Phylodynamic analysis of SARS-CoV-2 spread in Rio de Janeiro, Brazil, highlights how metropolitan areas act as dispersal hubs for new variants

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Abstract

During the first semester of 2021, all of Brazil has suffered an intense wave of COVID-19 associated with the Gamma variant. In July, the first cases of Delta variant were detected in the state of Rio de Janeiro. In this work, we have employed phylodynamic methods to analyse more than 1600 genomic sequences of Delta variant collected until September in Rio de Janeiro to reconstruct how this variant has surpassed Gamma and dispersed throughout the state. After the introduction of Delta, it has initially spread mostly in the homonymous city of Rio de Janeiro, the most populous of the state. In a second stage, dispersal occurred to mid- and long-range cities, which acted as new close-range hubs for spread. We observed that the substitution of Gamma by Delta was possibly caused by its higher viral load, a proxy for transmissibility. This variant turnover prompted a new surge in cases, but with lower lethality than was observed during the peak caused by Gamma. We reason that high vaccination rates in the state of Rio de Janeiro were possibly what prevented a higher number of deaths.

DATA SUMMARY

Four supplementary figures, one supplementary table and one supplementary file are available with the online version of this article. Raw short reads of the newly sequenced genomes are available at the GSA/NGDC database (https://ngdc.cncb.ac.cn/gsa/) under the BioProject PRJCA007589 and the assembled genomes are deposited at GISAID database (https://www.gisaid.org/) under the accession numbers listed in Table S1 (available in the online version of this article). Other genomic sequences used in the analyses are listed in https://doi.org/10.55876/gis8.220607kd. Epidemiological data for the state of Rio de Janeiro was obtained from https://www.saude.rj.gov.br/informacao-sus/dados-sus/2020/11/covid-19. Supplementary material and additional files such as scripts, data tables and the input files for BEAST can be accessed through Figshare: https://doi.org/10.6084/m9.figshare.19125863.v1
INTRODUCTION

Similarly to the rest of Brazil, the state of Rio de Janeiro, has suffered an intense wave of COVID-19 in the first semester of 2021. This surge was mostly associated with the substitution of previous prevalent lineages B.1.1.28, B.1.1.33 and P.2 by the lineage P.1 and its sublineages, grouped under the Variant of Concern Gamma [1–3]. As the number of COVID-19 cases started to decline in Brazil, the introduction of the more aggressive Variant of Concern Delta was detected in multiple states. While the first cases in Brazil were considered isolated ones or contained transmission, the introduction of Delta in Rio de Janeiro during June of 2021 led to the first community outbreak of the variant in Brazil [2]. Since then, Delta has spread across different Brazilian states and became the dominant variant circulating in the country [2, 4–7].

The state of Rio de Janeiro is the third most populous state of Brazil and the second most dense (399.15 hab km\(^{-2}\)), with an estimated population of more than 17.4 million people [8]. It has the fourth-highest Human Development Index in the country [9, 10] and almost half of this population lives in its homonymous capital, Rio de Janeiro (Fig. S1). The state is also deeply heterogeneous in social-economic indicators, as most of its industry is located in the cities neighbouring the capital. Previous works have shown that population density, proximity to international airports and city infrastructure influence the speed and routes in which SARS-CoV-2 spreads [11–18]. Understanding how this virus disperses within and between urban regions is crucial to elaborate efficient and science-driven public health policies to contain the pandemic. The continued genomic surveillance of SARS-CoV-2 lineages conducted in the state of Rio de Janeiro [2] is a rare opportunity to investigate and monitor the dispersal of the Delta variant since its first introduction. In this work, we employed phylodynamic methods to analyse more than 1600 Delta genomes collected between July and September and inferred the dispersal patterns and routes of the variant in the state.

METHODS

**Epidemiological analyses -** Daily number of confirmed COVID-19 cases, deaths and people vaccinated were obtained from the National Immunization Programme (Programa Nacional de Imunização, PNI), esus-VE and SIVEP-Gripe databases through the COVID-19 portal (https://www.saude.rj.gov.br/monitoramento/covid19.html). The number of tests for SARS-CoV-2 infection in Rio de Janeiro was considered consistent throughout 2021 [19].

