Epidemiology of COVID-19 after Emergence of SARS-CoV-2 Gamma Variant, Brazilian Amazon, 2020–2021

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The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Gamma variant has been hypothesized to cause more severe illness than previous variants, especially in children. Successive SARS-CoV-2 IgG serosurveys in the Brazilian Amazon showed that age-specific attack rates and proportions of symptomatic SARS-CoV-2 infections were similar before and after Gamma variant emergence.

The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Gamma (P.1) variant emerged in November 2020 and drove the second wave of coronavirus disease (COVID-19) in Brazil. Emergence of this variant in Manaus, the largest city in the Brazilian Amazon, was followed by a dramatic upsurge in deaths across the region in early 2021 (*1*,*2*). Gamma harbors amino acid substitutions in the angiotensin-converting enzyme 2 receptor–binding domain of the spike protein, which are thought to enhance host cell infectivity (*3*). This variant may be 1.7–2.4 times more transmissible than previously circulating variant lineages of SARS-CoV-2 (*3*) and can evade antibodies elicited by prior infection or vaccination (*4*,*5*).

During the first COVID-19 epidemic wave, symptoms were half as likely to develop in young children with SARS-CoV-2 infection than in adults >30 years of age, according to an ongoing population-based

cohort study in the Brazilian Amazon (*6*). However, patients hospitalized for COVID-19 during the Gamma-dominated second wave in Brazil tended to be younger and more likely to die (*7*), suggesting that Gamma might cause more severe illness, especially in children (*8*). To determine the epidemiology of COVID-19 after emergence of SARS-CoV-2, we compared age-specific COVID-19 attack rates and proportions of symptomatic SARS-CoV-2 infections in the cohort before and after spread of the Gamma variant in the Amazon. The National Committee of Ethics in Research, Ministry of Health of Brazil (CAAE no. 30481820.3.0000.5467), approved the study protocol. Written informed consent was obtained from study participants or their parents/guardians.

The Study

Follow-up of the Mâncio Lima cohort in the Brazilian Amazon (https://www.niaid.nih.gov/research/amazonian-international-center-excellence-malaria-research), which accounts for 20% of the town's 9,000 residents, started in April 2018 (Figure 1; Appendix Methods, https://wwwnc.cdc.gov/EID/article/28/3/21-1993-App1.pdf). The first COVID-19 case in Mâncio Lima was notified on April 29, 2020; as of April 30, 2021, a total of 1,797 laboratory-confirmed infections and 24 deaths were recorded (Figure 2, panel A).

We estimated overall and age-specific SARS-CoV-2 attack rates and the proportion of infections leading to clinically apparent COVID-19 during the first and second epidemic waves in Mâncio Lima. We tested 1,215 cohort participants, <1 to 93 (median 29) years of age, for IgG to the subdomain S1 of the SARS-CoV-2 spike protein (Euroimmun ELISA, EI 2606–9601 G; PerkinElmer, https://www.perkinelmer) during October–November 2020 (*6*) and April–May

2021 (Figure 2, panel A). We obtained information about sociodemographics, COVID-19 exposures, and history of recent illness and vaccination. As a simplifying assumption, we considered seropositive participants in 2020 to not be at risk for reinfection during the second wave, but we attempted to identify instances of antibody boosting, which might represent reinfection. We excluded IgG seroconversions in COVID-19–vaccinated participants because our serologic testing does not distinguish natural infection from vaccination (Figure 1; Appendix).

We collected nasopharyngeal specimens from patients seeking COVID-19 testing in August 2020 and April 2021 to genetically characterize local SARS-CoV-2 isolates (Appendix Methods) with nanopore sequencing on a MinION platform (Oxford Nanopore, https://nanoporetech.com), using the ARTIC V3 protocol (J.R. Tyson et al., unpub. data, https://www.biorxiv.org/content/10.1101/2020.09.04.2830 77v1). We used Pangolin version 3.1.5 (9) to classify SARS-CoV-2 lineages. The 14 isolates from August 2020 (6) were assigned to the B.1.1.33 lineage (10), and all 11 SARS-CoV-2 isolates from April 2021 were the

Oct–Nov 2020 serosurvey:
2,074 participants interviewed
1,674 blood samples collected (80.7%)

48 returning residents
63 new residents

25 refusals
11 deaths
187 moved out of town
88 not found

Apr–May 2021 serosurvey
1,874 participants interviewed
1,539 blood samples collected (82.1%)

Combined 2020 and 2021 serosurveys
1,408 participants interviewed in 2020 and 2021
1,215 participants tested for SARS-CoV-2 IgG in 2020 and 2021 (86.3%)

Outcomes, first wave, n = 1,215
SARS-CoV-2 IgG in Oct–Nov 2020 (n = 407; 33.5%)
Clinically manifest COVID-19 during the first wave (n = 229; 18.8%)

407 seropositive in 2020
79 seroconverted but vaccinated

Outcomes, second wave, n = 729
SARS-CoV-2 IgG seroconversion in Apr–May 2021 (n = 209; 28.7%)
Clinically manifest COVID-19 during the second wave (n = 98; 13.4%)

Figure 1. Study of epidemiology of COVID-19 after emergence of SARS-CoV-2 Gamma variant, Brazilian Amazon, 2020–2021. COVID-19, coronavirus disease; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Gamma variant (Appendix Table 1; GISAID accession nos. EPI_ISL_2987666–74, EPI_ISL_2988699, and EPI_ISL_2988700), which dominated the second wave.

Outcomes were 1) SARS-CoV-2 IgG positivity (2020 survey) or IgG seroconversion in the absence of COVID-19 vaccination (2021 survey), as proxies of SARS-CoV-2 infection, to estimate attack rates during the first and second waves, respectively; and 2) presence of $\geq$1 sign/symptom—new or increased fever, cough, shortness of breath, chills, muscle pain, loss of taste or smell, sore throat, diarrhea, or vomiting—within the past 6 months (6), self-reported by participants with serologic evidence of SARS-CoV-2 infection, as a proxy of clinically apparent COVID-19. We excluded 79 persons vaccinated for COVID-19 who seroconverted (Appendix Results, Figure 2). For each outcome, we used Stata 15.1 (StataCorp LLC, https://www.stata.com) to estimate adjusted relative risks, along with 95% CIs, and used mixed-effects Poisson regression models with random effects at the household level and robust variance (Appendix Methods). Statistical significance was defined at the 5% level.

