of Ukraine and the Institute of Molecular Biology and Genetics (IMBG) of NAS of Ukraine. IMBG carried out research under an agreement with the Public Health Center of the Ministry of Health of Ukraine. All patients had acute respiratory symptoms and were positive for SARS-CoV-2 tested by a specific qPCR test. The study group had 401 samples.

2.2. SARS-CoV-2 whole genome sequencing (WGS)

It was performed using an Ion S5 Plus next-generation sequencing system (Thermo Fisher Scientific, USA). Total RNA isolation was performed, using a Virus DNA/RNA isolation kit (ZymoResearch, USA), according to manufacturer protocol. cDNA synthesis was carried out with the use of a SuperScript IV VILO Master Mix (INVITROGEN, USA). The targeted libraries for sequencing were prepared with the help of the SARS-CoV-2 specific primers pools 1 and 2 from an Ion AmpliSeq SARS-CoV-2 Research Panel (Thermo Fisher Scientific, USA), barcoded with an Ion Xpress™ Barcode Adapters Kit and an Ion AmpliSeq Plus Library Kit (Thermo Fisher Scientific, USA), following the user's guides as it was described earlier [18]. Emulsion PCR was performed on a One Touch 2 machine (Thermo Fisher Scientific, USA), followed by enrichment and loading onto the Ion S5 530 chip. NGS was performed on Ion GeneStudio S5 Plus System, according to manufacturer protocols. Bioinformatic and statistical analysis were conducted by the Torrent Suite Software, using the SARS-CoV-2 Research Plug-in Package v1.3.0 (Thermo Fisher Scientific, USA). To assemble the SARS-CoV-2 genome, a reference-based IRMA plugin report was used.

Full length SARS-CoV-2 sequences have been submitted to the GISAID database (https://www.gisaid.org).

2.3. SARS-CoV-2 phylogenetic analysis

EpiCoV Pango lineage v4.0.5 (2022-04-13) tool to assign phylogenetic lineages to genetic sequences has been used. The 338 SARS-CoV-2 full length genomes obtained in our study with high coverage were analyzed using the GISAID online tools. Phylogenetic trees were constructed using the nextstrain/ncov tool (https://github.com/nextstrain/ncov) and visualized with Nextstrain auspice.us open-source project (https://docs.nextstrain.org/projects/auspice/en) [19,20].

2.4. Ukrainian dataset for SARS-CoV-2 and transmission analysis

To analyze the transmission history of the VOC Alpha, Delta and Omicron in Ukraine, all Ukrainian datasets (401 of our samples and 22 samples from GISAID database), containing both, the FASTA sequences and the corresponding epidemiological datasets of patients were created, using GISAID (https://www.gisaid.org) [20]. Exclusively, high coverage data of the Alpha (97 samples), Delta (250 samples) and Omicron (76 samples) variants were used.

We used the phylogenetic pipeline and approach described by Perera et al. [21]. The pipeline consisted of the following steps: (i) data extraction; (ii) Multiple Sequence Alignment (MSA); (iii) parameterization and phylogenetic inference; (iv) transmission tree generation; (v) data visualization.

Within each data set (Alpha, Delta and Omicron) we applied the TransPhylo R package [22], using the dated phylogeny obtained with TreeTime. The Ukrainian data sets appeared to be sampled at a lesser rate than in other studies of SARS-CoV-2 transmission analysis, suggesting a large number of unsampled sources to be remaining in the Ukrainian population. Hence, we corrected the algorithm and ran it for 100000000 Markov Chain Monte Carlo iterations, using the infrastructure of a MolDynGrid virtual laboratory (http://moldyngrid.org), based on Nordugrid ARC Middleware in the Ukrainian National Grid infrastructure (http://grid.nas.gov.ua) [23]. We assumed a Gamma distribution for the generation time with shape 1 and scale 0.01917, according to the phylogenetic pipeline recommendations (https://github.com/theLongLab/TransCOVID).

2.5. Statistical analysis

Statistical processing was performed, using OpenEpi online software (http://www.openepi.com), version 3.0 and Statistica 10 software. The significance of the difference in the frequencies SARS-CoV-2 lineages was evaluated in two by two tables according to calculated $\chi 2$. A two-sample independent *t*-test has been run on data from normally distributed numerical variables (days from COVID-19 onset to hospitalization) to determine the significance of the mean difference across two independent groups. The threshold value of statistical significance for all tests used was $p < 0.05$. The heuristic approach with more than 70% weekly average of maximums of new

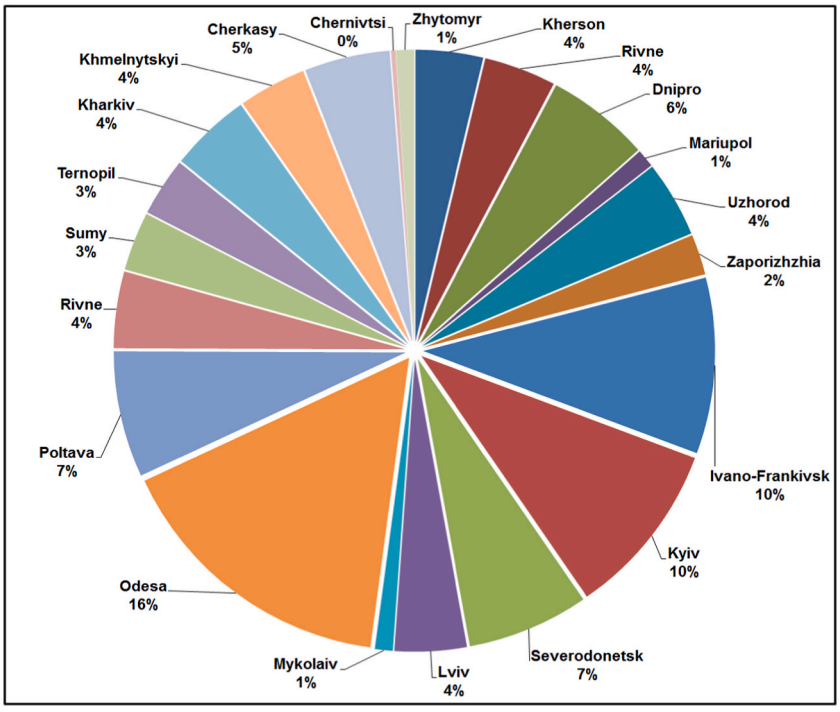

Fig. 1. Geographical distributions of the patients group (n = 401): the regions of Ukraine are represented by the name of the relevant regional center.

COVID-19 cases were used to analyze differences between epidemiological indexes at the peaks of three COVID-19 waves. Kruskal-Wallis and Dunn-Bonferroni post-hoc tests for multiple comparisons were used to determine the significant differences between investigated groups.

3. Results

3.1. SARS-CoV-2 genotyping

During the period from February 2021 to January 2022, 401 SARS-CoV-2 positive samples were collected. The majority of patients

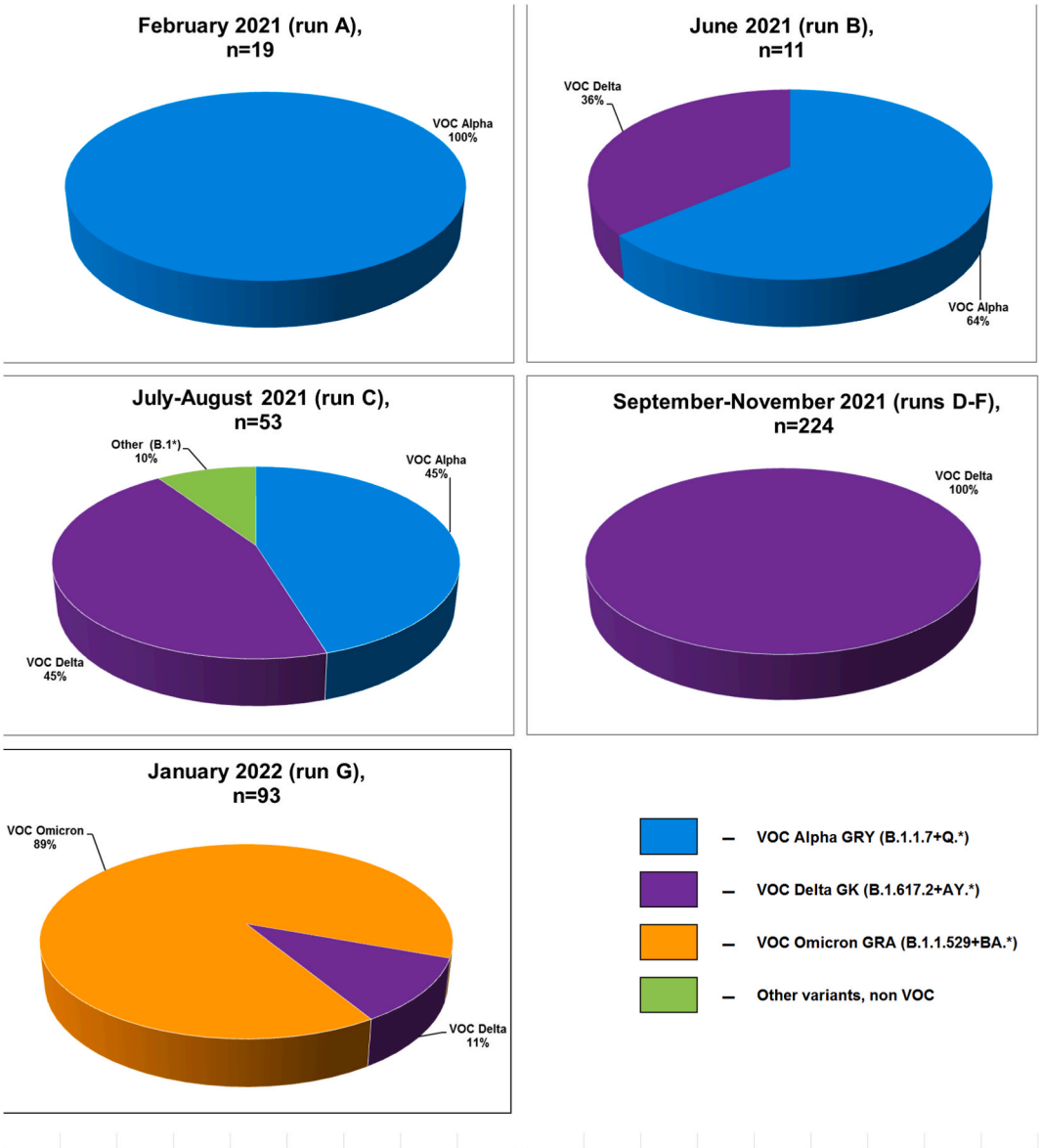

Fig. 2. The spectrum and SARS-variants frequencies in 7 NGS runs: Run A: 17 samples from Ivano-Frankivsk region (Ivano-Frankivsk, Nadvirna, Tysmenytsia, Kosovo, Kalush, Bohorodchany, Brushtyn) and 2 samples from Kyiv; Run B: 11 samples (Kyiv region - 7; Transcarpathian - 2; Kherson - 1; Sumy - 1); Run C: 54 samples (Cherkasy region - 4; Dnipropetrovsk region - 5; Kherson region - 4; Khmelnytsky region - 6; Kyiv region - 10; Volyn region - 7; Donetsk region - 4; Mykolaiv region - 1; Odesa region - 6; Poltava region - 5 Rivne - 2); Run D: 75 samples (Cherkasy - 5; Chernivtsi - 1; Ivano-Frankivsk - 13; Kyiv −1; Lviv - 7; Poltava - 9; Rivne - 6; Luhansk - 27; Ternopil - 4; Zaporizhia - 2); Run E: 75 samples (Dnipropetrovsk - 10; Kharkiv - 8; Kherson - 10; Kyiv - 9; Volyn - 9; Lviv - 9; Odesa - 10; Transcarpathian - 10); Run F: 74 samples (Dnipropetrovsk - 8; Mykolaiv - 3; Odesa - 39; Poltava - 5; Volyn - 3; Sumy - 5; Zaporizhia - 7; Zhytomyr - 4); Run G: 93 samples (Cherkasy - 10; Ivano-Frankivsk - 9; Kharkiv - 10; Khmelnytsky - 9; Kyiv - 10; Odesa - 9; Poltava - 9; Rivne - 6; Sumy - 7; Ternopil - 9; Transcarpathian - 5).

were hospitalized with moderate and severe COVID-19 symptoms (311 patients, 77.6%); for 81 patients there was no data on hospitalization; 9 patients underwent outpatient treatment.

