IJID Regions 14 (2025) 100503

Contents lists available at ScienceDirect

IJID Regions

journal homepage: www.elsevier.com/locate/ijregi

SARS-CoV-2 Omicron XBB infections boost cross-variant neutralizing antibodies, potentially explaining the observed delay of the JN.1 wave in some Brazilian regions

Luis Fernando Lopez Tort[1,2,#,*], Mia Ferreira de Araújo[1,#], Ighor Arantes[3,#], Jéssica SCC Martins[1], Marcelo Gomes[4,5], Felipe Cotrim de Carvalho[5], Walquiria Aparecida Ferreira de Almeida[5], Braulia Costa Caetano[1], Luciana R. Appolinario[1], Elisa Calvalcante Pereira[1], Jéssica Carvalho[1], Fábio Miyajima[6], Gabriel Luz Wallau[7,8], Felipe Gomes Naveca[3,9], Pedro Alves[10], Otávio Espíndola[11], Patricia Brasil[11], Paola Cristina Resende[1,‡], Gonzalo Bello[3,‡], Marilda Mendonça Siqueira[1,‡]

[1] *Laboratory of Respiratory Viruses, Exanthematous and Enteroviruses and Viral Emergencies, Oswaldo Cruz Institute, Rio de Janeiro, Brazil*
[2] *Laboratory of Molecular Virology, Biological Sciences Department,* CENUR Litoral Norte, Universidad de la República, *Salto, Uruguay*
[3] *Laboratory of Arbovirus and Hemorragic Viruses, Oswaldo Cruz Institute, Rio de Janeiro, Brazil*
[4] *Fiocruz, Presidency, Scientific Computing Program, Group of Analytical Methods in Epidemiological Surveillance, Rio de Janeiro, Brazil*
[5] *Department of Transmissible Diseases, General Coordination of Surveillance of Covid-19, Influenza and Other Respiratory Viruses, Secretariat of Health and Environmental Surveillance, Ministry of Health, Brasília, Brazil*
[6] *Analytical Competence Molecular Epidemiology Laboratory (ACME), Fundação Oswaldo Cruz (FIOCRUZ), Fortaleza, Ceará, Brazil*
[7] *Department of Entomology & Bioinformatics Center, Aggeu Magalhães Institute, FIOCRUZ, S/N Professor Moraes Rego Ave., Pernambuco, Brazil*
[8] *Department of Arbovirology, Bernhard Nocht Institute for Tropical Medicine, Who Collaborating Center for Arbovirus and Hemorrhagic Fever Reference and Research. National Reference Center for Tropical Infectious Diseases, Hamburg, Germany*
[9] *Laboratory of Ecology of Transmissible Diseases of Amazônia, Leônidas e Maria Deane Institute, Amazonas, Brazil*
[10] *Laboratory of Immunology of Viral diseases, René Rachou Institute, Minas Gerais, Brazil*
[11] *Laboratory of Clinical Research for Acute Febrile Illnesses, Evandro Chagas National Institute of Infectious Diseases, Rio de Janeiro, Brazil*

ARTICLE INFO

Keywords:
SARS-CoV-2
XBB and JN.1 variants
lineages evolution
Severe Acute Respiratory Infection Cases
Neutralizing Antibodies
Brazil

ABSTRACT

Objectives: The SARS-CoV-2 JN.1 lineage emerged in late 2023 and quickly replaced the XBB lineages, becoming the predominant Omicron variant worldwide in 2024. We estimate the epidemiologic impact of this SARS-CoV-2 lineage replacement in Brazil and we further assessed the cross-reactive neutralizing antibody (NAb) responses in a cohort of convalescent Brazilian patients infected during 2023.

Methods: We analyzed the evolution of SARS-CoV-2 lineages and severe acute respiratory infection (SARI) cases in Brazil between July 2023 and March 2024. We evaluated the cross-reactive NAb responses to the JN.1 variant in a cohort of convalescent Brazilian patients before and after infection with XBB.1* lineages.

Results: JN.1 replaced XBB with similar temporal dynamics across all country regions, although its epidemiologic impact varied between locations. The southeastern, southern, and central-western regions experienced a brief XBB wave around October 2023, shortly before the introduction of JN.1, without any immediate upsurge of SARI cases during viral lineage replacement. By contrast, the northeastern and northern regions did not experience an XBB wave in the latter half of 2023 and displayed a rapid surge in SARI cases driven by the emergence of the JN.1. We found that recent XBB infections in the Brazilian population significantly boosted cross-reactive NAb levels against JN.1.

Conclusion: The XBB wave observed in the second half of 2023 in some Brazilian states likely acted as a booster for population immunity, providing short-term protection against JN.1 infections and delaying the rise of SARI cases in certain regions of the country.

* **Corresponding author:** Tel.: 0055 21 25984220.
E-mail address: fernandolopeztort@gmail.com (L.F.L. Tort).
# Luis Fernando Lopez Tort, Mia Ferreira de Araújo and Ighor Arantes have contributed equally to this work.
‡ Paola Cristina Resende, Gonzalo Bello and Marilda Mendonça Siqueira share the senior authorship.

https://doi.org/10.1016/j.ijregi.2024.100503
Received 27 September 2024; Received in revised form 26 November 2024; Accepted 27 November 2024

Introduction

In response to the decline in COVID-19–related hospitalizations and intensive care unit admissions and the high rates of population immunity against the SARS-CoV-2, the World Health Organization has announced that this disease is now an established and ongoing health issue that no longer constitutes a public health emergency of international concern [1]. However, the unceasing circulation and ongoing evolution of SARS-CoV-2 leads to the constant emergence of novel viral lineages that challenge the immune barrier established by previous infections and vaccine boosters, particularly, since the emergence of the variant of concern (VOC) Omicron [2]. In the last years, immune evasion has reached a new threshold with the emergence of the Omicron BA.2 descendant lineages XBB and JN.1, which greatly reduced neutralization responses induced by SARS-CoV-2 wild-type monovalent (wild type) and bivalent (wild type + Omicron BA.5) vaccines [3–8]. The XBB variant resulted from a recombination between two BA.2 lineages (BJ.1 and BM.1.1.1) and was first identified in mid-August of 2022, and several XBB descendant lineages (XBB.1*) dominated viral transmissions across the world in 2023 [9–11]. Another substantially mutated BA.2 lineage, the BA.2.86, emerged in August 2023 and carries more than 30 mutations in the spike (S) protein compared with XBB and BA.2 variants [12]. BA.2.86 rapidly evolved, leading to the emergence of several descendant lineages, such as JN.1 that emerged in late 2023, which has only one additional mutation in the S protein (L455S) compared with BA.2.86 and it is one of the most immune-evading variants to date [13,14]. The JN.1 family (JN.1*) reached global dominance at the beginning of 2024 and represented more than 90% of the SARS-CoV-2 lineages detected worldwide as of June 2024 [11,15].