Most Mâncio Lima residents (54.2%, 95% CI 51.3%–57.1%) demonstrated serologic evidence of SARS-CoV-2 infection at the end of the study (first and second waves combined); sensitivity/specificity-adjusted prevalence (Appendix) was 65.0% (95% highest density interval [95% HDI] 58.5%–73.9%). This finding is consistent with the high COVID-19 attack rates observed in population-based studies in the Amazon (11–13). One third of study participants (33.5%, 407/1,215) were seropositive at the end of the first wave, and adjusted seroprevalence was 38.9% (95% HDI 33.2%–44.8%). Ten (0.8%) participants reported having been hospitalized between April 2020 and the first survey (blood sampling and questionnaire administration; missing information for 4 study participants), but we did not explicitly ask whether the cause of hospital admission was COVID-19; only 4 of 10 patients who reported hospital admissions (28, 66, 58, and 68 years of age) were seropositive. Among 729 initially seronegative participants, 209 (28.7%) seroconverted (adjusted prevalence 32.7%, 95% HDI 26.7%–38.9%) by the second visit but were not vaccinated (Figure 2, panel B; Appendix Results, Figure 1). We specifically asked for COVID-19–associated hospitalizations, and 7 (0.6%) participants (32, 32, 67, 58, 71, 3, and 81 years of age) reported hospital admissions during the second wave.

Of the 407 participants who were seropositive at the time of the first survey, 60 (14.7%, 95% CI 11.4%–18.6%) became negative (seroreverted) by April–May
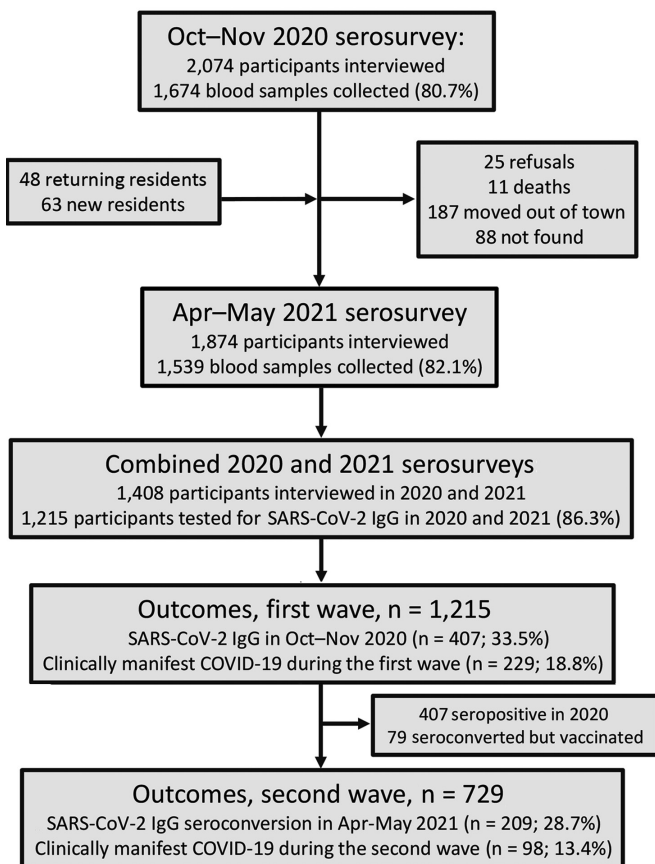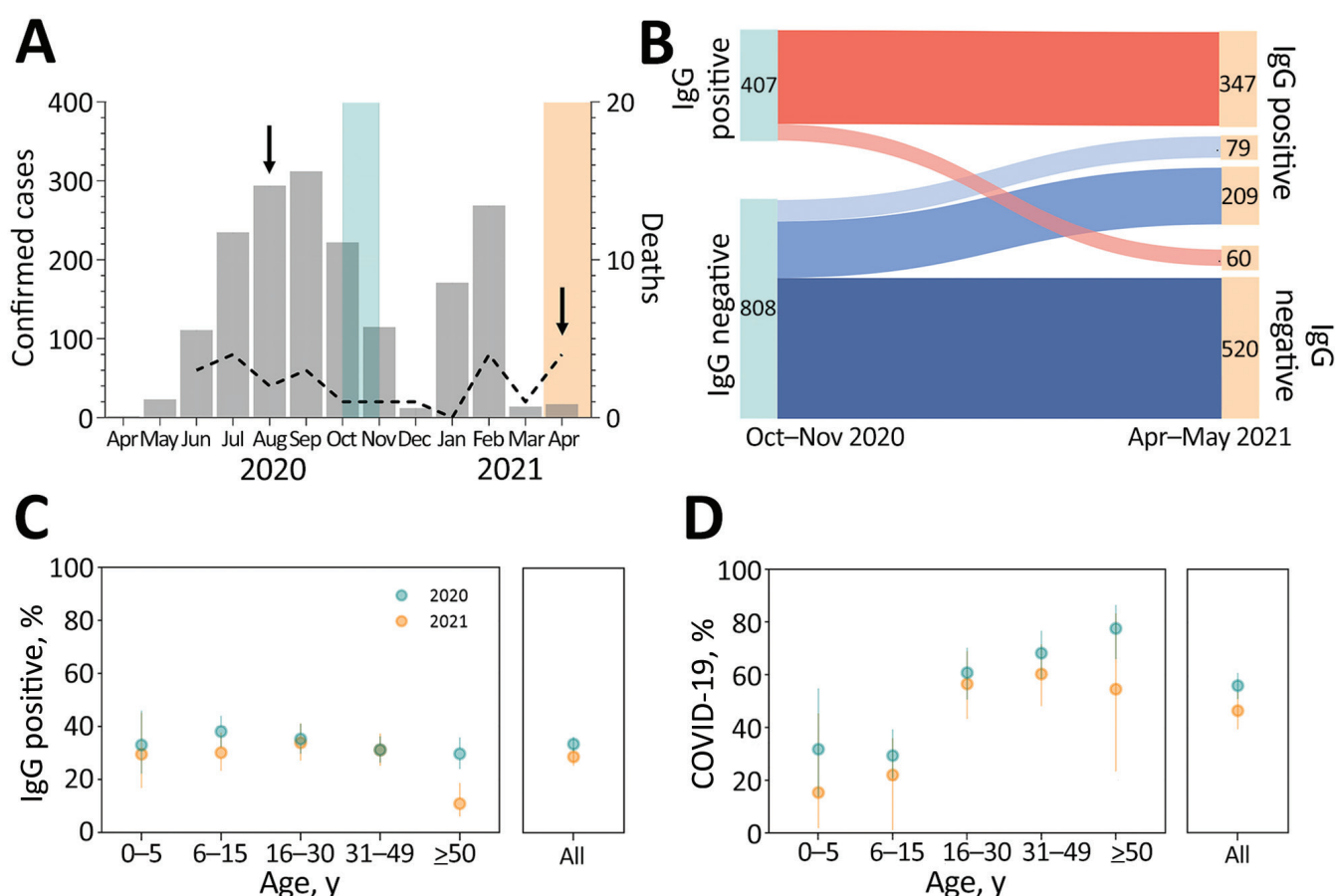
Figure 2. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and clinically apparent coronavirus disease (COVID-19) during the first and second epidemic waves, Brazilian Amazon, 2020–2021. A) Monthly cases (bars) and COVID-19–associated deaths (dashed line) notified in the municipality of Mâncio Lima, Brazil, during April 2020–April 2021. Light blue shading represents serosurveys conducted during October–November 2020; light peach shading represents serosurveys conducted during April–May 2021; arrows indicate dates of SARS-CoV-2 isolate collection for genomic surveillance. B) Distribution of study participants (n = 1,215) according to SARS-CoV-2 IgG detected in each serosurvey. The 288 for whom IgG seroconverted during April–May 2021 includes 79 vaccinated persons (light blue), who were not considered when estimating rates of seroconversion resulting from natural SARS-CoV-2 infection. C) Age-specific percentages of persons positive for SARS-CoV-2 IgG at the end of the first wave (October–November 2020; light blue dots) and of IgG seroconversions among initially seronegative persons by the second wave (April–May 2021; light peach dots). Error bars indicate 95% CIs. D) Age-specific percentages of SARS-CoV-2 infections that led to clinically apparent COVID-19 during the first wave (light blue dots) and second wave (light peach dots). Denominators correspond to the number of participants with serologic evidence of SARS-CoV-2 infection during the period. Error bars indicate 95% CIs.