SARS-CoV-2 RNAs were isolated from samples of nasopharyngeal epithelium of patients with COVID-19 from 19 different regions of Ukraine (Fig. 1), aged from 5 months to 90 years old.

The isolated RNA was analyzed by seven next generation sequensing (NGS) runs of SARS-CoV-2 (Fig. 2). For all 401 analyzed samples, whole genome sequencing (WGS) data was of sufficient quality to identify the virus clade. For most samples, the exact variant or lineage of the virus was identified.

The data presented in Fig. 1 shows the coverage of almost all regions of Ukraine. Noteworthy, Odesa, Ivano-Frankivsk and Kyiv regions are most represented in the study.

Fig. 2 shows the WGS data, sorted according to the sampling time. The obtained results showed that the VOC Alpha (lineage B.1.1.7) prevails in Runs A and B. In Runs C, D, E, and F, the different lineages, i.e., the VOC Delta is dominated, whereas in Run G the dominating lineage is the VOC Omicron.

The obtained WGS data were annotated to GISAID. The international online resource GISAID (https://www.gisaid.org) considers SARS-CoV-2 genomes, longer than 29000 nt, as “complete” and further assigns labels of “high coverage” to the genomes that contains les than 1% undefined bases and “low coverage” to genomes with >5% undefined bases. The “complete” genome sequences of SARS-CoV-2 samples were added to the GISAID. From 401 sequenced and analyzed samples there were accepted 345 (S1 Table). To determine the spectrum and frequency of VOC strain mutations as well as for phylogenetic analysis, our GISAID’s "high coverage" samples were used.

3.2. *Epidemiology of the three COVID-19 waves in Ukraine*

According to World Health Organization requirements, the Public Health Center of Ministry of Health (PHC) of Ukraine measured the main indicators of COVID-19, including the daily incidence, new cases of illness, hospitalization and mortality rates, age, etc., that all is presented in an open database on the PHC center website (https://cloud.phc.org.ua/index.php/s/L26pPBzdq8t8yRA). Epidemiological data have undulating fluctuations during the week due to the peculiarities of the registration of patients and their parameters on weekdays and weekends. We analyzed this data during the study period, plus/minus one month.

In the period between the beginning of 2021 to the beginning of 2022, three COVID-19 epidemic waves were registered (Fig. 3), according to open data from the Public Health Center of Ukraine. The maximal incidence rates were observed from the middle of March to the middle of April 2021; the next wave - from the middle of October to the middle of November 2021; the last one - from the middle of January to the middle of February 2022. It should be noted that there was a rather long “interwave” period in the summer of 2021 (between the first and second epidemic waves). The highest peak of the new COVID-19 cases per day was observed for the third wave, while the highest peaks of hospitalization and death of patients from COVID-19 were noted in the second wave.

According to our genotyping data (Runs A-G), these three COVID-19 waves corresponded to dominance of three distinct VOC variants of SARS-CoV-2: Alpha, Delta and Omicron (Fig. 4).

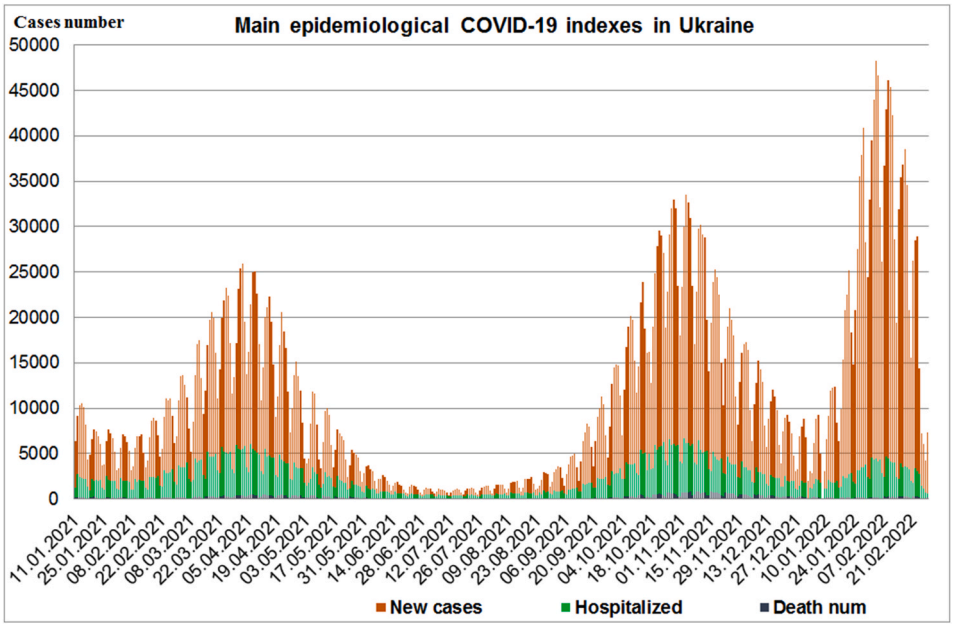

Fig. 3. Epidemiological data of COVID-19 (Main epidemiological indexes): new cases, hospitalization, mortality in Ukraine (open data) between February 2021 – beginning of 2022.

To confirm our data, we analyzed the GISAID SARS-CoV-2 genotyping data of other authors of patients from Ukraine during the investigation period – from the beginning of 2021 to the beginning of 2022 (S2 Table). These data indicate that the VOC Alpha dominates in the first wave, which was displaces by the VOC Delta in the second wave, which gave way to the VOC Omicron in the third epidemic wave. Therefore, they could call these COVID-19 waves the VOC Alpha, VOC Delta and VOC Omicron.

Each successive wave of COVID-19 from Alpha to Omicron has increased maximal peak levels in detecting new disease cases. If the VOC Alpha wave at its peak maximum has about 19–21 thousand new cases per day, then the Delta wave already has 26–27 thousand, which is about 30% higher than at the peak maximum of the Alpha wave (fig. 3 and S1 Fig.). VOC Omicron wave has shown 43–44 thousand new cases per day at the peak's maximum, which is more than two times higher (more than 200%) than VOC Alpha peak and more than 65% higher than VOC Delta peak. These data are consistent with the incidence data described by VOC in other countries, which indicate an increase in the transmissibility of VOC Delta compared to VOC Alpha by up to two to two and a half times [15] and an increase in the transmissibility of Omicron compared to Delta by hundreds of times [24].

To assess the statistically significant difference in epidemiological indicators (morbidity, hospitalization, mortality, etc.) between the three COVID-19 waves, they were taken their peak values for time periods that correspond to more than 70% of the maximum COVID-19 weekly average incidence in each wave, respectively. This data has shown significant differences ($p < 0.01$) between all three waves (Alpha, Delta and Omicron) in new cases detection rates according to Kruskal-Wallis and Dunn-Bonferroni post-hoc tests for multiple comparisons (Table 1).

However, despite significant increases in morbidity (new cases) in each new wave, hospitalization and mortality rates have different characteristics and dependencies on them (S2 Fig. and S3 Fig.). Hospitalization rates at peaks Alpha and Delta are practically the same in absolute units (cases per day). There are approximately 5500 and almost 6000 cases of maximum waves, respectively. Hospitalization of patients in the Omicron wave is just over 4000 cases at the peak of incidence. This difference seems insignificant, not even reaching 50%. However, if we take into account the real levels of new cases in every wave and time period of disease with more than 70% from incidence rate, then the reduction in hospitalization during the Omicron wave has significant differences ($p < 0.01$) compared to VOC Alpha and Delta waves (Table 1). Moreover, this index is more than three times lower compared with Alpha, and more than two times compared with Delta wave as a percentage of relative hospitalization to peak incidence wave rates (S4 Fig). These data indicate a significant reduction in hospitalization of patients during the VOC Omicron wave in Ukraine.

The next important indicator of a COVID-19 pandemic is the mortality of patients from this disease. Comparison of these indicators at the maximums of three waves in absolute units indicates that the VOC Alpha wave patients' death per day was 450–480 cases; the VOC Delta mortality maximum was 800–820 cases, while for VOC Omicron wave mortality was only 280–300 cases.

Based on these data, there was a slight increase in mortality in Delta wave compared to Alpha (about 50%) and a significant decrease in mortality with Omicron, which was more than 2.5 times lower, compared with Delta and about 40% compared with Alpha. According to the Kruskal-Wallis and Dunn-Bonferroni post-hoc test for multiple comparisons, there were found significant statistical differences in peak death levels between all three waves ($p < 0.01$) (Table 1). Calculation of the percentage from relative rates of mortality to the new cases of disease (incidence rate) in the peaks of different waves has shown that death numbers during VOC Omicron (S4 Fig.) were more than three and four times lower than during the Alpha and Delta waves, respectively.

The described epidemiological indexes were given for all age groups of Ukrainian patients. According to the PHC data for different age groups, all studied COVID-19 waves have their own age characteristics. Thus, the incidence of children (under 18 years old) (S5 Fig.) has increased more than two times with each successive wave at a maximum value. Peak indicators also indicate statistically significant increases in the incidence of children during the VOC Delta and Omicron waves ($p < 0.01$) by more than 2 and 4 times with Delta and Omicron, respectively, compared with the VOC Alpha wave.

Age indicators of morbidity and mortality in eight groups from birth to over 70 years for three waves (S6 Fig. and S7 Fig.) have shown changing levels of both morbidity and mortality in patients of different age groups during three different waves of COVID-19. Although childhood morbidity increased in each wave by more than 2 times, however, in the total number of cases at the Omicron peak, where the highest childhood morbidity was noted, this did not exceed 7–8% of the total number of registered new cases. The largest percentage of new cases in all COVID-19 waves are middle and older age groups over 30 years old. Moreover, for the group

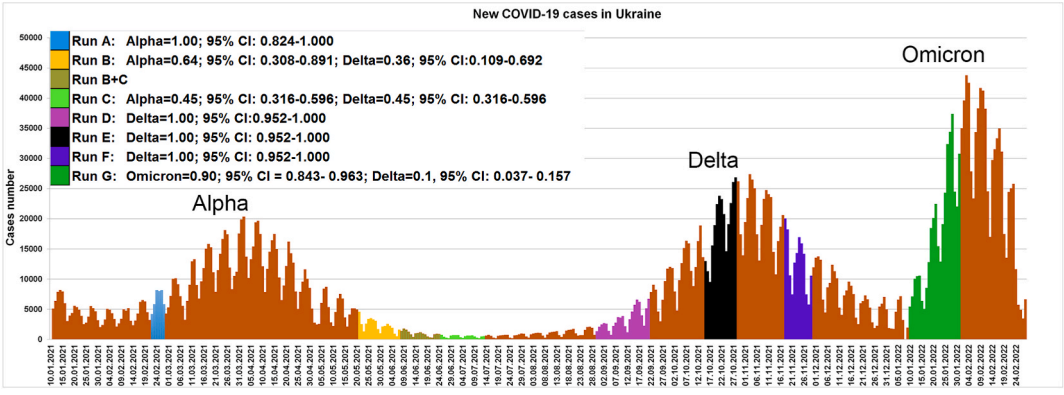

Fig. 4. Dates of sampling for the NGS run according to three COVID-19 waves in Ukraine.

Table 1
Comparative analysis of epidemiological indicators in three VOCs waves in Ukraine.

Groups	VOC	Median	25-th percentile	75-th percentile	p-value Alpha vs Delta	p-value Alpha vs Omicron	p-value Delta vs Omicron
New cases	VOC Alpha	13276	10408	16331	0.0001	<0.0001	0.0042
	VOC Delta	20691	16864	23922			
	VOC Omicron	31125	24440	37351			
Hospitalized	VOC Alpha	4359	3202	4912	0.2555	0.0021	<0.0001
	VOC Delta	4887	3680	5470			
	VOC Omicron	3284	2566	3935			
Death numbers	VOC Alpha	308	252	430	<0.0001	0.0009	<0.0001
	VOC Delta	631	461	727			
	VOC Omicron	192	142	265			
Children new cases	VOC Alpha	606	483	747	<0.0001	<0.0001	0.0014
	VOC Delta	1463	1144	1633			
	VOC Omicron	2872	2427	4056			

Note: p-values according to the Dunn-Bonferroni post-hoc test for multiple comparisons.

older than 70 years (70+), there is a slight increase in the number of cases in the Delta and Omicron waves compared to Alpha. Whereas for groups from 30 to 69 there is a significant increase in the incidence in the VOC Delta and VOC Omicron waves. In addition, the age group of young patients 20–29 years showed a very significant increase in the incidence rate (more than two and a half times) in the VOC Omicron wave compared to VOC Alpha. Seq data ages in Omicron are lower than in Delta (our data above).