In Brazil, the epidemiologic scenario regarding the sequential replacements of BA.5 by XBB.1* lineages in early 2023 and of XBB.1* by JN.1* lineages in late 2023 followed the same pattern observed at the global scale [16,17]. Nevertheless, the immediate epidemiologic impact of the spread of JN.1* lineages across different Brazilian regions have not been previously explored. In this study, we combined the data of SARS-CoV-2 sequences with information of severe acute respiratory infection (SARI) cases across different Brazilian states to investigate the potential rise in SARI cases in relation to the timing of XBB.1* replacement by JN.1* lineages. Moreover, we further assessed the cross-reactive neutralizing antibody (NAb) responses against JN.1 in a cohort of convalescent Brazilian patients infected with XBB.1* lineages.

Materials and methods

Sample collection and human cohorts

Nasopharyngeal swabs and serum samples were obtained from 29 individuals (Table S1) selected from a major study that aimed to investigate SARS-CoV-2 dynamics among patients and household contacts in a slum in Rio de Janeiro, Brazil [18,19]. The cohort of volunteers analyzed in the present study is composed of 10 individuals infected with XBB.1* lineages between January and September of 2023, including (i) six individuals with serum samples collected 25-255 days before and 20-40 days after the infection and (ii) four individuals with serum samples collected only 25-30 days after infection. Moreover, serum samples from 19 individuals infected with JN.1* lineages between January and February of 2024, collected 30-70 days after infection, were also included. Nasopharyngeal swabs were used to screen for SARS-CoV-2 by real-time reverse transcription-polymerase chain reaction (RT-PCR) 4Plex SC2/VOC Molecular Kit (Bio-Manguinhos, Rio de Janeiro, Brazil), or TaqPath COVID-19 RT-PCR Kit (Applied Biosystems, Waltham, Massachusetts, USA), according to the manufacturer's instructions. Viruses from selected individuals were sequenced by the COVID-19 Fiocruz Genomic Surveillance Network, and all genomic and epidemiologic data were uploaded to the EpiCoV database (Table S1) [20].

SARS-CoV-2 Brazilian genome sequences

A total of 6785 SARS-CoV-2 complete genome sequences recovered across different Brazilian states between July 1, 2023 and March 31, 2024 were newly generated by the COVID-19 Fiocruz Genomic Surveillance Network. All samples had real-time RT-PCR cycling thresholds below 30, indicating elevated viral load. SARS-CoV-2 genome sequences were generated using the Illumina COVIDSeq Test kit. Raw data were converted to FASTQ files at Illumina BaseSpace cloud, and consensus sequences were produced with the most up-to-date version of DRAGEN COVID LINEAGE. All genomes were evaluated for mutation calling and quality with the Nextclade 2.14.0 algorithm and were uploaded to the EpiCoV database of Global Initiative on Sharing All Influenza Data (https://gisaid.org/) (see the supplemental material) [21]. In addition, we downloaded all publicly available Brazilian sequences (n = 6216) collected between July 1, 2023 and March 31, 2024 that were submitted to the EpiCoV database until July 10, 2024, with complete collection date/location and lineage assignment. Whole genome consensus sequences were classified using the Phylogenetic Assignment of Named Global Outbreak Lineages (PANGOLIN) software v4.3 (pangolin-data version 1.21) [22].

SARS-CoV-2 isolates

Two isolates belonging to the XBB.1.9 (EpiCoV accession ID: EPI_ISL_18250802) and JN.1.1 (EpiCoVaccession ID: EPI_ISL_19225686) lineages were obtained from nasopharyngeal swabs and used as reference isolates in the plaque reduction neutralization test (PRNT) assay. Briefly, eligible SARS-CoV-2-positive samples—quantitative RT-PCR cycle threshold value below 25 and lineage confirmation by genomic sequencing—were submitted to virus isolation in Vero E6 cells, as previously described [23]. When cytopathic effect was observed, culture supernatants were collected in working stocks and an aliquot was submitted to quantitative RT-PCR, followed by whole genome sequencing for lineage confirmation and to guarantee that the genome sequence of the isolate was almost identical to the one of the clinical samples from which it was isolated. Once confirmed, the consensus sequences were deposited at the EpiCoV database on Global Initiative on Sharing All Influenza Data (www.gisaid.org) and titrated by plaque assay. All the aforementioned steps were conducted for multiple samples. The criteria for selecting these two isolates as reference samples were based on two main factors: (i) the genome sequence of the isolate exhibited more than 95% similarity to that of the original clinical sample, maintaining all the variant-defining mutations, and (ii) the viral titers were sufficiently high to allow the performance of PRNT assays.

PRNT assay

PRNT was used to determine the serum titers of SARS-CoV-2 neutralizing antibodies, as previously described by Pauvolid-Correa et al. (2022), with some modifications. Briefly, sera were treated at 56°C for 30 minutes and used in PRNT in Vero cells (ATCC, CCL 81) maintained in cell culture medium supplemented with fetal bovine serum, sodium bicarbonate, and antibiotics/antimycotics and cultivated at 5% CO_2 atmosphere and 37°C. Serum samples were tested in duplicates in serial twofold dilutions (1:10 to 1:320) for their ability to neutralize 40-60 plaque-forming units by each one of the two SARS-CoV-2 reference isolates: XBB.1.9 and JN.1.1. Sera were mixed with the respective SARS-CoV-2 isolates and incubated at 37°C for 1 hour. Afterward, the culture medium was removed from Vero cell monolayers on 12-well culture plates, which were inoculated with the virus-serum mixtures, incubated at 37°C for 1 hour, and overlaid with a pre-warmed cell culture medium containing 0.5% ultrapure agarose (Sigma-Aldrich). To ensure reproducibility between experiments and establish a baseline for neutralization activity, each neutralization experiment included an infection control (no serum) and a reference serum. After 48 hours of incubation,

plates were overlaid with a pre-warmed cell culture medium containing 0.5% ultrapure agarose and neutral red solution (Sigma-Aldrich) to visualize viral plaques, and, after 72 hours, plaque-forming units were counted through a transilluminator. The neutralization titer of a serum is calculated as the reciprocal value of the highest serum dilution that reduces the number of viral plaques by 90% ($PRNT_{90}$). A serum sample is considered reactive to a specific SARS-CoV-2 lineage when the $PRNT_{90}$ neutralization titer reaches a value of at least 10 against that isolate [24].

Data on hospitalizations for SARI

We extracted data about hospitalizations resulting from SARI attributed explicitly to SARS-CoV-2 (SARI-COVID) in Brazil during the period spanning from July 2023 to March 2024. This information was sourced from the Influenza Surveillance Information System (SIVEP-Gripe) database (https://opendatasus.saude.gov.br/dataset?tags=SRAG). To identify SARI cases, we used a set of four criteria which required individuals to exhibit (i) fever, including self-reported cases, (ii) cough or sore throat, (iii) dyspnea or oxygen saturation levels below 95% or experiencing respiratory discomfort, and (iv) hospitalization. Once an individual meets these criteria and is admitted to a hospital for SARI, their case must be reported and recorded as a distinct entry in the SIVEP-Gripe database. Confirmation of hospitalization for SARI-COVID was contingent on a positive result from the RT-PCR test for SARS-CoV-2. For our analysis, we considered all the records within the SIVEP-Gripe database that adhered to the criteria for defining a hospitalized SARI case. We excluded records related to non-hospitalized deaths from our examination.