2021 (Figure 2, panel B). Of the 347 persistently seropositive participants, antibody reactivity index increased by >2-fold for 46 (13.3%) by April–May 2021 (Appendix Figure 3); 18 (5.2%) of the 347 were not vaccinated and therefore may have experienced reinfection during the second wave. Only 4 (22.2%) of the 18 participants with possible reinfection reported clinical manifestations (Appendix Results); the rest were asymptomatic.

At the end of the first and second epidemic waves, antibody positivity and seroconversion rates were similar across age groups, except for adults >50 years of age, among whom there were proportionally fewer infections in the second wave than in the first wave (Figure 2, panel C; Appendix Table 2). A smaller

proportion of SARS-CoV-2–infected persons were symptomatic during the second wave (46.9% [95% CI 40.0%–53.9%]) than during the first wave (56.3% [95% CI, 51.3%–61.1%]; p = 0.034 by Yates-corrected χ^2 test) (Figure 2, panel D).

During the second wave, risk for SARS-CoV-2 infection was similar for young children and adults, but risk for symptomatic COVID-19 was lower for children than for adults. Similar trends were observed during the first wave (Appendix Tables 2, 3). After infection, clinical signs/symptoms were significantly less likely to develop in young children than in adults during both epidemic waves (Figure 2, panel D), even after we adjusted for potential confounders by using multiple Poisson regression (Appendix Table 3).

Conclusions

In this Brazilian Amazon cohort, we found no evidence that SARS-CoV-2 infections acquired during the second epidemic wave, dominated by the Gamma variant, produced more symptomatic illness than infections acquired during the first wave. Of note, symptomatic infections did not affect young children disproportionally more during the second wave. The explosive increase in illness and death in the Amazon during the second COVID-19 wave most likely reflects the rapid spread of a highly transmissible variant of concern, the regional and federal government's failure to enforce nonpharmaceutical interventions to curb community transmission of SARS-CoV-2, and limited availability of intensive care beds to cope with severe cases of COVID-19 (*14*).

About the Author

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Appendix

Supplementary Methods

The Mâncio Lima cohort study

The Mâncio Lima cohort study is part of the National Institutes of Health (NIH)-funded Amazonian International Center of Excellence for Malaria Research network, with the overall aim of investigating malaria epidemiology, vector biology and ecology, diagnostics, transmission biology, and clinical pathogenesis (https://www.niaid.nih.gov/research/amazonian-international-center-excellence-malaria-research). This population-based open cohort study was set up in 2018 to investigate a wide range of biologic and sociodemographic factors that drive malaria endemicity in the main urban transmission hotspot of Amazonian Brazil. The original study has since expanded to include SARS-CoV-2 antibody measurements (*1*).

We carried out a baseline population census in the town of Mâncio Lima between November 2015 and April 2016. We enumerated 9,124 permanent residents in the urban area, with ages ranging between <1 month and 105 years, distributed into 2,329 households (*2*). The cohort study participants are members of randomly chosen urban households in Mâncio Lima. We used simple probability sampling to draw 534 households from the list of those enumerated during the baseline census survey. We initially allowed for up to 2.9% non-localized or empty houses and refusals and aimed to enroll at least 20% of all households in the town. Because the target sample size was not reached during the first visit, we used a list of randomly chosen substitute households during the second visit to replace households that had declined participation or were not located (*1*). Because this cohort was set-up to evaluate a wide range of exposures and malaria-related outcomes in the same population, no formal a-priori sample size and power calculations were made.

The first study visit, between April and May 2018, targeted the 534 households drawn from the census listings; 1,391 residents from 354 households were located and agreed to participate. To achieve the desired sample size, 147 substitute households were randomly selected and approached during the second visit, in October-November 2018. The ongoing cohort is dynamic and new residents joining the household (those who moved in or were born between study visits) are enrolled during the follow-up visits. Study participants leaving the sampled households are retained in the cohort as long as they can be located by the field team and their new residences, which are labeled as new households, are situated in the urban area of Mâncio Lima. Six sequential house-to-house visits were carried out so far.

The median age of participants in the Mâncio Lima cohort study is 22 years (range, <1 to 103 years), with 51.3% of females. Among study participants ≥10 years of age, the literacy rate is 91.8%. Only 9.9% adult participants have a formal job. Most (80.0%) of study participants are supported by the Federal conditional cash transfer program called "Bolsa Família," a proxy of poverty.

Data and samples analyzed in the present study were obtained during serosurveys carried out in October-November 2020 and April-May 2021. The 2020 survey comprised 2,074 subjects distributed into 567 households, while the 2021 survey comprised 1,874 subjects distributed into 540 households. Overall, 1,408 individuals participated in both surveys; 1,215 (86.3%) of them (56.3% females) were tested for anti-SARS-CoV-2 antibodies on both occasions (main text, Figure 1). Compared to untested individuals, participants with antibody data in 2021 were significantly older (mean, 31.7 versus 26.4 years; $p < 0.001$, t-test), more likely to be females (56.3% versus 44.1%; $p < 0.001$, χ^2 test) and less likely to report at least one overnight stay outside Mâncio Lima within the past 6 months (29.1% versus 41.3%; $p < 0.001$, χ^2 test). No significant differences were observed regarding other covariates of interest.