Comparing the death rate in the three waves of COVID-19 (S7 Fig.) indicates that the highest rates are in the age group over 70 years. Mortality rates are almost half lower in the 60–69 age group and almost half lower than in the previous group, in the 50–59 age group. Moreover, the highest mortality rates are observed in the VOC Delta wave, and the lowest rate is in the Omicron wave.

It was analyzed age group data (S1 Table) of three different VOCs groups for sequenced samples. Statistical analysis (Kruskal-Wallis and Dunn-Bonferroni post hoc test) has shown significant differences ($p = 0.013$) between VOCs Alpha and VOC Omicron age groups at the median values of 56.3 and 46.2 years, respectively. Whereas the VOC Delta group had an intermediate median value (51.6 years) and had no significant difference between the two previous groups. There weren't found significant differences in sex index between the three VOC groups at approximately the same incidence of morbidity in both sexes during the investigation time.

Thus, these data suggest that SARS-CoV-2 genetic changes in the different VOC strains are sufficient to reveal the differences in infectious and epidemiological parameters in Ukrainian patients. This data indicate that these VOC changes ultimately aim to spread among different age groups, increase virus spread and rate, and reduce mortality.

Table 2
Mutations of SARS-Cov-2 in our VOC samples submitted to GISAID.

Mutations			VOC, *n*		
			Alpha, 47	Delta, 232	Omicron, 62
All types, n	Total		3098	9441	4270
	Unic		1780	1781	604
	Major unic*		26	31	78
Amino acid substitutions, n	**Total**		**1471**	**7636**	**2918**
	Unic		623	748	206
	Major unic*		**20**	**29**	**60**
	Missence	**Total**	**1388**	**7583**	**2915**
		Unic	608	731	203
		Major unic*	**18**	**29**	**60**
	Nonsence	**Total**	**83**	**53**	**3**
		Unic	15	17	3
		Major unic*	**2**	**0**	**0**
Inframe deletions, n	**Total****		**1616**	**1803**	**1309**
	Unic**		1146	1031	395
	Major unic*		**6**	**2**	**17**
Inframe insertions	**Total**		**11**	**2**	**43**
	Unic		11	2	3
	Major unic*		0	0	1
	Missence unic		7	2	3
	Nonsence unic		4	0	0

Notes: * - in ≥25% samples; ** - include NNN tracks (low coverage sequences).

Table 3
Summary results of identified lineages by WGS analysis with common mutations in detected VOC strains in Ukraine.

WHO name (Clade)	Lineage PANGO*	Number of samples	First date of detection	Last date of detection	Origine/ lineage	Main mutations (miss, nons//del) with freq>0,1
Alpha (GRY)	B.1.1.7	47	February 22, 2021	July 13, 2021	UK lineage of concern	**Spike_D614G, NSP12_P323L, Spike_S982A, Spike_P681H, N_R203K, N_D3L, Spike_T716I, Spike_N501Y, Spike_D1118H, NSP3_A890D, NS8_R52I, NS8_Q27stop, Spike_A570D//Spike_V70del, Spike_H69del, NSP6_G107del, Spike_Y144del, NSP6_S106del, NSP6_F108del**//*NSP3_T183I, NSP3_I1412T, NS8_Y73C, N_S235F,* N_G204L, NS8_K68stop, N_G204R
Delta (GK)	B.1.617.2	17	June 17, 2021	January 12, 2022	India lineage	**NSP12_G671S, Spike_D614G, Spike_L452R, N_D377Y, N_D63G, N_R203M, NSP12_P323L, M_I82T, NS3_S26L, Spike_P681R, Spike_T19R, Spike_T478K, NSP13_P77L, Spike_E156G, NS7a_T120I, NS7a_V82A, Spike_G142D//Spike_F157del, Spike_R158del**//*NSP4_T492I, N_G215C, NSP14_A394V, Spike_D950 N, NS7b_T40I, NSP3_P1228L, NSP4_V167L, NSP6_T77A, NSP3_A488S, NSP3_P1469S,* NSP2_K81 N, NS7a_P45L, Spike_T95I, Spike_A222V, NSP4_A446V, NSP14_T16I, NSP3_P822L, NSP6_T181I, NSP6_V149A, NS8_E19stop, NS8_W45L, NSP12_R197Q
	AY.4	14	September 02, 2021	January 20, 2022	UK lineage	
	AY.4.2.3	1	unknown	October 25, 2021	European lineage	
	AY.4.5	1	unknown	September 21, 2021	Lithuania lineage	
	AY.5.4	1	unknown	November 23, 2021	European lineage	
	AY.9.2	29	September 15, 2021	October 25, 2021	European lineage	
	AY.25	1	unknown	November 22, 2021	USA lineage	
	AY.29	1	unknown	November 22, 2021	Japane lineage	
	AY.34	1	unknown	October 25, 2021	multiple countries	
	AY.36	12	September 14, 2021	November 23, 2021	Nigeria, UK, USA	
	AY.43	12	August 30, 2021	January 12, 2022	European lineage	
	AY.46	4	July 06, 2021	November 19, 2021	Africa lineage	
	AY.68	1	unknown	July 05, 2021	European lineage	
	AY.82	1	unknown	September 16, 2021	Fiji lineage	
	AY.98.1	3	October 27, 2021	November 23, 2021	European lineage	
	AY.99	1	unknown	June 21, 2021	South Africa, Nigeria	
	AY.103	1	unknown	September 07, 2021	USA lineage	
	AY.106	2	unknown	June 22, 2021	Jordan and other	
	AY.107	1	unknown	June 17, 2021	USA lineage	
	AY.121	9	August 30, 2021	November 22, 2021	European lineage	
	AY.122	102	June 18, 2021	January 21, 2022	European lineage	
	AY.125	1	unknown	September 20, 2021	European lineage	
	AY.126	13	September 13, 2021	November 23, 2021	European lineage	
	AY.127	2	October 25, 2021	October 27, 2021	India and other	
	AY.129	1	unknown	October 25, 2021	European lineage	
Omicron (GRA)	BA.1	5	January 19, 2022	January 31, 2022	*multiple countries*	**E_T9I, M_A63T, N_G204R, N_P13L, N_R203K, NSP12_P323L, NSP14_I42V, NSP4_T492I, NSP5_P132H, Spike_D614G, Spike_D796Y, Spike_G339D, Spike_H655Y, Spike_N679K, Spike_N764K, Spike_N969K, Spike_P681H, Spike_Q954H, Spike_S373P, M_Q19E, Spike_K417N, Spike_T478K, Spike_N440K, Spike_S375F, Spike_G142D//N_E31del, N_R32del, N_S33del, NSP6_G107del, NSP6_S106del**//*Spike_S477 N, Spike_Q493R, Spike_Q498R, Spike_N501Y, Spike_E484A, Spike_A67V, Spike_G446S, Spike_L212I//Spike_H69del, Spike_N211del, Spike_V70del, NSP6_L105del*//NSP3_A1892T, NSP3_K38R, NSP6_I189V, Spike_L981F, Spike_N856K, Spike_S371L, Spike_T547K,
	BA.1.1	23	January 17, 2022	January 31, 2022	*multiple countries*	
	BA.1.1.1	1	January 21, 2022	Unknown	European lineage	
	BA.1.1.13	1	January 31, 2022	Unknown	UK lineage	
	BA.1.1.14	1	January 20, 2022	Unknown	European lineage	

(*continued on next page*)

Table 3 (*continued*)

BA.1.14	2	January 18, 2022	Unknown	Brazil and other	M_D3G, NSP3_L1266I, Spike_G496S, Spike_T95I, Spike_R346K, Spike_Y505H, NS3_T223I, NSP1_S135R, NSP3_G489S, Spike_D405 N, Spike_S371F, N_S413R, NSP13_R392C, NSP15_T112I, NSP4_L264F, Spike_A27S, Spike_T19I, Spike_T376A, NSP4_L438F, Spike_R408S, NSP4_T327I, NS3_H78Y, NSP3_T24I, Spike_V213G//NSP3_S1265del, Spike_Y144del, Spike_Y145del, Spike_V143del, NSP6_F108del, Spike_L24del, Spike_P25del, Spike_P26del
BA.1.17	1	January 21, 2022	Unknown	European lineage	
BA.1.17.2	3	January 20, 2022	January 21, 2022	*multiple countries*	
BA.1.18	2	January 21, 2022	Unknown	Europe, North America	
BA.1.19	4	January 17, 2022	January 21, 2022	European lineage	
BA.2	2	January 29, 2022	January 31, 2022	*multiple countries*	
BA.2.9	16	January 19, 2022	January 29, 2022	European lineage	
BA.3	1	January 20, 2022	Unknown	*multiple countries*	

Notes: mutation frequency (in order of decreasing frequency): **bold** - 1–0.9; *italic* - 0.9–0.7; normal – 0.7–0.1; * - according to https://cov-lineages.org./lineage_list.html.

3.3. Genetic characteristics of detected VOC strains in Ukraine

Analysis of SARS-CoV-2 WGS data showed that three VOC variants were identified (Alpha Delta and Omicron) according to GISAID classification and 39 SARS-CoV-2 lineages according to Pango Lineage classification (Pango v.4.0.6) in 341 samples (Tables 2 and 3). 2 samples were detected as B.1 and 2 samples - B.1.1.159 by Pango Lineage. They are not VOC SARS-CoV-2 samples.

Based on the analysis of the full-length SARS-CoV-2 genomes in our patients, it was found that the total number of mutations per 1 genome gradually increases from Alpha to Omicron. Most amino acid mutations are represented by missense mutations and deletions (Table 2). In Alpha samples, the ratio of missense mutations to deletions is approximately 1:1, in Delta ~4:1, in Omicron ~2:1.

The VOC Alpha is represented by a single B.1.1.7 lineage. During the study period, 47 samples of this strain were identified, starting from February 22, 2021–19 samples have been described earlier [18] and ending on July 13, 2021. It was detected 232 samples VOC Delta, which contains the highest number of lineages (25 lineages), among them a maternal lineage B.1.617.2 and 24 AY.x lineages. The first VOC Delta samples belonged to lineages B.1.617.2 and AY.122 were identified in Run B (June 17 and 18, 2021), the last – in our last Run G (January 2022).47 samples of this strain were identified.

So far, B.1.617.2 and AY.122 are still the most long-lived SARS-CoV-2 lineages in our country. Moreover, these lineages and AY.9.2 were the most widespread among VOC Delta lineages. VOC Omicron has 13 BA.x lineages in 62 SARS-CoV-2 samples.

All these lineages were identified in Run G in January 2022. Among them, BA.1.1 and BA.2.9 lineages are the most widespread.

Detected lineages, according to database data [12] have different world origins, such as European countries and the UK, USA, Africa and other world places including multiple countries of origin. In addition, the identified strains also have a different distribution/spread in different countries and continents.

For all mutations detected in SARS-CoV-2 virus samples, their frequencies have been calculated for each of three VOC strains (Table 2). Mutations with high frequency values (0.9–1.0) could characterize VOC strain alterations, while mutations with medium or low frequency values characterize the features of different lineages in VOC strain or the individual characteristics of specific samples of the SARS-CoV-2 viruses.

Genetic differences between VOC strains SARS-CoV-2 were detected in all types of SARS-CoV-2 virus proteins: structural (4 proteins: S, M, N, E), nonstructural proteins (16 proteins (NSP1-16)) and accessory proteins (11 proteins (NS, ORFx)) [25] in different combinations and frequencies as well as untranslated regions [26]. Unfortunately, today not all genetic alterations of SARS-CoV-2 have the described functions, impact on the virus structure and epidemiological parameters of COVID-19.

In all virus groups, lineages were first identified in different parts of the world and dominated in different countries, according to an international database. The VOC Alpha variant is represented in Ukraine by only one B.1.1.7 lineage which has UK origin. It has been detected in all investigated regions in Ukraine. The main mutations of the virus cover the following genetic elements of the virus, encoding only two structural proteins (from four): S, N; non-structural proteins: NSP3, NSP6, NSP9, NSP12, and only one accessory NS8 protein.