**Sequencing -** The Noel Nutels Central Laboratory (LACEN-RJ) and the Unidades de Apoio ao Diagnóstico da Covid-19 (UNADIG-RJ) received in 2021 more than 1.3 million nasopharyngeal swab samples from public health clinics and hospitals located in the state of Rio de Janeiro to detect the infection by SARS-CoV-2 with the RT-PCR method [20]. We biweekly retrieved samples available in both laboratory networks for genomic sequencing, selected randomly from the pool of positive diagnosis with CT values lower than 33. These samples were collected between 1 June and 12 September 2021. The genetic material was extracted at the Laboratory of Molecular Virology at Universidade Federal do Rio de Janeiro (LVM-UFRJ) using MagMAX Viral/Pathogen Nucleic Acid Isolation kits KingFisher automatic platform. The cDNA was annealed with 8.5 µl of viral RNA extracted from each sample and the Illumina COVIDSeq Test (Illumina) was used according to the manufacturer’s protocol to construct libraries at the DFA/LNCC Genomics Unit. An aliquot of 5 µl of each library was combined and purified and we used the TapeStation (Agilent) system for quality control. The NextSeq 500/550 Mid Output Kit v2.5 (300 Cycles) was used to generate reads of 2×149 bp in the NextSeq (Illumina). The DRAGEN COVID Lineage v3.5.1 pipeline was used for sequence analysis, consensus building, and variant calling. The study was approved by the Ethics Committee (30161620.1.000.5257 and 34025020.0.0000.5257). All newly sequenced and assembled genomes are publicly available at GISAID (Table S1) and GSA/NGDC database (BioProject PRJCA007589) databases. Finally, we used the PangoLEARN v1.2.103 model database to classify the lineage of our newly assembled genomes and removed from the dataset all non-Delta sequences (Fig. S2). The remaining genomes (n=1334) belonged to
samples obtained from patients with ages ranging from 1 to 100 years old, 44.6% being male and 55.4% female, and residing in 85 (of 92) cities of Rio de Janeiro (Fig. S3).

Comparison between viral load of Gamma and Delta samples - The relative quantification for the viral load (RQVL) was estimated based on the 2-ΔΔCT method [21]. For each viral target provided by the laboratory, only those with endogenous (input) gene data were selected for our study. As the relative quantification is a fold-change between the virus target and the endogenous gene, the laboratory bias was considered minimal.

Evolutionary analyses and phylogeographic reconstruction - We obtained from the GISAID database all genomes classified as lineage B.1.617.2 or its sublineages (Delta variant) that were submitted until 1 October and collected in the state of Rio de Janeiro, Brazil. We removed from this dataset any sequence that did not contain complete date information, was shorter than 29000 bp, had more than 1% of Ns, more than 0.05% of unique amino acid mutations ('high-coverage' in GISAID) or didn't have the locality/city information. We added to this dataset our newly sequenced genomes collected in the state, totalling 1602 genome sequences (Fig. S2). These sequences were independently aligned to the WH01 (EPI ISL 406798) sequence from Wuhan, China using MAFFT v.7 [22] with the --addfragments option. All genomes’ 3’ and 5’ ends were trimmed using the SeqKit toolkit [23]. Maximum likelihood trees were then inferred with IQTREE2 [24] using the GTR+F+I+G4 model selected by the ModelFinder algorithm [25] with ‘-mset mrbayes’ option and 1000 ultrafast-bootstrap replicates [26]. The outgroup (WH01) was then removed and root-to-tip distances were calculated using TempEst. Using Cook’s distance, we removed from the alignment the sequences that influenced most the root-to-tip correlation. This final dataset contained 1512 sequences and was used in all subsequent analyses. A new maximum likelihood tree was generated using the same parameters as before. This new tree was used as a fixed topology in the subsequent analyses (divergence dating and phylogeography).
We then investigated whether the outbreak of Delta in Rio de Janeiro originated from one or multiple introductions of the variant in the state. As representative sequences of the global diversity, we used the genomes belonging to the Delta clade in the SARS-CoV-2 global phylogeny (https://github.com/nextstrain/ncov) generated through the Nextstrain pipeline [27]. We further tested the origin and monophyly of lineages AY.99.1 and AY.99.2 by obtaining from GISAID the 20 oldest sequences of each of these lineages that were not from the state of Rio de Janeiro (ten global sequences and ten from Brazil) and adding them to the background dataset. The background genomes were combined to our dataset from Rio de Janeiro, aligned to the WH01 genome and the 3′ and 5′ ends of the new sequences were trimmed. A maximum likelihood tree was inferred as described before.