Variant-specific SARI cases

To estimate the number of SARS-CoV-2 infections driven by different variants, we combined the variant frequency data with the number of SARI cases over time (Figure S1). For these analyses, we included a total of 15 states from northern (Amazonas [AM] and Para [PA]), northeastern (Alagoas [AL], Ceara [CE], Paraiba [PB], Pernambuco [PE], Piaui [PI], Rio Grande do Norte [RN], and Sergipe [SE]), central-western (Goiás [GO] and federal district [DF]), southeastern (Minas Gerais [MG], Rio de Janeiro [RJ], and Sao Paulo [SP]), and southern (Parana [PR], Santa Catarina [SC] and Rio Grande do Sul [RS]) regions that displayed a significant number of SARI cases (n >100) and clearly defined wave peaks in the study period.

Data analysis

The Wilcoxon signed-rank and Mann–Whitney tests were used to assess the significant difference between the data sets of $PRNT_{90}$ titers values obtained. The chi-square test was used to compare the distribution of SARI-COVID cases by age among locations. Graph representations and statistical analysis were performed using GraphPad Prism software v10.2.3 (GraphPad Software, San Diego, CA, USA). A P <0.05 was considered to be statistically significant.

Results and discussion

We analyzed a total of 13,001 Brazilian SARS-CoV-2 genomes sampled across all Brazilian regions and states between July 1, 2023 and March 31, 2024 (Figure 1a and Figure S1). Viral sequences were mostly classified as XBB.1* (63%), JN.1* (31%), or XDR (5%) lineages. XDR is a recombinant clade between XBB.1 and JN.1 descendant lineages which harbors the S protein of JN.1 and display a transmissibility similar to that lineage and was then grouped within the family of viral lineages with a JN.1* S protein [28]. The JN.1* lineages were first detected in Brazil in October 2023 and achieved dominance (>50% of cases) by January 2024. This pattern was quite homogenous across all Brazilian regions and states, except for Ceara, located in the northeastern region, where the JN.1* lineages already achieved dominance by November 2023 (Figure 1b and Figure S2). This clear pattern of substitution of XBB.1* by JN.1* lineages across all Brazilian localities is consistent with the higher JN.1* transmissibility concerning XBB.1* lineages previously estimated in Brazil and elsewhere [13,28].

To assess the epidemiologic consequences of the dissemination of JN.1* lineages across different Brazilian states (Figure S3), we estimated the relative number of variant-specific infections by combining variant frequency data with the number of SARI-COVID cases over time. This analysis revealed four major epidemic patterns (Figure 1c). In some states from northeastern (CE, PI, PB, RN, and SE) and northern (AM and PA) regions, the JN.1* lineages started to circulate after a long time interval (>6 months) of low SARI incidence, and viral lineage replacement coincided with a sharp increase of SARI cases that peaked in December 2023 to January 2024. In the northeastern states of AL and PE, viral lineage replacement also coincided with a sharp increase of SARI cases in late 2023, but such a wave was driven by a mixture of XBB.1* and JN.1* lineages. The XBB variant wave started a couple of weeks earlier and reached a peak somewhat larger than the JN.1 variant wave. The northeastern state of BA displayed a wave of SARI cases driven by XBB.1* that peaked in November 2023, shortly followed by a wave of smaller size driven by JN.1* that extends from January to March 2024. Finally, states from central-western, southeastern, and southern regions experienced a wave of SARI cases driven by XBB.1* that peaked around October 2023, a subsequent spread of JN.1* lineages without an immediate upsurge of SARI cases, and a delayed wave of SARI cases driven by JN.1* around February to March 2024.

Given the age-related decline in immune protection [29–32], it could be hypothesized that age-stratified differences in SARI cases across Brazilian states may underlie the observed epidemiologic dynamics. To test this, we examined the distribution of age groups among SARI-COVID cases across locations (Figure S4). Although we detected statistically significant differences in age group distributions among regions (P <0.0001), this variation does not account for the disparate SARI dynamics observed. For instance, the age distribution of SARI cases in certain northeastern states (PB+PI+RN+SE) was comparable to that of the southern states (PR+RS+SC) (P = 0.12); however, their overall temporal patterns differed significantly. Conversely, certain northeastern states (PB+PI+RN+SE) and northern states (AM+PA) displayed quite different age distributions (P <0.0001) but exhibited similar SARI dynamics. Similarly, the southeastern states (RJ+SP+MG) and the southern states (PR+RS+SC) exhibited distinct age distributions of SARI cases (P <0.0001), despite exhibiting similar trends in case dynamics. This underscores that although age distribution is a critical factor in SARI analyses, it does not explain the observed regional disparities in case waves across Brazilian locations.

This pattern observed in the northeastern/northern regions resembles that previously described in Brazil during replacements of B.1* lineages by Gamma and of Delta by Omicron (BA.1), although the total number of SARI cases in the JN.1 wave was much lower than in previous waves [33–37]. In addition, the pattern of lineage replacement without an upsurge of SARI cases observed in the other country regions resembles that described during the substitution of VOC Gamma by Delta in Brazil [33,38]. These findings are consistent with the notion that SARS-CoV-2 lineage replacements are primarily driven by the relative transmissibility of viral lineages, whereas epidemic dynamics also depend on the underlying population immunologic landscape and, particularly, the proportion of susceptible hosts [2,39–42]. We hypothesized that the XBB variant wave that occurred in some Brazilian regions around October 2023 boosted the population cross-immunity and generated short-term protection against JN.1 infection, thus preventing an immediate upsurge of SARI cases during lineage turnover.

To test this hypothesis, we investigated the NAb responses induced by XBB.1* infections against the JN.1 variant in 10 Brazilian individu-