VOC Alpha Ukrainian samples have the most common combination with high frequency of S protein mutation: D614G, P681H, S982A, T716I, D1118H, N501Y, A570D, H69del, V70del, Y144del.

It was detected in VOC Alpha N protein mutation: D3L, R203K, S235F.

The most frequent mutations in non-structural proteins of VOC Alpha samples are: NSP3_A890D, NSP3_I1412T, NSP3_T183I, NSP6_S106del, NSP6_G107del, NSP6_F108del, NSP12_P323L.

VOC Alpha has some highly frequent mutations in accessory protein NS8: Q27stop, R52I, Y73C.

The Ukrainian VOC Alpha samples have differences in the spectrum of additional mutations, which have been found in virus samples (Table 2). Individual genetic alterations of SARS-CoV-2 virus samples require more careful analysis.

VOC Delta strain was the second VOC variant which was detected first on 2021.06.17 as maternal Indian strain B.1.617.2 and 2021.06.18 - AY.122 (European lineage) (Table 3). The most numerous lineages are AY.122 (102 samples) (European lineage), most widespread in Germany, Sweden, USA, Russia; AY.9.2. (29 samples) (European lineage), which, most widespread in Germany,

Netherland, UK, France, Sweden; B.1.617.2 (17 samples) is India lineage which most widely identified in USA, India, UK Turkey, Germany.

Analysis of genetic alterations of VOC Delta lineages has shown, that all Delta lineages have common mutations with high frequency in three structural proteins (Tables 2 and 3): S-, M – , N-protein, whereas VOC Alpha hasn't mutations in M protein. VOC Delta has more mutations in non-structural proteins (compared with VOC Alpha), common to all lineages named NSP3, NSP4, NSP6, NSP9, NSP12, NSP13, NSP14 and specific to some lineages shown non-structural proteins's mutations, which will be showed below. Moreover, VOC Delta has mutations in accessory proteins, which are absent in VOC Alpha: NS3, NS7a, NS7b.

WGS analysis has shown that VOC Delta has a different set of mutations in the S protein, which is quite different from the previous VOC Alpha. The VOC Delta S protein main high frequency mutations are: T19R, G142D, E156G, F157del, R158del, L452R, T478K, D614G, P681R, D950 N.

The T478K mutation has not been observed in previous VOC strains of SARS-CoV-2. It is found in combination with L452R.

High frequency N protein mutations were found in VOC Delta samples: D377Y, D63G, R203 M, G215C. N protein N_G215C mutation has become dominant in the VOC Delta.

Only one mutation in M protein I82T was found in VOC Delta samples.

The most significant VOC Delta mutations in nonstructural proteins are: NSP3_P1228L, NSP3_A488S, NSP3_P1469S, NSP4_T492I, NSP4_V167L, NSP6_T77A, NSP12_G671S, NSP12_P323L, NSP13_P77L, NSP14_A394V. Accessory proteins of VOC Delta SARS-CoV-2 in Ukrainian samples have shown high frequency mutations: NS3_S26L, NS7a_T120I, NS7a_V82A, NS7b_T40I.

These data indicate that the VOC Delta strain has more mutations. They were found both in proteins that already had mutations in the VOC Alpha strain and in other proteins of SARS-CoV-2.

The last VOC strain to enter at the beginning of 2022 to the country is VOC Omicron. During the first month from the start of Omicron circulation, 13 lineages were identified which have originated from multiple countries. The most widely identified two lineages are named BA.1.1 (23 samples) and BA.2.9 (16 samples). VOC Omicron has the highest mutational rate compared to previous VOC Alpha and Delta.

The VOC Omicron strain has mutations with high frequency in all four structural proteins of SARS-CoV-2: S, M, E, N, which may indicate a significant change in viral physiology [27]. Especially a huge number of mutations have been registered in S protein (listed in order of decreasing frequency (Tables 2 and 3): D614G, D796Y, G339D, H655Y, N679K, N764K, N969K, P681H, Q954H, S373P, K417 N, T478K, N440K, S375F, G142D, S477 N, Q493R, Q498R, N501Y, E484A, A67V, G446S, L212I, L981F, N856K, S371L, T547K, G496S, T95I. Among them there are some Alpha and Delta-associated mutations like D614G, G142D, T478K, N501Y, P681H and other.

VOC Omicron samples contained many different missense and nonsense mutations and new types of mutation – insertions. It has three different insertions: Spike_ins214EPE, M_ins39GTIT and Spike_ins152QLK. The insertion of Spike_ins214EPE was identified in 66.1% of samples. 17 different deletions were found with frequency more than 0.2 (in more than 5 samples). Five deletions in different proteins: N_E31del, N_R32del, N_S33del, NSP6_G107del and NSP6_S106del were found in all Omicron samples. The major Omicron AA substitutions are listed in Table 2.

The VOC Omicron specific mutations of other structural proteins (M, N, E proteins) are: M_A63T, M_Q19E, M_D3G, N_G204R, N_P13L, N_R203K, E_T9I. A lot of new common for most lineages of Omicron mutations have found in nonstructural proteins with high frequency: NSP3, NSP4, NSP5, NSP6, NSP12, NSP14: NSP3_A1892T, NSP3_K38R, NSP3_L1266I, NSP4_T492I, NSP5_P132H, NSP6_I189V, NSP12_P323L, NSP14_I42V. It was registered mutations in NSP1, NSP13, NSP15 and NSP16 with medium frequency levels, which may be inherent in certain lineages. Mutations in accessory proteins are almost absent in the VOC Omicron strain.

It should be noted that the VOC Alpha variant has the least number of baseline mutations compared to subsequent VOC Delta and Omicron variants.

In order to evaluate the fundamental/basic genetic differences (hot spot alterations) between different lineages within the VOC strains, it was selected as an example by analyzing SARS-CoV-2 virus samples with the lowest numbers of mutations and the most frequently detected lineages of the SARS-CoV-2 virus over the given time period. These characteristics are presented in the S3 and S4 Tables of the following subsection, along with the names and main functions of the presented proteins that have genetic alterations. This data could help to describe principal mutation differences and to assume genetic-phenotypic changes of the most widely identified lineages in Ukraine for the investigated period.

3.4. Genetic distinguishing features and regional profile of the spread of SARS-CoV-2 lineages in Ukraine

3.4.1. Genetic characterization of VOC Alpha lineages in Ukraine

VOC Alpha has only one B.1.1.7 lineage which was identified in 47 Ukrainian samples in a period from February 22, 2021 to July 13, 2021. High frequency mutations in VOC Alpha/B.1.1.7 lineage were described in Section 3.3.

In Tables 3 and it is presented Ukrainian sample from B.1.1.7 lineage and one of the samples from GISAID database. It should be noted that considered samples have the same set of mutations in different SARS-CoV-2 proteins. The S3 Table shows the SARS-CoV-2 proteins that have genetic changes in the main lineages with their functional names and main functions in the virus cycle [28]. High frequency mutations have been described earlier for VOC Alpha and are presented in Tables 2 and 3 Among mutations with moderate frequency it should be marked the following missense mutations: NS7a_V82A, NS3_G172C, NS7a_T120I, NS7b_T40I, Spike_T307I, Spike_T323I. It have been observed a large number of deletions with fairly low frequency in some nonstructural proteins, which may indicate the individual characteristics of the viruses: NSP6_C223del, NSP6_Y224del, NSP6_F225del, NSP6_G226del, NSP6_L227del, NSP6_F228del, NSP6_C229del, NSP6_L230del, NSP6_L231del, NSP6_N232del, NSP6_R233del, NSP6_Y234del, NSP3_L1096del, NSP3_V1097del, NSP6_Y214del, NSP6_C215del, NSP6_F216del, NSP6_L217del, NSP6_G218del, NSP6_Y219del, NSP6_F220del,

Table 4
VOC Delta lineages distribution by regions in Ukraine.

Region	N samples	Lineage																										
		B.1.617.2	AY.4	AY.4.2.3	AY.4.5	AY.5.4	AY.9.2	AY.25	AY.29	AY.34	AY.36	AY.43	AY.46	*AY.68*	AY.82	AY.98.1	*AY.99*	AY.103	*AY.106*	*AY.107*	AY.121	AY.122	AY.125	AY.126	AY.127	AY.129
Kyiv	18	*	–	–	–	–	–	–	–	–	*	–	–	–	–	–	–	–	–	–	*	*	–	*	*	–
Cherkasy	4	–	–	–	–	–	–	–	–	–	*	*	–	–	–	–	–	–	–	–	–	*	–	–	–	–
Zhytomyr	4	–	–	–	–	–	–	–	–	–	–	*	–	–	–	–	–	–	–	–	–	*	–	–	–	–
Dnipro	17	*	–	*	–	–	*	–	–	–	*	*	–	–	–	–	–	–	*	–	–	*	–	–	–	–
Sumy	4	–	–	–	–	–	–	*	–	–	–	–	*	–	–	–	–	–	–	–	–	*	–	–	–	–
Poltava	15	*	–	–	–	–	–	–	–	–	–	*	–	–	–	–	–	–	–	–	*	*	–	–	–	–
Kharkiv	7	–	–	–	–	–	*	–	–	–	*	–	–	–	–	–	–	–	–	–	–	*	–	–	–	–
Severo-donetsk	26	*	–	–	–	–	*	–	–	–	–	–	*	–	–	–	–	–	–	–	–	*	–	*	–	–
Zapo-rizhzhia	8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	*	–	–	–	*	–	–	–	–
Odesa	49	*	*	–	–	*	–	–	*	–	*	*	*	–	–	*	*	–	–	–	*	*	–	*	–	–
Mykolaiv	1	–	*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	*	–	–	–	–	–
Kherson	9	*	*	–	–	–	–	–	–	*	*	–	*	–	–	–	–	–	–	*	–	*	–	–	–	–
Lutsk	17	*	*	–	–	–	–	–	–	–	*	–	*	*	–	–	–	–	–	–	–	*	–	–	–	–
Lviv	11	–	*	–	–	–	–	–	–	–	–	*	–	–	–	–	–	–	–	–	*	*	–	–	*	–
Rivne	5	–	–	–	*	–	*	–	–	–	–	–	–	–	–	–	–	–	–	–	*	*	–	*	–	–
Ternopil	3	–	*	–	–	–	–	–	–	–	–	–	–	–	*	–	–	–	–	–	–	–	–	–	–	–
Khmelnytskyi	5	–	*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	*	–	–	–	–
Ivano-Frankivsk	13	*	*	–	–	–	*	–	–	–	–	*	–	–	–	–	–	–	–	–	–	*	–	*	–	–
Uzhgorod	16	*	*	–	–	–	–	–	–	–	–	*	–	–	–	–	–	–	–	–	*	*	–	–	–	*

Note: ***bold italic*** – lineages detected in interwave period only.

Table 5
VOC Omicron lineages distribution by regions in Ukraine.

Region	N samples	Lineage												
		BA.1	BA.1.1	BA.1.1.1	BA.1.1.13	BA.1.1.14	BA.1.14	BA.1.17	BA.1.17.2	BA.1.18	BA.1.19	BA.2	BA.2.9	BA.3.1
Kyiv	6	*	*	–	*	–	–	–	–	–	–	*	*	–
Cherkasy	10	*	*	*	–	–	–	*	–	*	–	–	*	–
Sumy	6	–	*	–	–	–	*	–	–	–	–	–	–	–
Poltava	5	*	*	–	–	–	–	–	–	–	*	–	–	–
Kharkiv	7	–	*	–	–	–	–	–	–	–	–	–	–	–
Odesa	4	–	*	–	–	–	–	–	–	–	–	*	*	–
Rivne	4	–	–	–	–	–	–	–	–	–	–	–	*	*
Ternopil	7	–	*	–	–	–	*	–	–	–	*	–	*	–
Khmelnytskyi	5	–	–	–	–	–	–	–	*	–	*	–	–	–
Ivano-Frankivsk	8	–	–	–	–	*	–	–	–	–	–	–	*	–

NSP6_C221del, NSP6_T222del, NSP6_F235del.

We suppose that medium and low frequency mutations in VOC Alpha in Ukrainian samples demonstrate virus evolution and counteract the pressure of external factors (in particular anti-epidemic actions in a specific country), including the characteristics of the state and features of the host's organisms.

3.4.2. Genetic characterization of VOC Delta lineages in Ukraine

Since the VOC Delta has circulated in the Ukrainian population for more than 6 months, its evolution has been more extensive. In this study, 25 different Delta lineages have been identified, which have been detected in all 19 investigated regions in Ukraine (Tables 3 and 4).