SARS-CoV-2 IgG antibody detection

We tested 1,215 paired plasma samples, obtained from the same study participants at a 6-month interval during the 2020 and 2021 surveys, for anti-SARS-CoV-2 IgG with a semiquantitative ELISA that uses the recombinant subdomain S1 of the Spike protein as antibody-capture antigen (EI 2606–9601 G; Euroimmun) (3). The assay has a sensitivity of

82.5% to 93.3% and specificity of 98.0% to 98.5% (*4,5*). Results from the 2020 survey were previously published (*6*).

We used a quantitative ELISA to investigate changes in specific antibody concentrations in selected paired plasma samples. To this end, we added to each microplate a standard curve with a serially diluted pool of 10 strongly positive plasmas (eight dilutions from 1:25 to 1:1:3,200). We defined that the pool had an antibody concentration of 100 arbitrary units (AU) at a 1:25 dilution. Antibody concentrations in test samples were interpolated using a four-parameter logistic regression model. Samples were tested at a 1:100 dilution and those with absorbance values outside the range of the standard curve (i.e., absorbances >3.363 or <0.527) were assigned antibody concentrations of 110 AU and 0.7 AU, respectively.

SARS-Cov-2 detection

To characterize SARS-CoV-2 lineages circulating during the first and second waves, we obtained two nasopharyngeal swab samples from 49 consecutive symptomatic patients (age range, 3–77 years) seeking COVID-19 testing in Mâncio Lima in August 2020 and again from 49 patients (age range, 4–86 years) in April 2021. Results obtained with the samples collected in 2020 were published elsewhere (*6*).

One swab collected between 21 and 29 April 2021 was used for point-of-care antigen-based diagnosis (ECO F COVID-19 Ag test FA0054; Ecodiagnostica, Corinto, Brazil) and the other was preserved in RNA/DNA Shield (Zymo Research, Irvine, CA) for RNA extraction. Template RNA was prepared using QIAamp Viral RNA mini kits (Qiagen, Hilden, Germany). We tested antigen-positive samples for SARS-CoV-2 RNA by reverse transcription PCR by using the China CDC protocol that targets the ORF1ab and N genes (XGEN Master COVID-19 kit, Mobius Life Science, Pinhais, Brazil). Target amplification was carried out as described (*7*).

SARS-CoV-2 genome sequencing

We selected 15 samples with cycle threshold <30 for whole-genome sequencing as part of a countrywide SARS-CoV-2 genomic surveillance project (*8*). Template RNA was converted to cDNA using the Protoscript II First Strand cDNA synthesis Kit (New England Biolabs, Cambridge, MA) and random hexamers. Whole-genome amplification was performed with multiplex PCR amplification using the SARS-CoV-2 primer scheme (V1 to V3) and Q5 High-Fidelity DNA polymerase (New England Biolabs, UK), by using ARTIC protocol

(https://www.protocols.io/view/ncov-2019-sequencing-protocol-bbmuik6w?version_warning = no). PCR products were cleaned-up using AmpureXP purification beads (Beckman Coulter, High Wycombe, UK) and quantified using the Qubit dsDNA High Sensitivity assay on the Qubit 3.0 instrument (Life Technologies, Thermo Fischer Scientific, USA). Amplicons from each sample were normalized and pooled in an equimolar fashion and barcoded using the EXP-NBD104 (1–12) and EXP-NBD114 (13–24) Native Barcoding Kits (Oxford Nanopore Technologies, UK). Concentrations of double-stranded DNA for the library-negative controls were below detection levels, indicating no contamination.

Nanopore sequencing on the MinION platform (Oxford Nanopore, Oxford, UK) was carried out libraries were generated using the SQK-LSK109 Kit (Oxford Nanopore) and were loaded onto an R9.4.1 flow-cell (Oxford Nanopore). RAMPART software from the ARTIC Network (https://artic.network/ncov-2019/ncov2019-using-rampart.html) was used to monitor the sequencing run in real time to estimate the coverage depth (target, 200×). With the Guppy software version 4.4.0 (Oxford Nanopore Technologies), fastq files were base-called, demultiplexed, and trimmed. Sequencing data were subjected to sequence quality controls and the consensus genomes were obtained by the mapping of fastq files to Wuhan-Hu 1 reference genome (GenBank Accession Number MN908947).

Assembled sequences of 11 isolates (out of 15) yielded at least 50% coverage of the SARS-CoV-2 genome, with at least 20× depth. Lineages were classified using the Pangolin COVID-19 Lineage Assigner software tool (http://pangolin.cog-uk.io/) and phylogenetic analysis using complete reference genomes. Sequencing statistics and lineage assignment information are provided in Appendix Table 1.

Estimating COVID-19 attack rates

We used IgG positivity during the first survey (October-November 2020) as a proxy of SARS-CoV-2 infection during the first wave. The crude antibody prevalence (%) in October-November 2020, a proxy of the COVID-19 attack rate between April and November 2020, was calculated as number of IgG positive persons (n = 407) divided by the number of participants tested (n = 1215) × 100, with exact binomial 95% confidence intervals. We used IgG seroconversion detected in the second survey as a proxy of SARS-CoV-2 infection during the second wave. The attack rate between surveys was calculated as the number of IgG

seroconversions in April-May 2021 in participants who had not been vaccinated (n = 209) divided by the number of participants who were IgG-negative during the first survey (n = 729). To this end, we excluded from both the numerator and the denominator the 79 participants who seroconverted but had been vaccinated until the date of the latest study visit (April-May 2021), as they might have developed SARS-CoV-2 antibodies upon vaccination rather than natural infection. COVID-19 vaccination in Mâncio Lima started in February 2021, with the inactivated vaccine CoronaVac (Sinovac Life Sciences, China) and the adenoviral-vectored vaccine ChAdOx1 nCoV-19 (Oxford University–AstraZeneca, UK). The initial target populations for COVID-19 vaccination were health professionals and persons >60 years of age.

To estimate the overall attack rate during the whole study period, we considered all participants with IgG antibodies detected in the first survey (n = 407) and all unvaccinated IgG seroconversions detected in the second survey (n = 209), giving a total of 616 cohort participants with serologic evidence of SARS-CoV-2 infection in the numerator. The denominator was 1215 - 79 = 1136 participants, as the 79 vaccinated subjects were excluded.

As well as crude antibody prevalence, we also present sensitivity and specificity adjusted prevalence estimates. We used a Bayesian framework that propagates uncertainty in the sensitivity and specificity estimates of the test (9). We used the validation data from Naaber et al. (4) in which 80 out of 97 PCR-confirmed SARS-CoV-2 cases tested positive on the Euroimmun IgG assay, and 98 out of 100 known negative samples tested negative. The point estimate along with the 95% highest density interval are presented.