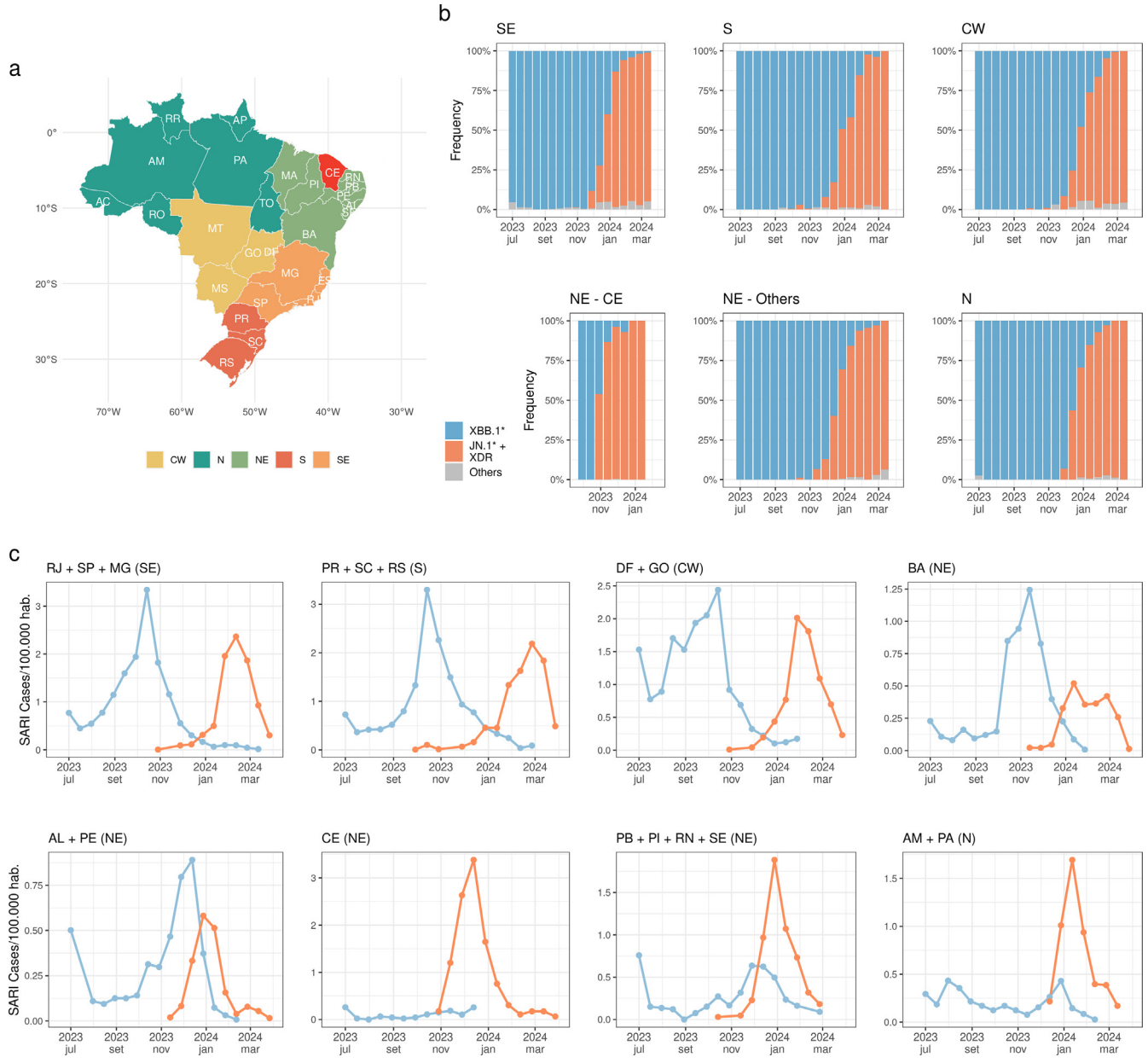

Figure 1. Space-time evolution of major SARS-CoV-2 variants and variant-specific SARI cases in Brazil between July 2023 and March 2024. (a) The map depicts Brazil's regions and states, highlighting the SE, S, CW, NE, and N regions in distinct colors as indicated in the legend at the bottom. The state of CE is highlighted in red. Each Brazilian state is labeled with its two-letter abbreviation. (b) Temporal fluctuations in the relative prevalence of XBB.1* and JN.1*+XDR lineages across Brazilian regions. (c) Estimated number of SARI cases caused by SARS-CoV-2 XBB.1* and JN.1*+XDR lineages through time across selected Brazilian states from southeastern (MG, RJ, and SP), southern (PR, SC, and RS), northeastern (AL, BA, CE, PB, PE, PI, RN, and SE) and northern (AM and PA) regions. States from the same region showing a similar pattern of temporal fluctuations of SARS-CoV-2 lineages (Figure S2) and SARI cases (Figure S3) were grouped for visual clarity. The Brazilian map was generated with the ggplot2 [25], sf [26], and geobr [27] R packages.
CE, Ceará; CW, central-western; NE, northeastern; N, northern; SE, southeastern; S, southern.

als who were longitudinally followed up. As expected, a significant (P <0.05) and large increase in $PRNT_{90}$ titers (29.6-fold) was observed between pre- and post-infection sera against XBB.1 (Figure 2a). We also observed a significant (P <0.05) increase (10.7-fold higher) in the $PRNT_{90}$ titers against JN.1 in post-infection sera compared with the pre-infection samples (Figure 2a). The $PRNT_{90}$ titers against JN.1 were 2.9-fold lower than those against XBB.1 after XBB.1* infection, whereas the $PRNT_{90}$ titers against JN.1 after JN.1* infection were 3.5-fold higher than those obtained after XBB.1* infection (Figure 2b). Despite this reduced neutralizing activity, neutralization of JN.1 was observed in most (90%) individuals infected with XBB analyzed. These results agree with recent studies showing a significant (~10-fold) increase in NAb responses against the JN.1 variant after XBB.1.5 vaccine booster or XBB.1* infection [43–48]. Those studies also showed that serum titers against JN.1 were around two- to sixfold lower than those against XBB.1.5, depending on population immune backgrounds.

The epidemic pattern of SARI cases in the southeastern/southern/central-western regions reveals a minimum time interval of 4-5 months between the XBB and JN.1 wave peaks (Figure 1c). This is consistent with the notion that cross-reactive immunity induced by XBB.1* infections wanes over time, fueling a JN.1 wave of SARI cases ~4-5 months later in those regions [49,50]. Interestingly, among the 19 individuals