Data from Tables 3 and 4 have shown that 7 VOC Delta lineages have been detected in the interwave period (06–08 2021). Among them, 4 lineages observed in different regions did not further spread in the VOC Delta wave: AY.68, AY.99, AY.106, AY.107. Whereas B.1.617.2 and AY.122, which were detected in the interwave period, were widely detected both in the VOC Delta wave and found in the VOC Omicron wave (January 2022).

Unfortunately, we cannot carry out an adequate analysis of the distribution of different Delta lineages in each individual region of Ukraine due to the abnormal distribution of the number of samples and dates of sampling of patients. However, grouping the regions into 4 larger parts, like the central part (Kyiv, Cherkasy, Zhytomyr Dnipro), east-northeast part (Sumy, Poltava, Kharkiv, Severodonetsk), southern part (Zaporizhzhia, Odesa, Mykolaiv, Kherson) and western-northwestern part (Lutsk, Lviv, Rivne, Ternopil, Khmelnytskyi, Ivano-Frankivsk, Uzhgorod) shows that the number of VOC Delta lineages in the central and east-northeast regions is 10–11 lineages, while in the western-northwestern and southern parts is 14–15 lineages, respectively.

One of the most highly detected AY.122 lineage (102 samples) was detected in all 4 parts of Ukraine and in 17 out of 19 studied regions. The next highly detected AY.9.2 lineage (29 samples), was found in only 5 of 19 regions, while the half smaller number B.1.617.2 lineage (17 samples) was found in 9 different regions of Ukraine in all 4 parts of the country. The next highly detected AY.4 lineage was found in 9 regions in only two parts of Ukraine: southern and western-northwestern parts. Three lineages, AY.126, AY.36 and AY.43 with approximately the same level of detection (13-12 samples) were detected in all 4 parts in different regions (5, 7, 8 regions respectively) without any dependence in the detected cases. Several lines more then 10 (Table 4) were revealed in only 1–2 samples during the entire time of the VOC Delta. To make a conclusion and a definite forecast on their distribution and origin is not realistic due to the small number of samples.in only Basic genetic alterations in SARS-CoV-2 samples detected in Ukrainian lineages of VOC Alpha and Delta are presented in the S3 Table.

It is well known that the maternal lineage in VOC Delta is B.1.617.2. Therefore, the differences we have been analyzed which are found in the other nine lineages (AY.x) are widely spread throughout the country according to our data, with the maternal B.1.617.2 as a reference strain. The progeny of the B.1.617.2 lineage have genetic alterations in all protein groups of SARS-CoV-2: structural proteins, non-structural and accessory proteins. The most studied S protein has only single differences in some lineages, which probably indicate the effective structure (combination of mutations) of this protein for a given SARS-CoV-2 strain in this human population at that time.

It is necessary to note the genetic differences of the AY.122 lineage, which entered Ukraine practically together with the B.1.617.2 lineage and spread most widely throughout all regions of the country for the longest time. The AY.122 has two missense mutations in NSP2, which encode an Endosome-associated protein: K81 N, K489 N. This protein has no mutations as in maternal lineage like most of the analyzed lineages.

The second protein is Primase (NSP8), which has unique for some lineages of VOC Delta mutation – NSP8_A25S. This protein initiates de novo replication and is able to synthesize RNA only de novo with a low fidelity on ssRNA templates [28].

Moreover, AY.122 has additional mutations in several proteins, which have some alterations in B.1.617.2 and other lineages: NSP4, NSP14, NS7a.

3.4.3. Genetic characterization of VOC Omicron lineages in Ukraine

VOC Omicron suddenly appeared in Europe and other worlds last autumn. It was characterized by high contagion, milder clinical manifestations and a high rate of spread among the young population. It was assumed that the virus acquired these characteristics due to the extremely large number of mutations in the Spike protein.

The VOC Omicron lineages were initially identified in Ukraine at the beginning of 2022. In our last Run G, in 62 samples, 13 different lineages of this VOC were identified (Table 2). The Ukrainian Omicron lineages belong to three different types – BA.1*, BA.2* and BA.3 and two different Nextstrain Clades (21K, 21L), which correspond to BA.1* and BA.2* respectively. The most represented lineages in the last Run G were BA.1.1 (37,1%) and BA.2.9 (25,8%).

The VOC Omicron variant is represented in Ukraine by 13 lineages, which have been detected in 10 investigated regions in Ukraine (tables 3 and 5).

Data from Tables 3 and 5 have shown that two weeks of collected samples from 10 regions of Ukraine had 13 different lineages of VOC Omicron descended from a BA.1 lineage like the BA.2 lineage with different regional distribution.

The most highly detected lineages are BA.1.1 (23 samples) and BA.2.9 (16 samples). These lineages were identified in all 4 parts except Khmelnytskyi region.

It is not possible to conduct a more detailed analysis of the VOC Omicron other lineages region distribution due to the small number of samples and the short time of investigation in the Omicron wave. What can definitely be said is that already at the beginning of the VOC Omicron wave, many different lineages entered Ukraine, which are likely to compete and change under the influence of selection factors in the pandemic.

The main genetic features of the most widely identified lineages of VOC Omicron in Ukraine are presented in the S4 Table. There are presenting lineage samples with basic mutation combinations.

The reference lineage for Omicron can be considered BA.1, as one of the first lineage in the SARS-CoV-2 B.1.1.529 phylogenetic tree.

The main genetic differences between BA.1 and the widespread BA.1.1 are additional mutations in the S protein, which in VOC Omicron has a huge number of mutations, named: R346K and Y505H. Other BA.1 associated lineages BA.1.17.2 and BA.1.19 have shown additional mutation as in S protein as in nonstructural protein NSP3, which interacts with other viral nonstructural proteins and RNA to form replication/transcription complexes [28]. BA.1.19 is the only one of the presented lineages which has a mutation in NSP16 encoded SARS-CoV-2 Methyltransferase, named L126F. Moreover, BA.1.17.2 has an additional mutation in Nucleocapsid protein (N protein) - S413I.

BA.2 or associated lineages like BA.2.9 have shown more diverse combinations of mutations compared to BA.1, and not only in the already existing proteins (NSP3, NSP5, NSP12, NSP14, S and E proteins), but also in those proteins that did not have mutations in BA.1. Among them are registered mutations in NSP1 (encoded Virulent factor) - S135R, NSP13 (encoded Helicase) - R392C. NS3 (Hole borer) - T223I and additional mutation in BA.2.9 - H78Y. New mutation combinations were also found in proteins that already had them, among them NSP3 - E156D, G489S; NSP4 (encoded Double membrane vesicle marker) - L264F, T327I, L438F. Three new mutations in one protein could play the critical role on this protein function in host cells, which nucleates and anchors viral replication complexes on double-membrane vesicles in cytoplasm. Moreover, BA.2 and BA.2.9 have shown new mutations in structural proteins S, M and E. But the amount of S protein mutations in these lineages has registered about a quarter less than BA.1.

The BA.3 lineage has shown genetic characteristics of both branches BA.1 and BA.2 and its own new genetic alterations like missense mutations N_P326L, NSP6_A88V.

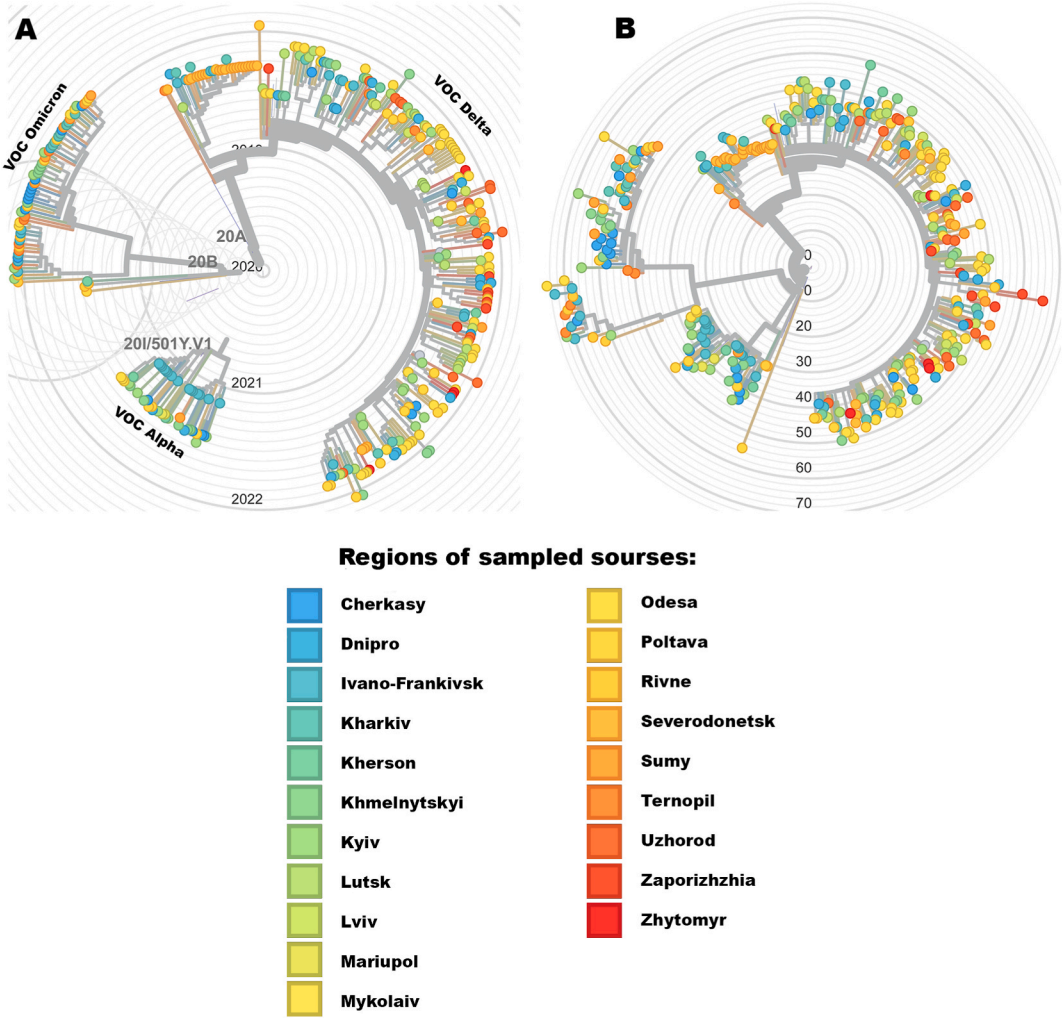

Fig. 5. Visualization of SARS-CoV-2 phylogenetics of 338 Ukrainian samples using Nextstraine Auspice software (https://auspice.us/). (A) clustered by the date of sample collection; (B) clustered by the genetic divergence.

3.5. SARS-CoV-2 phylogenetic analysis

The Nexstrain Auspice open-source software [6] was used for phylogenetic analysis of our samples (338 samples selected by this program for analysis out of 345 samples) (Fig. 5). The least divergences from the reference Wuhan genome have been found in the VOC Alpha and VOC Omicron lineages, which have had the greatest indexes of divergence. VOC Delta has been characterized by the greatest genetic diversity.

Phylogenetic analysis revealed that each of the VOCs - Alpha, Delta and Omicron appeared in Ukraine one after the other. Alpha was completely replaced by Delta in late summer 2021 Fig. 5A. With the advent of Omicron in Ukraine in early 2022, Delta has not completely disappeared Fig. 5A. Unfortunately, we have not been able to investigate the further evolution of the Omicron lineages in Ukraine due to the Russian military invasion of Ukraine.

The least divergences from the reference Wuhan genome have been found in VOC Alpha lineages (from 25 to 47 mutations per genome, Fig. 5B). VOC Delta has been characterized by the greatest genetic diversity (more than 20 different lineages, Fig. 5B). VOC Omicron had the greatest indexes of divergence (more than 60 mutations per genome, Fig. 5B).

These data show the presence of all widely distributed lineages of SARS-CoV-2 in all studied regions of Ukraine.

During the monitoring period, it was possible to trace the full complete evolutionary cycle of only 1 of the 3 VOCs - Alpha - from its appearance in the western regions of Ukraine to its almost complete disappearance. Phylogenetic analysis of Alpha showed that all “early” Alpha variants identified at the end of February 2021 (Ivano-Frankivsk region) spread later to all studied regions of Ukraine.