Data analysis

Data were transferred from tablets programmed with REDCap (10) to STATA 15.1 (StataCorp, College Station, TX) for analysis. Six multiple Poisson regression models (11) were built to identify factors associated with each binary outcome: (i) SARS-CoV-2 infection during the first wave (Appendix Table 2); (ii) clinically apparent COVID-19 during the first wave (Appendix Table 3), (iii) clinically apparent COVID-19 upon serologically documented SARS-CoV-2 infection during the first wave (Appendix Table 4); (iv) SARS-CoV-2 infection (using IgG seroconversion as a proxy) during the second wave, among participants who were seronegative during the first survey (October-November 2020; Appendix Table 2); (v) clinically apparent COVID-19 during the second wave among participants who were seronegative during

the first survey (October-November 2020; Appendix Table 3); and (vi) clinically apparent COVID-19 during the second wave among participants who were seronegative during the first survey (October-November 2020) and seroconverted by April-May 2021 (Appendix Table 4).

Note that models (ii) and (iii), as well as models (v) and (vi), have the same numerators (numbers of individuals with clinically apparent COVID-19 symptoms plus positive serology) but the denominators are different. Denominators in models (ii) and (v) are the entire susceptible population (n = 1027 participants with complete information in the first wave [model ii] and n = 729 with complete information in the second wave [model v]). Therefore, models (ii) and (v) explore the risk factors for serologically proven, symptomatic COVID-19 during the first and second waves in the entire study population. In contrast, models (iii) and (vi) explore the risk factors for symptomatic COVID-19 among individuals with serologically proven SARS-CoV-2 infection during the first and second waves. Denominators are the total number of seropositive participants at the end of the first wave (n = 359 [model iii]) and total number of seroconverters during the second wave (n = 209 [model vi]).

Because study participants are nested into households, which introduces dependency among observations, for each outcome we built mixed-effects Poisson regression models with random effects at the household level and robust variance. Individual covariates were age in October-November 2020 (categorical variable), sex (female versus male), laboratory-confirmed malaria within the past 12 months (no versus yes), overnight stay(s) away from Mâncio Lima within the past 12 months (no versus yes), and DENV seropositivity in the previous serosurvey (either October-November 2019 or October-November 2020; no versus yes). Household covariates were wealth index quintiles (6) and household size. Age, sex, and covariates associated with the outcome at a significance level <20% in unadjusted analysis were retained in multiple Poisson regression models. Participants with missing values were excluded from the adjusted models. Statistical significance was defined at the 5% level; relative risk (RR) estimates are provided along with 95% confidence intervals (CIs) to quantify the influence of each predictor on the outcome, while controlling for all other covariates (11).

Supplementary Results

SARS-CoV-2 attack rates during the first and second waves

We observed a higher attack rate between April and November 2020 (33.5%; 95% CI, 30.8%–36.2%) compared with that between November 2020 and April 2021 (28.7%; 95% CI, 25.4%–32.1%). However, differences in attack rate over time must not be overinterpreted because populations at risk are not entirely comparable during the first and second waves. We argue that SARS-CoV-2 has affected disproportionately the most exposed and most susceptible persons in our heterogeneous cohort population. High-risk participants were infected first and developed specific antibodies more rapidly; as a consequence, SARS-CoV-2 transmission during the first epidemic wave may have selectively removed high-risk individuals from the pool of seronegatives (12). Moreover, some high-risk population strata (health professionals and persons >60 years of age) were selectively vaccinated (see below). A proportionally larger fraction of individuals who remained seronegative after the first wave is expected to be either unexposed or little susceptible to SARS-CoV-2 infection, limiting virus spread during the second wave. This concept is illustrated in Appendix Figure 1.

IgG seroconversion after COVID-19 vaccination

Overall, 160 (13.2%; 95% CI, 11.4%–15.2%) study participants reported having been partially or fully vaccinated against COVID-19 until the date of the latest survey (April-May 2021). The locally available vaccines were the CoronaVac vaccine (administered to 64 participants; 40.0% of the vaccinees) and the ChAdOx1 nCoV-19 vaccine (administered to 89 participants; 55.6% of the vaccinees). Seven persons (4.4%) did not report the vaccine received. Most (78.8%) vaccinees were ≥60 years of age and 11.3% were health professionals. Among vaccinees, 94 (58.8%) had received a single vaccine dose and 59 (36.9%) had received both doses at the time of the latest survey; seven did not report the number of doses administered.

Seroconversion rates measured in April-May 2021 were much higher among vaccinees than in the general population. There were 107 study participants who were SARS-CoV-2 seronegative in 2020 and received one or more doses of a COVID-19 vaccine in 2021. Of them, 79 seroconverted (73.8%; 95% CI, 64.5%–81.4%). Considering participants with known vaccine administered, seroconversion rates were similar for recipients of the CoronaVac vaccine (73.7%; 95% CI, 56.8%–85.6%, n = 38) and the ChAdOx1 nCoV-19 vaccine (76.6%; 95% CI, 64.3%–

85.5%, n = 64) (Appendix Figure 2). Estimated seroconversion rates were 72.5% (95% CI, 60.4%–81.9%; n = 94) for a single vaccine dose and 75.6% (95% CI, 58.6%–87.2%; n = 59) for two vaccine doses.

Increased antibody concentration in paired sequential plasma samples

The majority of study participants with SARS-CoV-2 IgG antibodies detected in October-November 2020 remained seropositive in April-May 2021 (347/407, 85.3%). Appendix Figure 3 shows that 46 of those persistently seropositive persons had a substantial increase in antibody reactivity (>2-fold increase in reactivity index) between surveys, consistent with a boosting antibody response due to a new infection or vaccination. As shown in Appendix Figure 4, 28 (70.9%) of the 46 participants with increased antibody levels reported having been vaccinated after the first survey and 18 (39.1%) remained unvaccinated. We conclude that 18 participants (5.2%), out of 347 persons with persisting SARS-CoV-2 antibodies, had an antibody boosting consistent with SARS-CoV-2 reinfection during the second wave. Their ages range between 1 and 75 years (mean, 25.4 years). Interestingly, only 4 (22.2%) of them reported clinical symptoms suggestive of COVID-19 since November 2020. By using a quantitative ELISA, we estimate that antibody concentrations increased, on average, 8.5-fold among the study participants with serologic evidence of a new infection during the second epidemic wave (Appendix Figure 4).