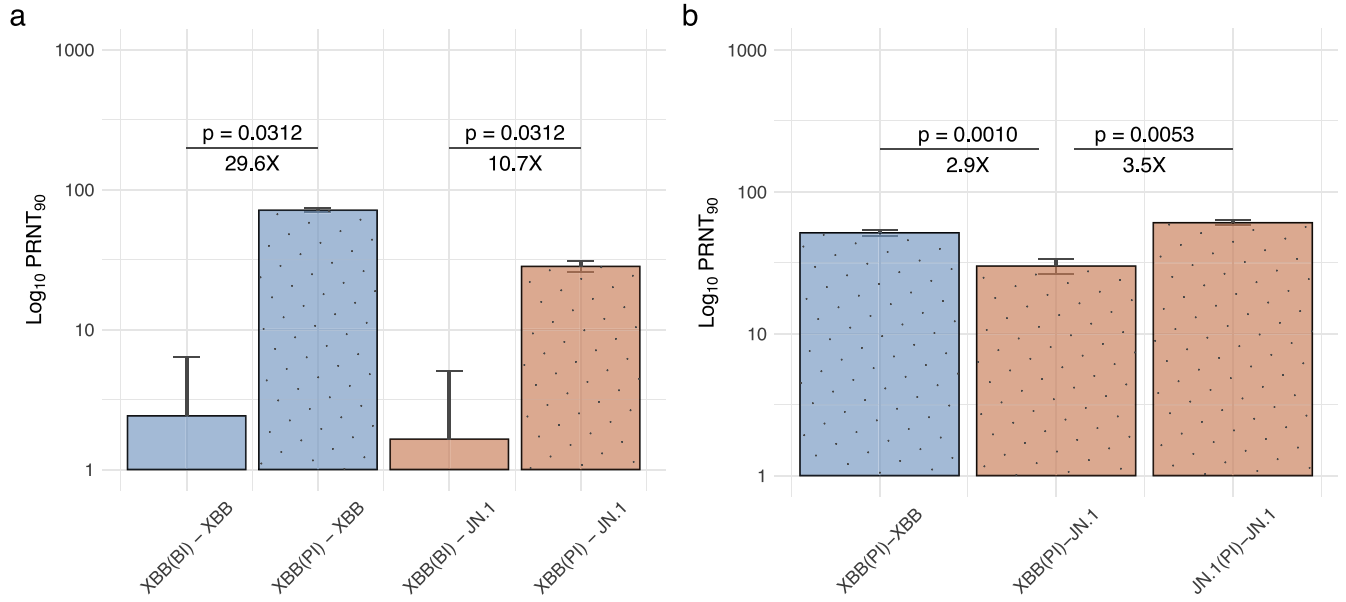

Figure 2. Neutralizing antibodies titers against XBB.1 and JN.1 variants of SARS-CoV-2 in Brazilian individuals who experienced an infection with XBB.1* or JN.1* lineages. (a) Neutralizing antibodies activity was measured by PRNT90 against XBB.1 and JN.1 variants in serum samples from a cohort of volunteers (n = 6) who were infected with XBB.1* lineages. Paired serum samples collected before infection—XBB(BI)—and after infection with XBB.1*—XBB(PI)—were tested for each patient. (b) PRNT90 against XBB.1 and/or JN.1 variants in a set of serum samples from individuals infected with XBB.1* (n = 10) or JN.1* (n = 19) lineages. Samples were collected at 25-255 days BI and 20-70 days PI. The y-axis shows the Log_{10} $PRNT_{90}$ values. Fold-change in the geometric mean titer (GMT) values and statistical significance are shown. Wilcoxon signed-rank (A) and Mann–Whitney tests (B) were used to calculate the *P*-values. $PRNT_{90}$ below the limit of detection (<10) were set to 1. XBB(BI)-XBB = sera obtained BI with XBB.1* and tested against XBB.1. XBB(PI)-XBB = sera PI with XBB.1* and tested against XBB.1. XBB(BI)-JN.1 = sera obtained BI with XBB.1* and tested against JN.1. XBB(PI)-JN.1 = sera obtained PI with XBB.1* and tested against JN.1, JN.1(PI)-JN.1 = sera obtained PI with JN.1* and tested against JN.1. The bars are color-coded based on the isolate used in each category, with blue representing XBB* isolates and orange for JN.1 isolates. PI sera bars are depicted with a dotted pattern.
BI, before infection; PI, post-infection; PRNT90, 90% plaque reduction neutralization titers.

from the southeastern state of RJ infected with the JN.1 variant analyzed in our study, seven had a documented previous SARS-CoV-2 infection (Table S1). This includes three individuals who were infected during the period of XBB dominance between March and September 2023. The time interval between previous XBB infections and JN.1 reinfections ranges from 5.3 to 10.8 months. These findings corroborate that individuals previously infected with XBB.1* lineages could be reinfected with JN.1* lineages and further support a minimum time interval of about 5 months between successive infections by those Omicron lineages in the Brazilian population immune landscape.

The dynamics of SARI cases in the northeastern state of BA exhibited a singular pattern characterized by a short delay between the peak of the XBB.1* wave (November 2023) and the increase of the JN.1* wave (January 2024). Moreover, the JN.1* wave exhibited a prolonged plateau of cases rather than a distinct peak, lasting from January to March 2024. BA is the fifth largest Brazilian state by area and is bordered by states from the northeastern, southeastern, northern and central-western regions. Therefore, BA is located at the crossroads of regions within the country that exhibit distinct SARI-COVID case dynamics. Given its geographic position, we propose that the atypical pattern of SARI-COVID cases in Bahia may have resulted from the mixing of varying intra-state dynamics across distinct municipalities. The JN.1* wave in BA may have emerged in January 2024 in municipalities that had not experienced the XBB.1* wave in the preceding months, subsequently spreading to regions that were affected by the XBB.1* wave observed in November 2023. Unfortunately, we lack data on SARI-COVID cases at the municipality level to test this hypothesis.

Despite the small number of subjects analyzed, our findings support that XBB.1* infections produced substantial cross-reactive NAb responses against the JN.1 variant in the Brazilian immune background. The precise level of NAbs required for protection against JN.1 infection is difficult to estimate; however, some evidence support that such cross-reactive responses induced by XBB may be protective. A recent report showed that immunity provided by natural infection against reinfection with JN.1 was strong (83% effectiveness) among those who were infected within the last 6 months with variants such as XBB.1* [51]. Other studies also showed that the XBB.1.5 monovalent mRNA vaccine booster conferred some protection against symptomatic infections and hospitalizations caused by the JN.1 variant [52–56]. In Brazil, the XBB.1.5 monovalent mRNA vaccine was first introduced by the end of May 2024 [57]. Our findings, along with previous studies, emphasize the importance of boosters with updated XBB.1.5 monovalent vaccines to enhance immune responses and reduce the onward transmission of the JN.1 variant that is currently prevalent in Brazil, particularly, in priority groups of the population.

Our study has some limitations. First, we estimated the relative number of variant-specific infections assuming that SARS-CoV-2 genomic surveillance was random and truly reflected the variant frequency within states and that the infection-hospitalization ratio (SARI cases/total cases) was the same for different Omicron variants. Second, due to the small sample size of individuals infected with XBB, we could not assess the potential impact of age or immune background (history of infections and vaccinations) on neutralization efficiency. The age of individuals infected with XBB ranges between 40 and 89 years (Table S1), pointing out that cross-reactive responses were not restricted to young adults. Moreover, all individuals infected with XBB included in our study were fully vaccinated (monovalent wild-type vaccines), and a significant fraction (70%) also have a documented previous infection (Table S1), thus indicating that hybrid immunity was an important component of the immune background of the studied population. Third, serum samples were collected within 2 months post-XBB.1* infection and we do not

have information about cross-reactive neutralization efficiency against JN.1 after longer periods since infection.

Conclusion

The JN.1* lineages consistently replaced the XBB.1* lineages circulating in all Brazilian states since late 2023 but with variable epidemiologic impact across regions. In those Brazilian states that experienced a XBB variant wave in the second half of 2023, shortly before the introduction of JN.1, lineage replacement occurred without a concurrent rise in the number of SARI cases. In contrast, Brazilian states without a recent XBB variant wave in the second half of 2023 experienced an upsurge of SARI cases concurrent with the expansion of the JN.1 variant. Recent infections with XBB.1* lineages induce a considerable production of cross-reactive NAbs that are effective against JN.1 in the Brazilian population's immune background. Together, these results support the notion that the XBB variant wave observed in the second half of 2023 in some Brazilian states probably acted as a natural booster of the population immunity, providing short-term protection against JN.1 infections and delaying the rise of SARI cases in those country regions.

Declarations of competing interest

The authors have no competing interests to declare.

Funding

This study received funding support from *Carlos Chagas Filho* Foundation for Research Support in the State of Rio de Janeiro (FAPERJ) (FAPERJ E-26/211.125/2021), National Council for Scientific and Technological Development (CNPq), Ministry of Science, Technology and Innovations (MCTI), and the Latin American Biotechnology Center (CABBIO) (CNPq/MCTI N° 17/2021, 423857/2021-5). P.C.R. had support from CNPq (Productivity fellowship 311759/2022-0). I.A. had support from FAPERJ (grant SEI-260003/019669/2022). G.L.W. had support from CNPq (Productivity fellowship 307209/2023-7). P.B. had support from FAPERJ (E-26/200.935/2022) and CNPq (311562/2021-3).