Phylogenetic analysis of the Delta revealed that this VOC came to Ukraine not at once, but from recurrent sources, both from the East and from the West or South. The first detection of the VOC Delta in June 2021 showed the presence of only 1 lineage (B.1.617.2), although variants of this lineage were quite distant in origin. As in the case of Alpha, various variants of the Delta have become widespread throughout the country, at least in all regions studied. A month later, a very wide range of "early" Delta were detected (B.1.617.2, AY.122, AY.43, AY 46 and others), indicating that these variants were brought to Ukraine from different regions of the world. An important role in the variability of the Delta was played by the fact that in the summer Ukraine showed a certain decline in

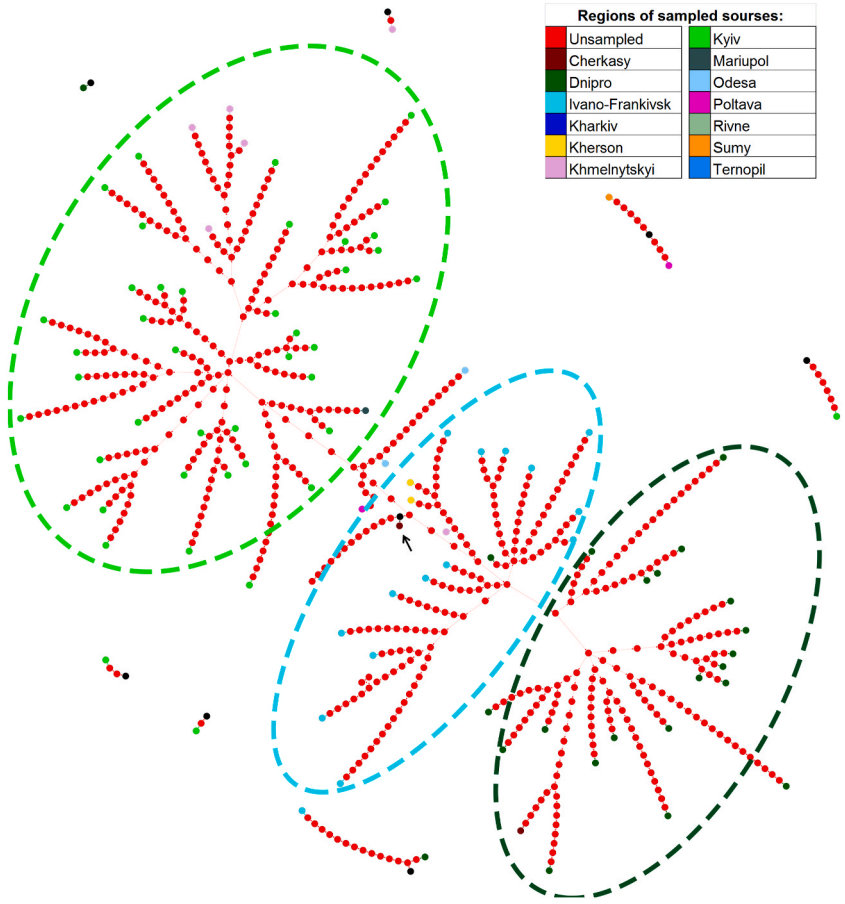

Fig. 6. The transmission network of the VOC Alpha Ukrainian samples (n = 97). Multi-colored nodes – the same sources from the regions shown in the legend, black node – theoretically calculated primary source of the network; red nodes - unsampled sources; light green oval - Kyiv transmission cluster, dark green oval – Dnipro transmission cluster, light blue oval - Ivano-Frankivsk transmission cluster; black arrow – potentially first node in the transmission network. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

the pandemic, and this allowed Ukrainians to visit some countries around the world on vacations. Already in autumn, the evolution of Delt variants has been observed; an increase in the spectrum of lineages and the emergence of evolutionarily "late" Delta lineages (more than 20 lineages of different origins).

The limited phylogenetic analysis of Omicron, which was first detected in Ukraine in early January 2022, was due to a very short observation period - a few weeks only. Nevertheless, it was found that, unlike Alpha and Delta, Omicron was represented in Ukraine from the very beginning by many lineages, and Ukraine immediately received both the original (BA.1 lineage) and "later" lineages of Omicron (BA.2 and BA.3). Therefore, the background of Omicron's genetic diversity in Ukraine allows us to predict its rapid spread across regions and rapid evolution appropriate for this strain in the world, which is already a reality now [29,30].

3.6. Reconstruction of SARS-CoV-2 transmission through the phylogenetic inference of unsampled sources of infection

Using the VOC Alpha, Delta, and Omicron datasets for all Ukrainin samples from the GISAID database described in the Materials and Methods, it was conducted a phylogenetic analysis of the pathways of spread of unselected/undiagnosed sources of infection during the three waves of the COVID-19 pandemic in Ukraine [20–22]. The resulting transmission trees were tested for statistical validity as well as consistency with publicly available epidemiological and other data. We considered BEAST-generated data that had estimated sample sizes (ESS) greater than 200 as statistically significant.

3.6.1. VOC Alpha transmission history in Ukraine

The Ukrainian VOC Alpha dataset was sampled in the period of mid February 2021 to mid-July 2021 from 10 regions of Ukraine. The results of the transmission network of the VOC Alpha of Ukrainian samples are shown in Fig. 6.

The calculated "sources" of SARS-CoV-2 infection (potentially basic samples) in the VOC Alpha network is the sampled node marked by black circles (one of them is close to the center of the transmission tree with a black arrow).

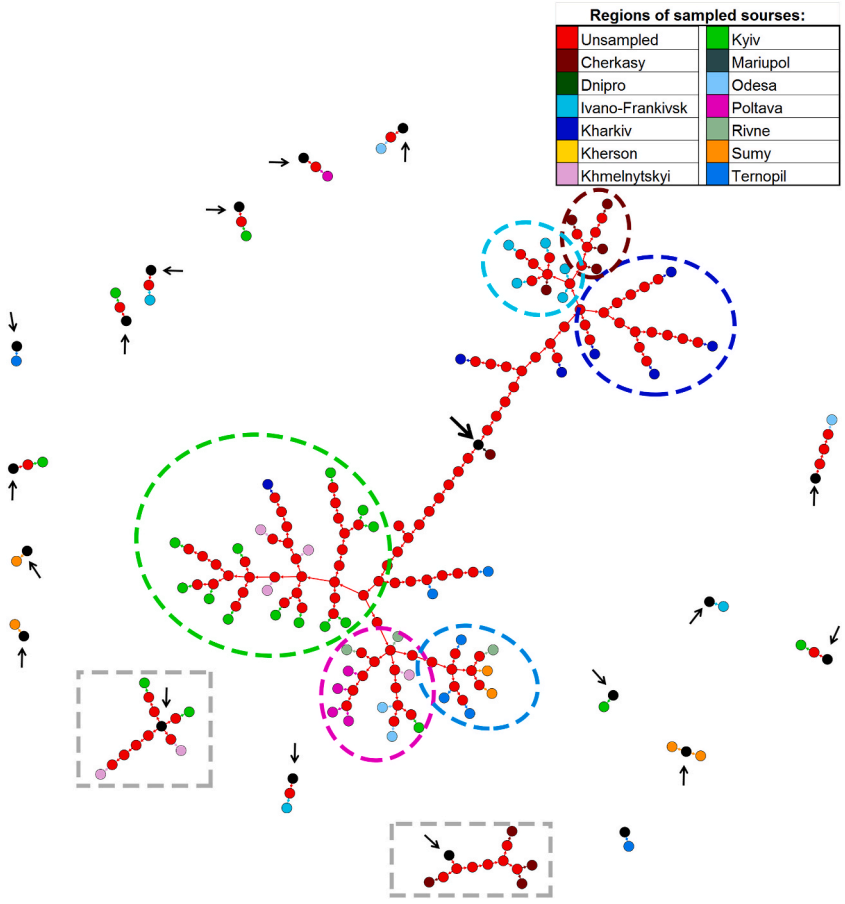

Fig. 7. The transmission network of the Omicron Ukrainian samples (n = 76). Multi-colored nodes – the same sources from the regions shown in the legend, black nodes – theoretically calculated primary sources of the networks, red nodes - unsampled sources; purple oval - Poltava and Odesa regions' cluster, blue oval - Ternopil-Rivne-Sumy cluster, dark blue oval – Kharkiv cluster, brown oval - Cherkasy cluster, light blue oval - Ivano-Frankivsk cluster; black arrow – potentially first node in the transmission network. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

It was calculated that our VOC Alpha transmission network consists of a large number of unsampled sources (87.42% shown in red – Fig. 6) in contrast to the sampled sources. The major samples are from Kyiv region (light green) – 5.58%, Dnipro (dark green) – 2.98%, and Ivano-Frankivsk (light blue) – 1.95%. The statistical analysis revealed that the number of the sampled sequences was not enough for the high significance of the transmission tree. It should be noted the clustering of nodes in three area clusters shown by ovals of the corresponding colors in Fig. 6 from a potentially basic sample (black circle) with two primary networks and one secondary network. The first primary network is from unsampled sources with registered transmissions to Ivano-Frankivsk (dominant samples) and Kherson. Second, the primary network is from unsampled sources with transmissions to Kyiv (dominant samples) and Khmelnytskyi. Secondary network is from the primary Ivano-Frankivsk cluster to unsampled sources with transmissions to the Dnipro cluster. The high level of granularity was not obtained for the studied regions of Ukraine due to the lack of sequences from these regions and a high level of pathogen genome divergence. Despite this significant limitation, some information on the transmission and distribution of VOC Alpha was obtained by reproducing these relationships through the inference of unselected sources of infection, indicating a very dynamic spread of this variant of virus in the Ukrainian regions from many different sources.

3.6.2. VOC Delta transmission history in Ukraine

The VOC Delta dataset was the largest (250 samples) and the most heterogenic in genetic variances (over 15 clusters), locations (19 regions) and sampling period (mid June 2021 – mid January 2022). Based on these characteristics, the highest sampling rate is necessary to build a high-quality transmission tree. Unfortunately, according to our preliminary calculations, it was established that the transmission trees for Delta in Ukraine would contain at least 95% of unassembled nodes. Therefore, the analysis of Delta transmission history would be uninformative and unreliable due to very low sampling rate in Ukraine and high VOC Delta lineage variability.

3.6.3. VOC Omicron transmission tree

The VOC Omicron dataset was the most homogenic one with the shortest sampling period (11–29 January 2022) and 10 distinct locations (Fig. 7). The main advantage for transmission history analysis was that all samples were collected almost at the very beginning of the spread of this VOC in our country.

The predicted source of infection in our Omicron network is an unsampled black node marked by the big black arrow transmitted from the sampled node from Cherkasy (the center of the transmission diagram).

As for VOC Alpha, the VOC Omicron transmission network contains an increased number of unsampled sources compared to the sampled sources. However, this ratio is significantly better (66.96% red circles) than for Alpha (87.42% red circles – Fig. 7), which indicates a better sampling rate (multi-colored circles). We can discriminate the primary transmission clusters for two branches. One branch contains the nodes in areas such as Kyiv (light green oval) from which the largest part of the sampled nodes are represented (8.70%). A high level of detailisation was difficult to achieve for other regions due to the low representation of patients' samples from those regions. However, the approach which has been used to analyze the transmission network made it possible to separate several more clusters. The clusters close to the Kyiv transmission network are the southeastern mixed networks - Poltava and Odesa (purple oval) and the northwestern mixed network - Ternopil-Rivne-Sumy (blue oval). Another branch contains the clusters that have a different origin from Kyiv. Those are: 1) the transmission network of the east of the country – Kharkiv region (dark blue oval); 2) the close to Kharkiv cluster of the southeast - Cherkasy region (brown oval) and, interestingly, 3) the transmission network of the western region - Ivano-Frankivsk region (light blue oval) connected with Cherkasy region.

Moreover, the formations of two small separate clusters (outlined by gray rectangles in Fig. 7), as well as a large number of non-clustered samples, all of which had different theoretically calculated primary sources (black nodes, marked by small black arrows in Fig. 7), were observed. One of the small clusters combined the samples of the Cherkasy region and the mixed network of Kyiv and Khmelnitsky regions.