Predictors of SARS-CoV-2 infection and clinically apparent COVID-19 during the first and second epidemic waves

Participants living in crowded households (≥7 people) were at increased risk of SARS-CoV-2 infection during both the first and second waves. Female sex and affluence (highest wealth index quintile) were significantly associated with an increased risk of infection only during the first wave, while age ≥50 years predicted a decreased risk of infection only during the second wave (Appendix Table 2).

We considered the following self-reported symptoms to define clinically apparent COVID-19: new or increased fever, cough, shortness of breath, chills, muscle pain, loss of taste or smell, sore throat, diarrhea, or vomiting within the past 6 months. Children ≤5 years of age tended to be at lower risk of clinically apparent COVID-19 than adults during both waves, although statistical significance was not reached in most comparisons (Appendix Table 3). In

addition, affluence and household crowding were associated with a significantly increased risk of clinically apparent COVID-19 during the first wave (Appendix Table 3). The previously described association between a positive DENV IgG serology and subsequent risk of clinically apparent COVID-19 (*6*) reached statistical significance only during the first wave.

We next used multiple Poisson regression models to identify the predictors of clinically apparent COVID-19 among study participants with serologically proven SARS-CoV-2 infection during the first wave (IgG seropositivity in October-November 2020; n = 359 after excluding persons with missing information) and the second wave (IgG seroconversion in April-May 2021; n = 209). We further confirm that, during both waves, study participants >15 years of age tended to be similarly more likely to develop symptoms, once infected with SARS-COV-2, than young children (Appendix Table 4).

Some of the symptoms used to define clinically apparent COVID-19 may be found in other locally prevalent infectious diseases, such as malaria, dengue and common upper your upper respiratory tract. Malaria is unlikely to be a confounder in this population (Appendix Tables 2 and 4; see also reference *6*), but the annual dengue transmission season (November to April) overlapped with the second SARS-CoV-2 wave in 2020–21. As a consequence, the proportion of symptomatic SARS-CoV-2 infections during the second wave may have been slightly overestimated due to dengue symptoms reported by our study participants.

COVID-19 severity during the first and second waves

We found no evidence that SARS-CoV-2 infections acquired during the second epidemic wave, dominated by the Gamma variant, are more likely to be symptomatic in our study population. In contrast, a recent study has shown that, among people hospitalized in Brazil due to COVID-19, the median age of patients decreased (63 years vs 59 years), with a relative increase of 18% in the proportion of patients younger than 60 years during the second wave (the period from week 44 in 2020 to week 21 in 2021) compared with the first wave (weeks 8 to 43 in 2020). The in-hospital mortality increased from 33·1% to 40·6% during the same period (*13*).

There are several factors that may have contributed to these results. First, we can hypothesize that individuals at increased risk of infection may have been preferentially infected during the first wave. In addition, individuals >60 years were among the early targets of mass vaccination campaigns. As individuals who have been vaccinated or experienced natural

infection are less likely to develop severe disease once (re)infected during the second wave, some differences in age-specific hospitalization rates are expected. In other works, individuals at high risk (including those vaccinated in early 2021) were selectively removed from the "susceptible pool" (Appendix Figure 1).

Second, individuals admitted to overwhelmed hospitals during the second epidemic wave, which was particularly intense in Brazil, are likely to have, on average, a more severe disease than those admitted during the first wave. The number of hospital admissions mirrors the number of available beds, not necessarily the number of patients who required intensive care. Patients with more threatening clinical conditions are expected to be selectively admitted when few hospital beds are available.

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Appendix Table 1. Characteristics of new SARS-CoV-2 genome sequences from Mâncio Lima, Amazonian Brazil, April 2021

Sample #	Average read depth (×)	Wuhan-Hu 1 genome coverage with 20× depth	Number of reads mapped	Bases covered >10×	Bases covered >25×	Pangolin lineage	Nextstrain clade
17	316	81%	20,387	26,883	24,194	P.1	20J (Gamma, V3)
13	329	79%	15,937	25,952	23,860	P.1	20J (Gamma, V3)
16	284	89%	35,228	28,341	27,451	P.1	20J (Gamma, V3)
22	117	95%	72,433	29,013	28,825	P.1	20J (Gamma, V3)
1	262	90%	34,923	28,234	27,237	P.1	20J (Gamma, V3)
38	83	53%	8,945	19,996	15,573	P.1	20J (Gamma, V3)
44	106	90%	45,431	28,073	27,394	P.1	20J (Gamma, V3)
39	365	94%	49,869	28,932	28,494	P.1	20J (Gamma, V3)
15	278	88%	46,836	28,542	27152	P.1	20J (Gamma, V3)
28	259	55%	7,492	19,600	18,295	P.1	20J (Gamma, V3)
23	257	81%	39,176	26,937	24,983	P.1.1	20J (Gamma, V3)

Appendix Table 2. Predictors of SARS-CoV-2 infection during the first and second epidemic waves in the Mâncio Lima cohort, Amazonian Brazil