Ethical approval

This study was approved by the Brazilian National Committee of Ethics in Research (CONEP) (protocol number: 30639420.0.0000.5262), and signed written informed consent was obtained from all participants.

Acknowledgments

The authors would like to thank the Biosafety Laboratory Level 3 platform (BSL3) at Pavilhão *Helio Peggy Pereira*, Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro, Brazil.

Author contributions

Dr. Luis Fernando Lopez Tort: study design, data analysis, data interpretation, figures, writing – original draft. **Dra. Mia Ferreira de Araújo:** study design, data analysis, data interpretation, figures, writing – original draft. **Dr. Ighor Arantes:** study design, data analysis, data interpretation, figures, writing – original draft. **MSc. Jéssica SCC Martins:** data analysis, data interpretation. **Dr. Marcelo Gomes:** data collection, data analysis, data interpretation. **Dr. Felipe Cotrim de Carvalho:** data collection, data analysis. **Dra. Walquiria Aparecida Ferreira de Almeida:** data collection, data analysis. **Dra. Braulia Costa Caetano:** data analysis, data interpretation, writing review & editing. Dra. **Luciana R. Appolinario:** data collection, data analysis, data interpretation. **Dra. Elisa Calvalcante Pereira:** data collection, data analysis, data interpretation. **BSc. Jéssica Carvalho:** data collection, data analysis. **Dr. Fábio Miyajima:** data collection, data analysis, data interpretation, funding acquisition. **Dr. Gabriel Luz Wallau:** data collection, data analysis, data interpretation, writing – review & editing, funding acquisition. **Dr. Felipe Gomes Naveca:** data collection, data analysis, data interpretation, funding acquisition. **Dr. Pedro Alves:** data collection, data analysis, data interpretation, writing – review & editing, funding acquisition. **Dr. Otávio Espíndola:** data collection, data analysis, data interpretation, writing – review & editing. **Dra. Patricia Brasil:** data collection, data analysis, data interpretation, writing – review & editing, funding acquisition. **Dra. Paola Cristina Resende:** study design, data analysis, data interpretation, funding acquisition, resources, writing – review & editing. **Dr. Gonzalo Bello:** conceptualisation, study design, data curation, validation, writing – review & editing, funding acquisition. **Dra. Marilda Mendonça Siqueira:** supervision, validation, funding acquisition, resources, writing – review & editing. Dr. Luis Fernando Lopez Tort, Dra. Mia Ferreira de Araújo & Dr. Ighor Arantes have contributed equally to this work. Dra. Paola Cristina Resende, Dr. Gonzalo Bello & Dra. Marilda Mendonça Siqueira share the senior authorship.

Data availability

This study's conclusions derive from examining 13,001 SARS-CoV-2 genomes from Brazil, which have been made publicly accessible via the EpiCoV database from the Global Initiative on Sharing All Influenza Data. These genomes were collected after July 1, 2023, and submissions were recorded up until March 31, 2024. The data can be accessed at https://doi.org/10.55876/gis8.240719ev.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijregi.2024.100503.

References

[1] ORGANIZATION WORLD HEALTH. Emergency Committee on the COVID-19 pandemic. *Statement on the fifteenth meeting of the IHR*; 2005 *https://www.who.int/europe/news/item/05-05-2023-statement-on-the-fifteenth-meeting-of-the-international-health-regulations-(2005)-emergency-committee-regarding-the-coronavirus-disease-(covid-19)-pandemic* 2023 [accessed 04 July 2024].

[2] Markov PV, Ghafari M, Beer M, Lythgoe K, Simmonds P, Stilianakis NI, et al. The evolution of SARS-CoV-2. *Nat Rev Microbiol* 2023;**21**:361–79. doi:10.1038/s41579-023-00878-2.

[3] Tan CW, Zhu F, Chia WN, Young BE, Yeoh AYY, Althaus T, et al. Distinctive serotypes of SARS-related coronaviruses defined by convalescent sera from unvaccinated individuals. *Hlife* 2023;**1**:26–34. doi:10.1016/j.hlife.2023.07.002.

[4] Bekliz M, Essaidi-Laziosi M, Adea K, Hosszu-Fellous K, Alvarez C, Bellon M, et al. Immune escape and replicative capacity of Omicron lineages BA;1, BA.2, BA.5.1, BQ.1, XBB.1.5, EG.5.1 and JN.1.1. bioRxiv. 29 October 2024. https://doi.org/10.1101/2024.02.14.579654 [accessed 04 July 2024].

[5] Favresse J, Gillot C, Cabo J, David C, Dogné JM, Douxfils J. Neutralizing antibody response to XBB.1.5, BA.2.86, FL.1.5.1, and JN.1 six months after the BNT162b2 bivalent booster. *Int J Infect Dis* 2024;**143**:107028. doi:10.1016/j.ijid.2024.107028.

[6] Jeworowski LM, Mühlemann B, Walper F, Schmidt ML, Jansen J, Krumbholz A, et al. Humoral immune escape by current SARS-CoV-2 variants BA.2.86 and JN.1, December 2023. *Eurosurveillance* 2024;**29**:2300740. doi:10.2807/1560-7917.ES.2024.29.2.2300740.

[7] Wang X, Jiang S, Ma W, Zhang Y, Wang P. Robust neutralization of SARS-CoV-2 variants including JN.1 and BA.2.87.1 by trivalent XBB vaccine-induced antibodies. *Signal Transduct Target Ther* 2024;**9**:123. doi:10.1038/s41392-024-01849-6.

[8] Li P, Liu Y, Faraone JN, Hsu CC, Chamblee M, Zheng YM, et al. Distinct patterns of SARS-CoV-2 BA.2.87.1 and JN.1 variants in immune evasion, antigenicity, and cell-cell fusion. *mBio* 2024;**15**:e0075124. doi:10.1128/mbio.00751-24.

[9] Yue C, Song W, Wang L, Jian F, Chen X, Gao F, et al. ACE2 binding and antibody evasion in enhanced transmissibility of XBB.1.5. *Lancet Infect Dis* 2023;**23**:278–80. doi:10.1016/S1473-3099(23)00010-5.

[10] Tamura T, Ito J, Uriu K, Zahradnik J, Kida I, Anraku Y, et al. Virological characteristics of the SARS-CoV-2 XBB variant derived from recombination of two Omicron subvariants. *Nat Commun* 2023;**14**:2800. doi:10.1038/s41467-023-38435-3.

[11] GISAID initiative. Lineage Frequency. EPIco, https://gisaid.org; 2024 [accessed 04 July 2024].

[12] Uriu K, Ito J, Kosugi Y, Tanaka YL, Mugita Y, Guo Z, et al. Transmissibility, infectivity, and immune evasion of the SARS-CoV-2 BA.2.86 variant. *Lancet Infect Dis* 2023;**23**:e460–1. doi:10.1016/S1473-3099(23)00575-3.