4. Discussion

This paper describes the WGS (whole genome sequence) of SARS-CoV-2, derived from samples of Ukrainian patients with COVID-19. These samples were collected for almost one year from 19 of 25 regions in Ukraine. Coverage of not all regions is associated with the problems of transporting samples during the lockdown. Unfortunately, the sample collecting and investigations in IMBG were interrupted by the Russian military invasion of Ukraine.

To establish the relationship between the genetic characteristics of SARS-CoV-2 strains and lineages with their biological properties, epidemiological indicators of the disease waves in Ukraine were analyzed. There were three waves of COVID-19 during the study period that were represented by three out of five VOC strains, named Alpha, Delta and Omicron. These three VOC strains were the cause of the three powerful epidemic waves of COVID-19 in the world as well [19,31,32]. It should be noted that approximately 43, 140, and 46 days elapsed from the moment the first sample of the Alpha, Delta, and Omicron strains was detected to the peak of the epidemic wave, respectively (S2 Table). Whether this is due only to the infectious properties of different VOC strains, or their competition between different strains of viruses, which can be observed between Alpha and Delta waves, or seasonal and anti-epidemic factors is not yet known exactly [33,34].

4.1. Mutation's influences on biological properties of SARS-CoV-2 VOC strains

It should be noted that significant genetic differences were observed both between different VOC strains and between lineages of

the same VOC strain, especially for VOC Delta and VOC Omicron compared to VOC Alpha and the Wuhan original virus.

The large number of deletions in Alpha samples (1616 deletions in 47 samples, Table 2) is due, inter alia, to the presence of genome regions with a low coverage level (primer bias). Therefore, it is not the total number of mutations that are important for the evolution of VOCs, but those mutations that are present in many samples (major mutations). Based on this, we detect that the number of major mutations slightly increased from 26 in Alpha to 31 in Delta, and dramatically increased to 78 in Omicron. Also, it could be concluded that missence mutations were the key source of evolution of the studied VOCs, compared to other types of mutations. Thus, the ratio of major missense mutations to deletions in Alpha is 3:1 (18 major missense mutations, 3 major deletions). In Delta, this proportion is almost 15:1, in Omicron - 3.5:1. Notably, despite the significant prevalence of the major missense mutations, a significant increase in the major deletions were observed in VOC Omicron (up to 17), as well as the appearance of 1 major insertion. This indicates that the evolution of VOC Omicron and its unique epidemiological properties did not occur in the same way as in previous VOCs. The SARS-CoV-2 Omicron genome was significantly more affected by genomic rearrangements (deletions/insertions), which likely resulted in less pathogenicity, but faster viral replication and better immune escape, as other authors have already discussed [35,36].

Prior to the appearance of VOC Alpha at the beginning of 2020, the differences in the mutational profiles of the virus across continents were reported already. Mutations were mostly present in the NSP2, NSP3, Spike, and ORF9 SARS-CoV-2 proteins [37], both common and unique continental mutations were detected [38].

The high frequency of S protein mutations in VOC Alpha has a high influence on SARS-CoV-2 functions. D614G is the most early and frequent SARS-CoV-2 mutation, which was found in late February 2020 and it is present in all VOC strains and now predominate globally in the world (reaching near 100%) [39]. It leads to increases in virion spike density and increasing SARS-CoV-2 infectivity [40–42] and enhances viral replication [43]. P681H is located proximal to the S1/S2 furin cleavage site and influences virus tropism by increasing S1/S2 cleavage [44]. It is present in the VOC Alpha and Omicron. S982A and D1118H mutations are located in the S2 part and present in the Alpha variant. They don't have a significant influence on reducing susceptibility to any of the antibodies [45].

H69del and V70del in S protein have been found in multiple independent lineages of SARS-CoV-2. Their presence increases infectivity associated with enhanced incorporation of cleaved spike into virions [46]. Next deletion Y144del is associated with resistance to several S-binding neutralizing antibodies but does not appear to reduce the neutralizing activity of vaccinated persons [45]. It has been confirmed that Y144del blocks the binding sites of neutralizing antibodies [47].

Among S protein mutations in B.1.1.7 the key resistant mutation for monoclonal antibody against the Wuhan strain is N501Y, whose presence increases ACE2-binding [48] and increases virus replication in human upper airway cells and enhances SARS-CoV-2 transmission [49]. The combination of D614G and N501Y was found to alter the transmissibility and virulence of SARS-CoV-2 [6].

Observed N protein SARS-CoV-2 mutations D3L, R203K in VOC Alpha/B.1.1.7 strain affect on antagonizing to host innate immunity [50].

The role of many of these deletions in NSP6 of VOC Alpha/B.1.1.7 lineage is under investigation now, but it is known that deletions of NSP6 in 106–108 showed proliferation advantages for SARS-CoV-2 samples [47].

It is known that nonstructural protein 6 (NSP6) 106–108 mutations are common to most VOC, including VOC Delta and Omicron. They lead to antagonizing host-responsiveness to interferons [51,52]. NSP12_P323L reached a global prevalence of 95%, which is slightly lower than that of S_D614G and often appears simultaneously [53]. It is located in the interface domain of the RNA-dependent RNA polymerase. P323L is a necessary alteration that led to the epidemiological success of SARS-CoV-2 [54].

VOC Alpha alterations in accessory protein NS8 (Q27stop, R52I, Y73C) observed in investigated samples could change IFN-I antagonists inducing an impairment in the host immune response [55].

The emergence of the VOC Alpha/B.1.1.7 variant has raised concerns of reduced vaccine efficacy and increased re-infection rates [56]. The majority of mutations influencing by virulence and properties of SARS-CoV-2, were detected in the Spike (S) protein, for example, D614G, N501Y, E484K, K417 N/T, L452R, and P681R [57]. Several mutations in the S protein contribute to the impaired recognition of the virus by the host immune system and to mismatch of the S protein with neutralizing antibodies, namely L452R, N501Y, S371L, K417 N, N440K, G446S, E484A, Q493R, G496S, Q498R, N856K, and N969K [58–61].

VOC Alpha was the first strain, replacing the primary strain almost completely, according to the NEXTSTRAIN data [12]. Then, the VOC Delta and Omicron strains displaced VOC Alpha; they were detected in almost 100% COVID-19 cases, when the new disease wave covered any continent.

The VOC Delta strain includes more than 200 different lineages that differ genetically from the parent strain (B.1.617.2), indicating an active evolution within the Delta strain.

Analysing the lineage composition of the VOC Delta strain, it was found that in June 2021 (interwave period) seven different lineages came to Ukraine from different sources simultaneously. Four out of seven were not distributed further in Ukraine. Altogether, during the epidemic Delta wave, 25 new lineages were found.

The combination of VOC Delta S protein mutations: E156G, F157del, R158del was present in more than 90% of the sequences reported from the USA and UK in October 2021 and increased cell-to-cell fusion [52]. It has been shown that a combination of E156G, F157del, R158del and L452R S protein mutation exhibited higher infectivity of SARS-CoV-2 [62,63].

VOC Delta contains one mutation in S protein named L452R that causes monoclonal antibody resistance [58] and increases virus transmissibility and infectivity [64]. It is present in VOC Epsilon, and Kappa variants of SARS-CoV-2.

VOC strains T478K and L452R mutations in S protein are probably associated with the infectivity and pathogenesis of the SARS-CoV-2 variant [65].

S protein mutation at the 681 position occurs already in VOC Alpha and Omicron. They have P681H, but VOC Delta has P681R. It has shown that P681R mutation is a hallmark of the virological phenotype of the B.1.617.2/Delta variant and is associated with enhanced SARS-CoV-2 pathogenicity [59].

The Ukrainian VOC Delta samples have differences in the spectrum of additional mutations in each lineage and inside them, which have been found in virus samples and in other countries [53].

N protein G215C dominant mutation in VOC Delta plays the stabilizing role in conserved transient helices in the disordered linker serving as protein-protein interaction interfaces [66].

Many mutations in nonstructural and accessory proteins are unique to the VOC Delta strain. Their effects on protein structure and virus epidemiology requires additional research [67].

The most widespread lineage in VOC Delta AY.122 has two missense mutations in NSP2: K81 N, K489 N. They could influence interaction with host proteins and be involved in mitochondrial biogenesis [28,68].

Apparently, in addition to the basic combination of mutations characteristic of maternal B.1.617.2, new mutations give this line evolutionary advantages in the spread, infection and reproduction of the virus in the human body, which requires additional research [69].

In Ukraine, VOC Omicron was registered later than in Europe and the world (NEXTSTRAIN); two different branches of Omicron - BA.1 and BA.2 entered Ukraine simultaneously.

There are several S protein mutations in VOC Omicron causing monoclonal antibody resistance: S371L, K417 N, N440K, G446S, E484A, Q493R, G496S, Q498R, N501Y, N856K, N969K [60,61,70]. But analysis of T-cell immunity has shown the maintenance of effective T-cell immunity against VOC Omicron despite mutations [71]. Moreover, due to the described combination of mutations in the RBD domain of S protein, VOC Omicron has stronger binding to the ACE receptor compared with VOC Alpha and Delta [72].

VOC Omicron samples three different insertions in S and M proteins. The insertion of Spike_ins214EPE was predicted a dramatic decline in binding affinity of HLA-DRB1 [71].

It should be noted that evolutionary changes in the VOC Omicron lineages are observed to a greater extent in changes in S protein mutation combination and a number of non-structural proteins (NSP1, NSP4, NSP13, NSP15), which potentially affect both the viral cycle, potential functional consequences and the host-virus interaction [29,30].

Eventually, the puzzling mutational pattern of VOC Omicron combines contradictory properties which may either decrease (virological properties) or increase (immunological escape/facilitation) the transmission of this variant in the human population [35].

4.2. The most frequently detected lineage of VOC Delta in Ukraine is AY.122.

The AY.122 was detected first among the VOC Delta strain lineages in Ukraine. It is the most frequently detected lineage in the VOC Delta in Ukraine. From the sequence and epidemiological data on the detection and distribution, the AY.122 lineage has a pan-European origin; it was spread in the summer of 2021 in Eastern Europe, including Ukraine, according to the GISAID data [12].

The hypothesis of the common origin of AY.122 in Eastern Europe is supported by the predominance in Ukraine (and in other countries) of a subtype with the unique combination of two mutations - NSP2_K81 N + NS7a_P45L (Table 6). AY.122 with NSP2_K81 N + NS7a_P45L combination was detected at a minor frequency in Western Europe.

According to NEXTSTRAIN data, AY.122 has been detected in the world up to the second Omicron wave (June 2022). Of note, it was also detected in the first half of the Omicron wave in Ukraine in January 2022. It could be assumed that AY.122 could successfully compete with VOC Omicron. However, Omicron was detected in 100% of COVID19 samples in the second half of the epidemic wave, in February 2022 (S2 Table). VOC Omicron shows a higher genetic variability than VOC Delta. According to the NEXTSTRAIN data, the VOC Delta strain was replaced by VOC Omicron in 2022, both BA.1 and BA.2.

4.2. Other factors influencing the course of COVID-19

It should be noted that in order to spread Omicron lineages more quickly and accelerate virus evolution, the incubation period of SARS-CoV-2 has changed from 5.8 days for VOC Alpha to 3.03 days for Omicron BA.1 according to Japanese data [73,74]. Netherlands data has shown the incubation period of SARS-CoV-2 for VOC Delta was 4.1 days and for VOC Omicron - 2.8 days [75] from the onset of the first symptoms to hospitalization.

One of the changing epidemiological indicators of COVID-19 is the age of patients. It is known that children and young adults possess several protective mechanisms, contributing to the lower number of COVID-19 incidences and the favorable course of this disease. The following should be noted: a faster and stronger innate immune response to the virus, fast synthesis of the protective cross-reactive antibodies and quick T cell response [76,77]. Clinical paediatric patients' data from Ukraine confirm a moderate course of

Table 6
AY.122 distribution in Ukraine and the world (GISAID data).