Covariates	Models for the 2020 serosurvey							Models for the 2021 serosurvey						
	Unadjusted (n = 1,027)*				Adjusted (n = 1,027)†			Unadjusted (n = 729)*				Adjusted (n = 729)†		
	n	RR	95% CI	P	RR	95% CI	P	n	RR	95% CI	P	RR	95% CI	P
Individual level														
Age group														
0–5	55	1.00	Reference		1.00	Reference		44	1.00	Reference		1.00	Reference	
6–15	235	0.89	0.62, 1.30	0.570	0.89	0.64, 1.26	0.531	166	0.98	0.59, 1.64	0.948	0.99	0.63, 1.56	0.975
16–30	222	0.94	0.65, 1.37	0.761	0.96	0.70, 1.31	0.783	183	1.17	0.71, 1.92	0.542	1.12	0.73, 1.73	0.596
31–49	307	0.78	0.54, 1.14	0.205	0.79	0.56, 1.12	0.185	235	1.09	0.66, 1.79	0.744	1.04	0.67, 1.61	0.854
>50	208	0.76	0.51, 1.15	0.196	0.79	0.53, 1.16	0.220	101	0.39	0.19, 0.81	0.011	0.38	0.18, 0.77	0.008
Sex														
Female	572	1.00	Reference		1.00	Reference		395	1.00	Reference		1.00	Reference	
Male	455	0.85	0.71, 1.00	0.054	0.86	0.74, 0.99	0.031	334	0.96	0.76, 1.20	0.696	0.95	0.76, 1.17	0.609
Recent malaria														
No	961	1.00	Reference					709	1.00	Reference		1.00	Reference	
Yes	63	0.99	0.70, 1.41	0.966				18	1.52	0.86, 2.69	0.145	1.56	0.98, 2.47	0.059
Overnight out of town														
No	767	1.00	Reference		1.00	Reference		527	1.00	Reference		1.00	Reference	
Yes	256	1.14	0.95, 1.37	0.176	1.11	0.89, 1.38	0.375	200	1.27	1.00, 1.62	0.052	1.28	0.96, 1.70	0.091
Past dengue														
No	650	1.00	Reference		1.00	Reference		433	1.00	Reference				
Yes	377	1.14	0.96, 1.36	0.149	1.12	0.93, 1.35	0.222	296	0.94	0.74, 1.21	0.638			
Household level														
Wealth index quintile														
1 (poorest)	198	1.00	Reference		1.00	Reference		164	1.00	Reference		1.00	Reference	
2	197	1.20	0.91, 1.59	0.199	1.21	0.79, 1.87	0.384	154	1.05	0.76, 1.45	0.760	1.09	0.66, 1.79	0.744
3	206	1.40	1.03, 1.91	0.029	1.41	0.91, 2.19	0.128	143	0.82	0.56, 1.19	0.301	0.85	0.52, 1.41	0.539
4	211	1.17	0.86, 1.58	0.313	1.17	0.73, 1.86	0.513	142	0.97	0.67, 1.41	0.893	1.01	0.62, 1.63	0.971
5 (most affluent)	215	1.62	1.20, 2.17	0.001	1.60	1.04, 2.46	0.034	126	1.18	0.81, 1.73	0.384	1.25	0.74, 2.10	0.398
Household size														
1–3	375	1.00	Reference		1.00	Reference		269	1.00	Reference		1.00	Reference	
4–6	499	1.37	1.11, 1.70	0.004	1.38	1.04, 1.82	0.025	372	1.20	0.91, 1.59	0.199	1.20	0.84, 1.72	0.313
≥7	153	1.83	1.38, 2.41	<0.0001	1.87	1.23, 2.85	0.004	88	1.67	1.17, 2.39	0.005	1.68	1.00, 2.82	0.048
AIC	1483.2				1468.4			944.2				938.8		

Abbreviations: AIC, Akaike information criterion; CI, confidence interval; and RR, relative risk.

"Past dengue" refers to dengue fever seropositivity in the previous survey (2019 serology for 2020 models and 2020 serology for 2021 models).

*Totals may vary for some covariates due to missing data.

†The adjusted model corresponds to the following STATA syntax: *mepoisson outcome indevars housevars || household: vce(robust) irr*. Relative risks are calculated for individual (*indevars*) and household-level covariates (*housevars*) included in the fixed-effects component.

Appendix Table 3. Predictors of clinically apparent COVID-19 during the first and second epidemic waves in the Mâncio Lima cohort, Amazonian Brazil.

	Models for the 2020 serosurvey							Models for the 2021 serosurvey						
	Unadjusted (n = 1,027)*				Adjusted (n = 1,027)†			Unadjusted (n = 729)*				Adjusted (n = 729)†		
Covariates	n	RR	95% CI	P	RR	95% CI	P	n	RR	95% CI	P	RR	95% CI	P
Individual level														
Age group														
0–5	55	1.00	Reference	·	1.00	Reference	·	44	1.00	Reference	·	1.00	Reference	·
6–15	235	1.16	0.47, 2.84	0.744	1.14	0.47, 2.74	0.771	166	1.57	0.37, 6.73	0.543	1.44	0.44, 4.75	0.548
16–30	222	2.31	0.97, 5.53	0.060	2.24	0.97, 5.22	0.060	183	4.52	1.16, 17.68	0.030	3.88	1.04, 14.48	0.044
31–49	307	2.07	0.87, 4.95	0.102	1.97	0.86, 4.54	0.111	235	4.43	1.13, 17.36	0.033	3.98	1.15, 13.78	0.029
>50	208	2.31	0.94, 5.64	0.067	2.40	1.02, 5.68	0.046	101	1.46	0.30, 6.97	0.638	1.29	0.27, 6.10	0.744
Sex														
Female	572	1.00	Reference		1.00	Reference		395	1.00	Reference		1.00	Reference	
Male	455	0.87	0.68, 1.11	0.253	0.86	0.71, 1.04	0.127	334	0.87	0.60, 1.25	0.451	0.87	0.62, 1.23	0.427
Recent malaria														
No	961	1.00	Reference					709	1.00	Reference				
Yes	63	1.21	0.76, 1.92	0.431				18	1.27	0.44, 3.68	0.654			
Overnight out of town														
No	767	1.00	Reference					527	1.00	Reference				
Yes	256	1.06	0.81, 1.38	0.683				200	1.30	0.88, 1.91	0.189			
Past dengue														
No	650	1.00	Reference		1.00	Reference		433	1.00	Reference		1.00	Reference	
Yes	377	1.25	0.98, 1.61	0.074	1.31	1.00, 1.72	0.050	296	0.73	0.49, 1.08	0.117	0.75	0.49, 1.13	0.171
Household level														
Wealth index quintile														
1 (poorest)	198	1.00	Reference		1.00	Reference		164	1.00	Reference		1.00	Reference	
2	197	1.32	0.84, 2.10	0.231	1.26	0.65, 2.47	0.492	154	1.40	0.80, 2.45	0.245	1.32	0.58, 3.01	0.508
3	206	1.88	1.15, 3.05	0.011	1.85	1.00, 3.43	0.050	143	0.78	0.40, 1.54	0.475	0.82	0.36, 1.87	0.636
4	211	1.30	0.80, 2.11	0.283	1.24	0.64, 2.40	0.523	142	1.23	0.68, 2.22	0.500	1.26	0.58, 2.74	0.565
5 (most affluent)	215	2.48	1.57, 3.92	0.000	2.37	1.29, 4.35	0.005	126	1.66	0.89, 3.11	0.110	1.74	0.77, 3.95	0.183
Household size														
1–3	375	1.00	Reference		1.00	Reference		269	1.00	Reference		1.00	Reference	
4–6	499	1.63	1.21, 2.20	0.001	1.67	1.13, 2.47	0.010	372	1.37	0.91, 2.07	0.136	1.36	0.83, 2.25	0.224
>7	153	2.58	1.74, 3.83	0.000	2.72	1.54, 4.80	0.001	88	0.47	0.17, 1.29	0.143	0.49	0.14, 1.69	0.259
AIC	1,061.5				1,036.9			580.4				573.3		

Abbreviations: AIC, Akaike information criterion; CI, confidence interval; and RR, relative risk.

"Past dengue" refers to dengue fever seropositivity in the previous survey (2019 serology for 2020 models and 2020 serology for 2021 models).

*Totals may vary for some covariates due to missing data.