[13] Kaku Y, Okumura K, Padilla-Blanco M, Kosugi Y, Uriu K, Hinay AA, et al. Virological characteristics of the SARS-CoV-2 JN.1 variant. *Lancet Infect Dis* 2024;**24**:e82. doi:10.1016/S1473-3099(23)00813-7.

[14] Zhang L, Dopfer-Jablonka A, Cossmann A, Stankov MV, Graichen L, Moldenhauer AS, et al. Rapid spread of the SARS-CoV-2 JN.1 lineage is associated with increased neutralization evasion. *iScience* 2024;**27**:109904. doi:10.1016/j.isci.2024.109904.

[15] Planas D, Staropoli I, Michel V, Lemoine F, Donati F, Prot M, et al. Distinct evolution of SARS-CoV-2 Omicron XBB and BA.2.86/JN.1 lineages combining increased fitness and antibody evasion. *Nat Commun* 2024;**15**:2254. doi:10.1038/s41467-024-46490-7.

[16] Arantes I, Gomes M, Ito K, Sarafim S, Gräf T, Miyajima F, et al. Spatiotemporal dynamics and epidemiological impact of SARS-CoV-2 XBB lineage dissemination in Brazil in 2023. *Microbiol Spectr* 2024;**12**:e0383123. doi:10.1128/spectrum.03831-23.

[17] Dashboard FIOCRUZ. SARS-CoV-2 genomic surveillance in Brazil. *Oswaldo Cruz Foundation. Fiocruz´s Genomics Network* 2024. https://www.genomahcov.fiocruz.br/dashboard-en/ [accessed 04 July 2024].

[18] Lugon P, Fuller T, Damasceno L, Calvet G, Resende PC, Matos AR, et al. SARS-CoV-2 infection dynamics in children and household contacts in a slum in Rio de Janeiro. *Pediatrics* 2021;**148** e20211050182. doi:10.1542/peds.2021-050182.

[19] Fernanda Borges da Silva M, Guaraldo L, Ferreira Pinto Santos H, Soares Bastos L, Lema Suarez Penetra S, Campos Vargas de Moraes I, et al. Cohort profile: follow-up of a household cohort throughout five epidemic waves of SARS-CoV-2 in Rio de Janeiro. *Brazil. Cad Saúde Publ* 2024;**40**:e00152023. doi:10.1590/0102-311XEN152023.

[20] FIOCRUZ. SARS-CoV-2 Genomic surveillance in Brazil, https://www.genomahcov.fiocruz.br/en/; 2021 [accessed 04 July 2024].

[21] Aksamentov I, Roemer C, Hodcroft E, Neher R. Nextclade: clade assignment, mutation calling and quality control for viral genomes. *J Open Source Softw* 2021;**6**:3773. doi:10.21105/joss.03773.

[22] O'Toole Á, Scher E, Underwood A, Jackson B, Hill V, McCrone JT, et al. Assignment of epidemiological lineages in an emerging pandemic using the pangolin tool. *Virus Evol* 2021;**7**:veab064. doi:10.1093/ve/veab064.

[23] Pauvolid-Corrêa A, Caetano BC, MacHado AB, Ferreira MA, Valente N, Neves TK, et al. Sera of patients infected by earlier lineages of SARS-CoV-2 are capable to neutralize later emerged variants of concern. *Biol Methods Protoc* 2022;**7**:bpac021. doi:10.1093/biomethods/bpac021.

[24] Deshpande GR, Sapkal GN, Tilekar BN, Yadav PD, Gurav Y, Gaikwad S, et al. Neutralizing antibody responses to SARS-CoV-2 in COVID-19 patients. *Indian J Med Res* 2020;**152**:82–7. doi:10.4103/ijmr.IJMR_2382_20.

[25] Wickham H. *ggplot2: Elegant Graphics for Data Analysis*. New York: Springer-Verlag New York; 2016.

[26] Pebesma E. Simple features for R: standardized support for spatial vector data. *R J* 2018;**10**:439–46. doi:10.32614/RJ-2018-009.

[27] Pereira RHM. geobr: LS of OSDS of BrazilG repository, https://github.com/ipeaGIT/geobr; 2019 [accessed 04 July 2024].

[28] Arantes I, Ito K, Gomes M, Cotrim De Carvalho F, Ferreira De Almeida WA, Khouri R, et al. Rapid spread of the SARS-CoV-2 Omicron XDR lineage derived from recombination between XBB and BA.2.86 subvariants circulating in Brazil in late 2023. *Microbiol Spectr* 2024. doi:10.1128/spectrum.01193-24.

[29] Collier DA, Ferreira IATM, Kotagiri P, Datir RP, Lim EY, Touizer E, et al. Age-related immune response heterogeneity to SARS-CoV-2 vaccine BNT162b2. *Nature* 2021;**596**:417–22. doi:10.1038/s41586-021-03739-1.

[30] Gallerani E, Proietto D, Dallan B, Campagnaro M, Pacifico S, Albanese V, et al. Impaired priming of SARS-CoV-2-Specific naive $CD8^+$ T cells in older subjects. *Front Immunol* 2021;**12**:693054. doi:10.3389/fimmu.2021.693054.

[31] Chen Y, Li C, Liu F, Ye Z, Song W, Lee ACY, et al. Age-associated SARS-CoV-2 breakthrough infection and changes in immune response in a mouse model. *Emerg Microbes Infect* 2022;**11**:368–83. doi:10.1080/22221751.2022.2026741.

[32] Zhu HH, Sun GQ, Wu JY, Fan SQ, Zhu YY, Wang ZC, Liao XF. Antibody responses to an inactivated SARS-CoV-2 vaccine in individuals aged from 50 to 102 years. *Front Immunol* 2023;**14**:1212988. doi:10.3389/fimmu.2023.1212988.

[33] Arantes I, Bello G, Nascimento V, Souza V, da Silva A, Silva D, et al. Comparative epidemic expansion of SARS-CoV-2 variants Delta and Omicron in the Brazilian State of Amazonas. *Nat Commun* 2023;**14**:2048. doi:10.1038/s41467-023-37541-6.

[34] COVID-19 Fiocruz Genomic Surveillance Network Arantes I, Gomes Naveca F, Gräf T, Miyajima F, Faoro H, et al. Emergence and spread of the SARS-CoV-2 variant of concern Delta across different Brazilian regions. *Microbiol Spectr* 2022;**10**:e0264121. doi:10.1128/spectrum.02641-21.

[35] Naveca FG, Nascimento V, de Souza VC, Corado AL, Nascimento F, Silva G, et al. COVID-19 in Amazonas, Brazil, was driven by the persistence of endemic lineages and P.1 emergence. *Nat Med* 2021;**27**:1230–8. doi:10.1038/s41591-021-01378-7.

[36] Gräf T, Bello G, Naveca FG, Gomes M, Cardoso VLO, da Silva AF, et al. Phylogenetic-based inference reveals distinct transmission dynamics of SARS-CoV-2 lineages Gamma and P.2 in Brazil. *iScience* 2022;**25**:104156. doi:10.1016/j.isci.2022.104156.