Divergence dates were estimated using BEAST v.1.10.4 employing the strict clock model with a uniform prior (max substitution rate of 1e⁻³, min 6e⁻⁴), the GTR substitution model with empirical frequencies and the Gamma +Invariant sites model, and the Coalescent-Exponential Growth tree prior. The MCMC was run using the BEAGLE library through a single chain of 100 000 000 with sampling every 10 000th and a burn-in of 15% of trees. Values of ESS were higher than above 200, with the exception of ‘joint’ (ESS=78), ‘prior’ (65), ‘treeLength’ (71), ‘exponential.growthRate’ (98), ‘clock.rate’ (165), ‘meanRate’ (165) and ‘coalescent’ (64) parameters. The consensus tree was summarized with Tree Annotator and used as the fixed topology and branch lengths for the phylogeographic reconstruction of the spread of SARS-CoV-2 in Rio de Janeiro. To accomplish this, we used the BEAST software and the Relaxed Random Walk model with Cauchy’s distribution [28]. The location of each sequence was encoded as a random coordinate draw within the geographical limits of the city in which it was sampled. The MCMC ran again using the BEAGLE library through a chain of 100000000 with sampling every 10000th and a burn-in of 15% of trees. Dispersal routes were extracted from the consensus tree using the seraphim package [29] and plotted using the ggplot2 package, both in R software. Base map was obtained from Instituto Brasileiro de Geografia e Estatística (IBGE, https://www.ibge.gov.br/geociencias/organizacao-do-territorio/malhas-territoriais/15774-malhas.html).

RESULTS AND DISCUSSION

On 16 June 2021, the first case of the Delta variant was confirmed in Rio de Janeiro, and a fast increase of the variant’s frequency in the state was reported [2]. At the time, Rio de Janeiro was on the decline of COVID-19 cases caused by the outbreak of Gamma variant in the country [2, 31]. The fast spread of Delta observed in Rio de Janeiro incited concern for a new COVID-19 surge. Analysing epidemiological data publicly available, we observed that there was indeed an increase of cases in the state (Fig. 1a), with a maximum of 23086 cases in a week at the beginning of August (week 32). This number corresponds to approximately 73% of cases from the first peak (end of March, week 11) of the bimodal wave caused by Gamma and is very similar to the number of
cases in the second peak (early May, week 19). However, the rate in which the number of cases increases is substantially higher in the Gamma, the increase in deaths caused by this new variant was much smaller, with the 890 lives lost (week 33) being less than half of the maximum observed in the first semester of 2021 \((n=2016, \text{week 11; Fig. 1b})\). While recent research suggests that Delta causes a higher number of hospitalizations and aggravated symptoms than previous lineages [32, 33], we believe the advances in vaccination throughout the state has reduced the lethality from 7.6\% (late March, week 13) to the approximately 3\% at the peak of the Delta surge. Interestingly, in the last 4 weeks of the temporal series analysed, there was a small increase in mean lethality (max of 6.5\%, 37\textsuperscript{th} week) even though cases and deaths were decreasing. This increase was observed in all age groups above 40 years and infants until 4 years (Fig. 1c), with a decrease in lethality in some of the age groups in the last week. We could not identify the factor determining this pattern. Age group proportions in the number of cases was similar throughout 2021 while the distribution in deaths saw first a decrease in the proportion of older age groups followed by normalization, possibly caused by health authorities prioritizing vaccination in such groups first (Fig. S4, Fig. 1d).

This surge is concomitant to the jump in frequency of Delta from 67\% \((n=156/232, 95\% \text{CI}=61.2–73.2\%)\) to 89\% \((n=130/146, 95\% \text{CI}=83.9–94.1\%)\) of the samples sequenced in this work (Fig. 2, Table S2, weeks 30–31). Substitution of Gamma, Alpha (B.1.1.7) and Beta (B.1.351) by Delta has been reported around the globe, being the dominant variant until the emergence of Omicron (B.1.1.529 and sublineages). While the replacement of Gamma by Delta occurred at an alarming rate, we detected at least 12 weeks of both variants co-circulating in the state. The presence of both lineages allows for coinfection events, which are the requirement for variant recombination. Events of recombination between variants were detected not only in the state of Rio de Janeiro during the period analysed [34], but also in several countries and times [35–37]. While the role of recombination in originating new Variants of Concern or Variants of Interest [38, 39] is still being discussed, genomic surveillance should be enforced during periods of lineage replacement.