Region	VOC Delta (n)	AY.122 (n)	AY.122 (%)	AY.122 with K81 N + P45L (n)	AY.122 with K81 N + P45L (%)
World	4416813	215442	4.88	122392	56.81
Europe	2329729	164372	7.06	103205	62.79
Ukraine	354	173	48.87*	156	90.17**
Russia	8205	7302	88.99	6313	86.46

Notes: Fisher Exact statistics was used for OR; 2-tail p-values were calculated; * - the freaquency of AY.full122 in Ukraine is significantly higher then in Europe (OR = 12.59; 95% CI = 10.16–15.60, $p < 0.0000001$) and in the world (OR = 18.64; 95% CI = 15.13–22.96, $p < 0.0000001$), and significantly lower then in Russia (OR = 0.12; CI = 0.09–0.15, $p < 0.0000001$); ** - the freaquency of AY.122 with K81 N + P45L combination in Ukraine is significantly higher then in Russia (OR = 3.49; 95% CI = 1.633–7.466, $p = 0.0002$), in Europe (OR = 13.21; 95% CI = 6.194–28.17, $p < 0.0000001$) and in the world (OR = 16.94; 95% CI = 7.946–36.13, $p < 0.0000001$).

illness and a good prognosis for children [78]. According to a population-based study in Ukraine, long COVID prevalence has been associated with age, female sex, unhealthy lifestyle and increased inflammatory markers disease [79].

Apparently, at the beginning of the COVID-19 pandemic (at the beginning of 2020) SARS-CoV-2 virus was detected mainly in the elderly population in different countries of the world [80,81]. It can be explained that the above-mentioned mechanisms worked well and the incidence among children and young people was very low, or symptoms of the disease were mild, and children were not tested. There is an increase in the incidence of the younger population and children, especially with VOC Omicron [81,82]. Anyway, despite a significant increase in the incidence of the young population, hospitalization and mortality rates are significantly reduced in the Omicron wave compared to the Delta wave. These Omicron genetic changes resulted in faster and easier spread of the disease, and more mild disease without severe chronic disease [17,82,83]. The decrease in hospitalization and mortality in Omicron is observed in Ukraine as well. However, the specific genetic changes leading to such an outcome of COVID19 among young people and children are not determined yet. This requires further research.

4.3. Anti-epidemic strategy in Ukraine

Every country in the world has its own approach to anti-epidemic strategies against SARS-CoV-2, from a zero-tolerance policy in China to different types of lockdowns [84]. In Ukraine, on 9 December of 2020, the adaptive quarantine was introduced by the Cabinet of Ministers of Ukraine by Resolution No. 1236, the recommendations were updated many times during the pandemics (https://www.kmu.gov.ua/npas/pro-vstanovlennya-karantinu-ta-zaprovadzhennya-obmezhuvalnih-protiepidemichnih-zahodiv-1236-091220). The main indicator to make stronger measures was the occupancy rate of hospital beds in the COVID-special medical centers. Based on the hospitalization rate and other epidemic indicators, four zones were introduced, from green to red with different degrees of anti-epidemic restrictions. This was done in order to control the burden on the Ukrainian medical system, with the aim of maintaining the function of providing the full medical care, hospitalization and treatment of all patients with severe symptoms.

One of the main approaches to stop the spread of the disease is the mass vaccination of the population against SARS-CoV-2. Unfortunately, Ukraine is a country with a low level of vaccinations in the population [85]. The mass vaccination started in March 2021, when the VOC Alpha epidemic wave was intensively spreading in the country. At the end of October 2021 (maximum of the VOC Delta wave), only 18.1% of the population was fully vaccinated in Ukraine. At the end of January 2022, this level was almost twice as high and amounted to 35.6% of fully vaccinated Ukrainians (https://covid19.gov.ua, https://index.minfin.com.ua/ua/reference/coronavirus/vaccination/ukraine/). It was analyzed the open Public Health Center data about hospitalization of vaccinated and unvaccinated patients (S8 Fig.) under the VOC Delta and Omicron waves (https://cloud.phc.org.ua/index.php/s/L26pPBzdq8t8yRA).

A significant reduction ($p < 0.01$) in the rate of hospitalization of unvaccinated patients was observed in the VOC Omicron wave, compared with the VOC Delta wave. Whereas for vaccinated patients, there is a significant increase ($p < 0.01$) in hospitalization levels during the Omicron wave, compared with the Delta wave. Importantly, Ukrainians traveling to other countries had a full course of vaccination against COVID-19 in accordance with international standards and rules. No doubt, the introduction of the new strains VOC Delta and VOC Omicron into the country could be carried out by vaccinated persons as well.

4.4. Differences in time-lapse of SARS-CoV-2 infection in Ukraine and Western Europe

Of note, the same VOC waves, from Alpha to Omicron were registered several months earlier in Western European countries. According to the data from the European Centre for Disease Prevention and Control (https://www.ecdc.europa.eu/en/covid-19), the first VOC Alpha was detected in September 2020 in the UK; the VOC Alpha COVID-19 wave came in the middle of December 2020. As opposed to Ukraine, the first Alpha was detected in the middle of February 2021, epidemic wave was in March–April 2021.

The same situation is observed with the VOC Delta and Omicron waves. The first Delta in Western Europe was detected in April 2021. The Delta wave was in the middle of May 2021. The first Delta in Ukraine was detected in the middle of June 2021 (S2 Table), the VOC Delta wave in Ukraine was in the middle of October–November 2021.

The first Omicron was found in Europe in April 2021, the epidemic wave was in the middle of December 2021. The first VOC Omicron was detected in Ukraine in the middle of December 2021, whereas the VOC Omicron wave was in the middle of January–February 2022.

Table 7
Comparison of divergence: "early" variants of VOC lineages, the variants at the beginning of the corresponding waves in Europe and Ukrainian VOC lineages (GISAID data).

VOC	Europe "early"		Europe wave		Ukraine all	
	n	Mutations per genome	n	Mutations per genome	n	Mutations per genome
Alpha	100	25.34	1500	26.43	97	27.92
Delta	100	33.29	1500	32.3	247	34.87
Omicron	100	44.92	1500	55.92	75	66.71

Note: Europe "early" – first high coverage genomes located in Europe; Europe wave - high coverage genomes at the beginning of the corresponding waves in Europe: VOC Alpha - from December 15, 2020; VOC Delta - from May 10, 2021; VOC Omicron - from December 20, 2021; Ukraine all – all Ukrainian full coverage genomes of each VOC.

A comparison of the average number of mutations per genome among "early", late European and Ukrainian variants of SARS-CoV2 VOC lineages, according to GISAID data, is presented in Table 7.

According to Table 7 data, the number of mutations per genome increases from VOC Alpha to VOC Omicron in all groups. The greatest increase in the rate of mutations is observed at VOC Omicron in Ukraine.

4.5. The analysis of phylogenies and the spread of SARS-CoV-2 in Ukraine

The WHO-affiliated GISAID database contains the results of full-genome sequencing of the SARS-COV-2 virus obtained from various platforms, including Illumina, ThermoFisher Scientific, and Oxphord Nanopore. However, despite this diversity of platforms and research centers involved in this investigation, the sample collection of SARS-COV-2 from Ukrainian patients from different regions cannot be considered normally distributed. This feature is likely related to both the difficulties of collecting and delivering material in a pandemic and funding issues. A number of researchers have analyzed the data available in GISAID to obtain a phylogenetic tree of SARS-COV-2 in Ukraine [86]. However, when interpreting the results, it is necessary to take into account the peculiarities of the available sample distribution and the difficulties associated with it.

The use of a very efficient algorithm for building transmission trees [21], made it possible to investigate the history of transmissions for very small data sets. From the three sets, we managed to build reasonably plausible transmission trees for two virus strains, Alpha and Omicron.

The branched and clustered transmission patterns for the VOC Alpha and VOC Omicron appear to be consistent with the epidemiological data, suggesting very rapid spread of each of the VOCs across different regions of Ukraine. According to our previously published data [18], the first cases of COVID-19 in Ukraine, caused by VOC Alpha, were detected in the Ivano-Frankivsk region. Therefore, the national transmission network may have started from there. Recurrent sources of the VOC Alpha spread, which intensified the outbreak of the pandemic, were newly arrived patients in other cities of the country, especially in the Kyiv region and the East (Donetsk region). This is confirmed by the presence of three quite separate and branched clusters on our transmission diagram (Fig. 6). Separation of these clusters could be linked to the effectiveness of quarantine restrictions within the country in the form of "red quarantine zones", which were introduced in the first half of 2021.

The VOC Delta strain shows the biggest number of different lineages which come to Ukraine from abroad. The absence of significant clusters and the large quantity of different small clusters probably indicates effective anti-epidemic quarantine zones within the country, with the frequent importation of new SARS-CoV-2 lineages from abroad.

Regarding the beginning of the spread of the VOC Omicron, the diagram clearly shows at least three possible sources of simultaneous entry of this variant into Ukraine. They associated with Christmas and New Year trips, probably, to ski resorts in Uzhorod region and outside Ukraine: 1) metropolitan (Kyiv), connected with the center of aviation and railway international communication in Kyiv; 2) Western Ukrainian with centers in Ivano-Frankivsk and Rivne, from where transmissions were recorded in the southern, southeastern and northern regions of the country; 3) Eastern (Kharkiv), based on the proximity to the Eastern border of the region and the visa-free regime with the Russian Federation (Fig. 7). Several very different sources of the VOC Omicron entering Ukraine may explain the extremely rapid spread of this variant.

The obtained results indicate the significant differences in the infectious properties of the SARS-CoV-2 virus and suggest the continuation of viral evolution, under the influence of natural selection and the pressure of anti-epidemic actions [3,5,7,10,16,42].

However, it is still not possible to give the final answer whether the new highly pathogenic and highly virulent strains of the SARS-CoV-2 virus will appear, since the pandemic is still ongoing. There is always a chance of the emergence of highly pathogenic and lethal strains of the virus, with highly virulent properties. Therefore, in-depth research into the genetic structure of the virus shall be continued, in order to deal successfully with the current COVID-19 pandemic.

5. Conclusions

Three COVID-19 epidemic waves were analyzed caused by VOC strains Alpha, Delta and Omicron during the 2021 – beginning of 2022.

Each VOC strain has its own genetic alterations and epidemic characteristics. Genetic alterations increased from VOC Alpha to VOC Omicron, whereas epidemiological indexes have shown individual indicators. Although VOC Omicron had the highest peak in new case detection, hospitalization and death rates were the lowest among the three VOC waves.

The VOC Alpha strain was represented by one B.1.1.7 lineage, which was detected in all the studied regions. The VOC Delta strain was represented by 25 lineages, which had features of distribution by region and time period. The VOC Omicron was represented by 13 lineages of both BA.1 and BA.2 clades.

Based on a phylogenetic analysis, it has been shown that the VOC Delta of SARS-CoV-2 remained in Ukraine for quite a long time, constantly evolving. The VOC Delta is characterized by high virulence and a very wide range of lineages. The VOC Alpha was represented by only one lineage, B.1.1.7. VOC Alpha showed less variability than VOC Delta, and was completely replaced by the latter. Even if the VOC Delta evolved into the very efficient lineage AY.122, it was anyway replaced by the VOC Omicron in Ukraine.

Using a high-throughput pipeline for the analysis of infection transmissions, the pathways and rates of emergence of different VOCs of SARS-CoV-2 in regions of Ukraine were evaluated, even for limited data sets.

The evolution of SARS-CoV-2 virus and high levels of morbidity in the world continue to be registered today, so the identification and determination of the genetic characteristics of SARS-CoV-2 is required for the successful fight against the COVID-19 pandemic.

Observed multiple virus sourses with the limited distribution between regions indicates the high efficiency of the anti-epidemic

policy pursued by the Ministry of Health of Ukraine to prevent the spread of the epidemic, despite the low level of vaccination of the Ukrainian population.

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Ethics declarations

Review and/or approval by an ethics committee was not needed for this study because it was approved by the Ministry of Health of Ukraine in agreement number 31/4056/21 between Public Health of the Ministry of Health of Ukraine and the Institute of Molecular Biology and Genetics (IMBG) of NAS of Ukraine.

Data availability statement

Data associated with this study has been deposited into a publicly available repository: Pandemic coronavirus causing COVID-19. GISAID database. Available from: https://www.epicov.org/epi3/frontend#3a964b.

CRediT authorship contribution statement

Ganna V. Gerashchenko: Writing – original draft, Conceptualization. **Nataliya V. Hryshchenko:** Methodology, Investigation. **Nataliia S. Melnichuk:** Validation. **Tetiana V. Marchyshak:** Validation. **Serhii Yu Chernushyn:** Software, Methodology. **Irina V. Demchyshina:** Validation. **Ludmyla M. Chernenko:** Validation. **Igor V. Kuzin:** Validation. **Zenovii Yu Tkachuk:** Conceptualization. **Vladimir I. Kashuba:** Writing – review & editing, Supervision, Conceptualization. **Mykhailo A. Tukalo:** Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e25618.

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