[37] Faria NR, Mellan TA, Whittaker C, Claro IM, da Candido DDS, Mishra S, et al. Genomics and epidemiology of the P.1 SARS-CoV-2 lineage in Manaus, Brazil. *Science* 2021;**372**:815–21. doi:10.1126/science.abh2644.

[38] Giovanetti M, Fonseca V, Wilkinson E, Tegally H, San EJ, Althaus CL, et al. Replacement of the Gamma by the Delta variant in Brazil: impact of lineage displacement on the ongoing pandemic. *Virus Evol* 2022;**8** veac024. doi:10.1093/ve/veac024.

[39] Meijers M, Ruchnewitz D, Eberhardt J, Łuksza M, Lässig M. Population immunity predicts evolutionary trajectories of SARS-CoV-2. *Cell* 2023;**186**:5151–64 .e13. doi:10.1016/j.cell.2023.09.022.

[40] Bushman M, Kahn R, Taylor BP, Lipsitch M, Hanage WP. Population impact of SARS-CoV-2 variants with enhanced transmissibility and/or partial immune escape. *Cell* 2021;**184**:6229–42 .e18. doi:10.1016/j.cell.2021.11.026.

[41] Dyson L, Hill EM, Moore S, Curran-Sebastian J, Tildesley MJ, Lythgoe KA, et al. Possible future waves of SARS-CoV-2 infection generated by variants of concern with a range of characteristics. *Nat Commun* 2021;**12**:5730. doi:10.1038/s41467-021-25915-7.

[42] Gomes MGM, Ferreira MU, Corder RM, King JG, Souto-Maior C, Penha-Gonçalves C, et al. Individual variation in susceptibility or exposure to SARS-CoV-2 lowers the herd immunity threshold. *J Theor Biol* 2022;**540**:111063. doi:10.1016/j.jtbi.2022.111063.

[43] Chalkias S, McGhee N, Whatley JL, Essink B, Brosz A, Tomassini JE, et al. Interim report of the reactogenicity and immunogenicity of severe acute respiratory syndrome coronavirus 2 XBB–containing vaccines. *J Infect Dis* 2024;**230**:e279–86. doi:10.1093/infdis/jiae067.

[44] Nguyenla XH, Bates TA, Trank-Greene M, Wahedi M, Tafesse FG, Curlin ME. Evaluating humoral immunity elicited by XBB.1.5 monovalent COVID-19 vaccine. *Emerg Infect Dis* 2024;**30**:1282–3. doi:10.3201/eid3006.240051.

[45] Springer DN, Camp JV, Aberle SW, Deutsch J, Lammel O, Weseslindtner L, et al. Neutralization of SARS-CoV-2 omicron XBB.1.5 and JN.1 variants after COVID-19 booster-vaccination and infection. *J Med Virol* 2024;**96**:e29801. doi:10.1002/jmv.29801.

[46] Wang Q, Guo Y, Bowen A, Mellis IA, Valdez R, Gherasim C, et al. XBB.1.5 monovalent mRNA vaccine booster elicits robust neutralizing antibodies against XBB subvariants and JN.1. *Cell Host Microbe* 2024;**32**:315 –321.e3. doi:10.1016/j.chom.2024.01.014.

[47] He X, Yu J, Jiang J, Liu J, Qi Q, Liu D, et al. Heterogeneous hybrid immunity against Omicron variant JN.1 at 11 months following breakthrough infection. *Signal Transduct Target Ther* 2024;**9**:180. doi:10.1038/s41392-024-01898-x.

[48] Abul Y, Nugent C, Vishnepolskiy I, Wallace T, Dickerson E, Holland L, et al. Broad immunogenicity to prior SARS-CoV-2 strains and JN.1 variant elicited by XBB.1.5 vaccination in nursing home residents. *GeroScience* 2024. doi:10.1007/s11357-024-01346-2.

[49] Wang X, Zhang M, Wei K, Li C, Yang J, Jiang S, et al. Longitudinal analysis of humoral and cellular immune response up to 6 months after SARS-CoV-2 BA.5/BF.7/XBB breakthrough infection and BA.5/BF.7-XBB reinfection. *Vaccines (Basel)* 2024;**12**:464. doi:10.3390/vaccines12050464.

[50] Khoury DS, Cromer D, Reynaldi A, Schlub TE, Wheatley AK, Juno JA, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat Med* 2021;**27**:1205–11. doi:10.1038/s41591-021-01377-8.

[51] Chemaitelly H, Coyle P, Ben Kacem MA, Ayoub HH, Tang P, Hasan MR, et al. Protection of natural infection against reinfection with SARS-CoV-2 JN.1 variant. *J Travel Med* 2024;**31** taae053. doi:10.1093/jtm/taae053.

[52] Link-Gelles R, Ciesla AA, Mak J, Miller JD, Silk BJ, Lambrou AS, et al. Early estimates of updated 2023–2024 (monovalent XBB.1.5) COVID-19 vaccine effectiveness against symptomatic SARS-CoV-2 infection attributable to co-circulating Omicron variants among immunocompetent adults–Increasing community access to testing program, United States, September 2023 – January 2024. *MMWR Morb Mortal Wkly Rep* 2024;**73**:77–83. doi:10.15585/mmwr.mm7304a2.

[53] preprint Tartof SY, Slezak JM, Frankland TB, Puzniak L, Hong V, Ackerson BK, et al. BNT162b2 XBB1.5-adapted vaccine and COVID-19 hospital admissions and ambulatory visits in US adults. medRxiv. 28 December 2023. http://doi.org/10.1101/2023.12.24.23300512 [accessed 04 July 2024].

[54] Nunes B, Humphreys J, Nicolay N, Braeye T, Van I, Holm Hansen C, et al. Monovalent XBB.1.5 COVID-19 vaccine effectiveness against hospitalisations and deaths during the Omicron BA.2.86/JN.1 period among older adults in seven European countries: a VEBIS-EHR Network Study 3. *Expert Rev Vaccines* 2024;**23**:1085–90. doi:10.1080/14760584.2024.2428800.

[55] Ma KC, Surie D, Lauring AS, Martin ET, Leis AM, Papalambros L, et al. Effectiveness of updated 2023–2024 (Monovalent XBB.1.5) COVID-19 vaccination against SARS-CoV-2 Omicron XBB and BA.2.86/JN.1 lineage hospitalization and a comparison of clinical severity – IVY Network, 26 hospitals, 18 October 2023-9 March 2024. *Clin Infect Dis* 2024. doi:10.1093/cid/ciae405.

[56] Chong C, Wee LE, Jin X, Zhang M, Malek MIA, Ong OB, et al. Risks of SARS-CoV-2 JN.1 infection and COVID-19 associated emergency-department (ED) visits/hospitalizations following updated boosters and prior infection: a population-based cohort study. *Clin Infect Dis* 2024;**79**:1190–6. doi:10.1093/cid/ciae339.

[57] Ministry of Health of Brazil. Posted news in the web site of the Ministry of Health of Brazil in 24 May 2024*Minist Health Launches New Vaccin Campaign Covid-19*; 2024 *https://www.gov.br/saude/pt-br/assuntos/noticias/2024/maio/ministerio-da-saude-lanca-nova-campanha-de-vacinacao-contra-covid-19* [accessed 04 July 2024].