The same pattern of Delta gradually replacing the variant Gamma was observed in all of the state's administrative regions (Figs S5 and S6). Because Delta was first detected in each region in different weeks of 2021, it would be expected that the highest number of cases and deaths would not occur synchronously between regions. On the contrary, we observed that all regions reach the peak in cases simultaneously, between weeks 31 and 33. When comparing the waves caused by Delta, it is possible to visualize that the regions of Médio Paraíba and Noroeste Fluminense barely had an increase in the number of cases or deaths, while the Costa Verde and Norte Fluminense suffered more with the introduction of the new variant (Figs S7 and S8). Interestingly, the lethality of COVID-19 in the city of Rio de Janeiro is significantly higher in the Metropolitana region than in others (Fig. S9), a result previously reported [40, 41]. These studies propose that the high social and economic inequality within the city of Rio de Janeiro in association with better diagnosis and notification systems resulted in this discrepancy when compared to other regions or cities.
To investigate how Delta surpassed Gamma in the state of Rio de Janeiro, we compared the relative quantification of viral load (RQVL = 2^{-\Delta CT}) of samples infected by variants circulating in the period analysed in this study. We observed that samples from Delta exhibited significantly higher RQVL values than Gamma (Wilcoxon test, p.value=0.01; Fig. S10a). While a few samples from Gamma accumulated more than 90% of the circulating viruses, more samples from Delta were required to represent the same amount of the viral load than Gamma (Fig. S10b). For example, 1% of the samples (n=4) classified as Gamma harboured approximately 96% of all viruses circulating in the state at the analysed period. On the other hand, 1% of Delta samples corresponded to 42% of the viral load suggesting an elevated number of supercarriers within Delta lineage. Viral load of Delta tends to be high until the seventh day, while viral load from Gamma samples in our database seems to decrease from the fifth day (Fig. S11). The Delta variant is known for its higher transmissibility [42, 43] and elevated viral loads, even when compared to the Alpha variant [32, 44, 45]. Altogether, our results add the information that Delta also exhibits a remarkable enhancement in viral load values when compared to Gamma. This could have facilitated the rapid spread of Delta in the state of Rio de Janeiro [46] and sheds light on possible mechanisms underlying the successive lineage replacement of both variants.

We have identified at least nine independent introductions of Delta in Rio de Janeiro, but 98% of the genomic sequences from the state have originated from a single introductory event of the AY.99 lineage (Fig. S12). Within the state, it has diverged into lineages AY.99.1 and AY.99.2 between May and June of 2021 (Fig. 3), a result supported by the study of [47]. Both lineages have spread across the state from this point onwards, with AY.99.2 being dominant. This dispersal has occurred in two stages in the state of Rio de Janeiro (Fig. 4). Until July, most transmissions were detected within the heavily populated city of Rio de Janeiro, with a small number of long-distance dispersals to the other macro regions of the state. From August onwards, these introductions in new cities/regions culminated in a high number of local and short-distance transmissions. The predominance of these lineages, specially AY.99.2, in Rio de Janeiro and the southeastern region of Brazil [4] may be attributed to a founder effect followed by density-dependent exclusion of other lineages [48].
A fast spread of the founder lineage (AY.99,1 and 2) may have triggered the transient immunity of the population, 'consumming' the available hosts before other lineages could establish. A partial suppression of available hosts may also be the reason for why Delta didn't cause such a drastic surge in COVID-19 cases in Rio de Janeiro and Brazil when compared to other countries [49], as Brazil was still recovering from the wave caused by the Gamma variant.

These results consolidate the important role of large and dense urbanized areas as dispersal hubs immediately after introducing a new SARS-CoV-2 variant or lineage [11–18]. Therefore, identification and surveillance of these hubs are of fundamental value to early control new variants that may emerge in the future. The results also suggest that non-medical interventions such as mass screening [50–53], use of masks [54–56], social distancing and lockdowns [57–59]; in metropolitan areas might result in better long-term effects on pandemic control than when applied on small cities, because it may reduce the number of seeding events on small cities [60–62].

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Conflicts of interest
The author(s) declared that there are no conflicts of interest.

Ethical statement
The study was approved by the Ethics Committee (30161620.0.1001.5257 and 34025020.0.0000.5257). Research protocol was approved without informed consent in accordance with Brazilian National Health Council’s Resolution 510/2016. All samples were residual COVID-19 clinical diagnostic samples de-identified before receipt by the researchers.

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