†The adjusted model corresponds to the following STATA syntax: *mepoisson outcome indevars housevars || household: vce(robust) irr*. Relative risks are calculated for individual (*indevars*) and household-level covariates (*housevars*) included in the fixed-effects component.

Appendix Table 4. Predictors of clinically manifest COVID-19 upon SARS-CoV-2 infection during the first and second epidemic waves.

Covariates		Models for the 2020 serosurvey						Models for the 2021 serosurvey						
		Unadjusted (n = 359)*			Adjusted (n = 359)†			Unadjusted (n = 209)*			Adjusted (n = 209)†		
	n	RR	95% CI	P	RR	95% CI	P	n	RR	95% CI	P	RR	95% CI	P
Individual level														
Age group														
0–5	21	1.00	Reference	.	1.00	Reference	.	13	1.00	Reference	.	1.00	Reference	.
6–15	88	1.28	0.57, 2.89	0.545	1.29	0.59, 2.81	0.518	50	1.40	0.38, 5.12	0.613	1.35	0.42, 4.35	0.615
16–30	87	2.47	1.14, 5.36	0.022	2.45	1.15, 5.22	0.020	62	3.36	1.00, 11.33	0.050	3.30	0.97, 11.27	0.057
31–49	100	2.55	1.17, 5.54	0.018	2.57	1.22, 5.41	0.013	73	3.75	1.10, 12.74	0.034	3.73	1.11, 12.54	0.034
≥50	63	2.83	1.30, 6.20	0.009	2.87	1.35, 6.08	0.006	11	3.51	0.95, 13.00	0.060	3.26	0.84, 12.58	0.087
Sex														
Female	214	1.00	Reference		1.00	Reference		116	1.00	Reference		1.00	Reference	
Male	145	0.99	0.84, 1.18	0.941	1.00	0.85, 1.17	0.999	93	1.03	0.79, 1.35	0.828	1.03	0.80, 1.31	0.835
Recent malaria														
No	336	1.00	Reference		1.00	Reference		200	1.00	Reference				
Yes	22	1.23	0.91, 1.66	0.181	1.22	0.92, 1.63	0.173	8	0.70	0.31, 1.59	0.393			
Overnight out of town														
No	256	1.00	Reference					143	1.00	Reference				
Yes	102	0.96	0.80, 1.14	0.626				66	1.03	0.78, 1.37	0.814			
Past dengue														
No	222	1.00	Reference		1.00	Reference		132	1.00	Reference		1.00	Reference	
Yes	137	1.16	0.98, 1.38	0.084	1.17	0.97, 1.41	0.096	77	0.81	0.60, 1.10	0.183	0.81	0.58, 1.12	0.198
Household level														
Wealth index quintile														
1 (poorest)	64	1.00	Reference		1.00	Reference		54	1.00	Reference		1.00	Reference	
2	71	1.11	0.79, 1.56	0.535	1.11	0.74, 1.67	0.624	48	1.03	0.68, 1.57	0.888	1.03	0.58, 1.86	0.911
3	76	1.30	0.93, 1.82	0.122	1.29	0.89, 1.87	0.185	33	0.70	0.41, 1.22	0.211	0.72	0.38, 1.34	0.299
4	65	1.15	0.82, 1.61	0.429	1.14	0.76, 1.69	0.528	38	1.03	0.66, 1.61	0.905	1.03	0.59, 1.80	0.920
5 (most affluent)	83	1.54	1.13, 2.11	0.007	1.51	1.07, 2.14	0.020	36	1.22	0.75, 1.98	0.416	1.24	0.68, 2.27	0.477
Household size														
1–3	107	1.00	Reference		1.00	Reference		64	1.00	Reference		1.00	Reference	
4–6	185	1.12	0.93, 1.36	0.238	1.14	0.91, 1.41	0.252	110	1.26	0.92, 1.72	0.151	1.24	0.88, 1.75	0.218
>7	67	1.32	1.03, 1.69	0.029	1.33	0.99, 1.77	0.055	35	0.29	0.11, 0.78	0.013	0.29	0.09, 0.91	0.034
AIC	645.6				640.9			344.4				339.5		

Abbreviations: AIC, Akaike information criterion; CI, confidence interval; and RR, relative risk.

"Past dengue" refers to dengue fever seropositivity in the previous survey (2019 serology for 2020 models and 2020 serology for 2021 models).
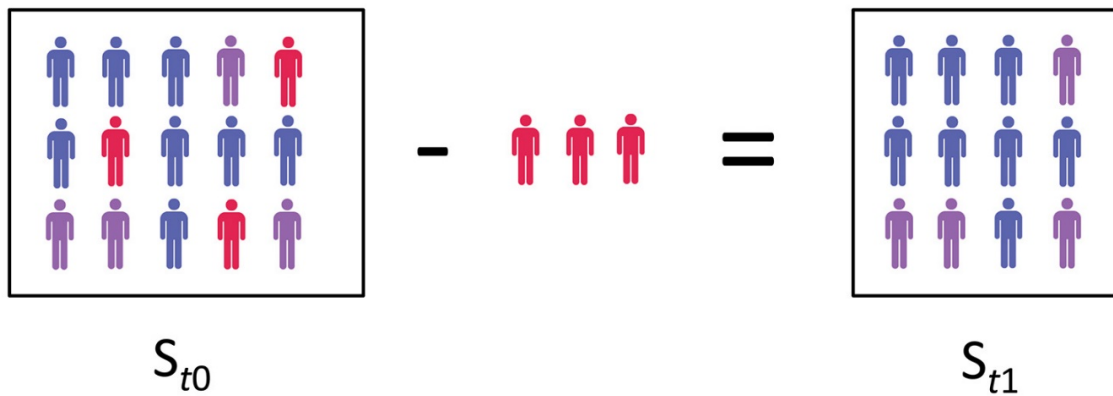
*Totals may vary for some covariates due to missing data.

†The adjusted model corresponds to the following STATA syntax: *mepoisson outcome indevars housevars || household: vce(robust) irr*. Relative risks are calculated for individual (*indevars*) and household-level covariates (*housevars*) included in the fixed-effects component.

Appendix Figure 1. Selective removal of high-risk cohort participants from the seronegative (= fully susceptive) pool as the COVID-19 pandemic develops. We represent a population with heterogeneous risk of infection (from blue to red) from which high-risk individuals (red) are selectively removed by infection, from the fully susceptible population S, between times t_0 and t_1. This decreases the average susceptibility to infection in the cohort of seronegatives left behind. As a consequence, the rate of new seroconversions tends to decrease unless viral transmissibility increases over time. if this selection process also affects the susceptibility to disease upon infection, the proportion of infections leading to clinical manifestations and severe disease may also decrease with time unless more virulent virus variants are introduced in the population.
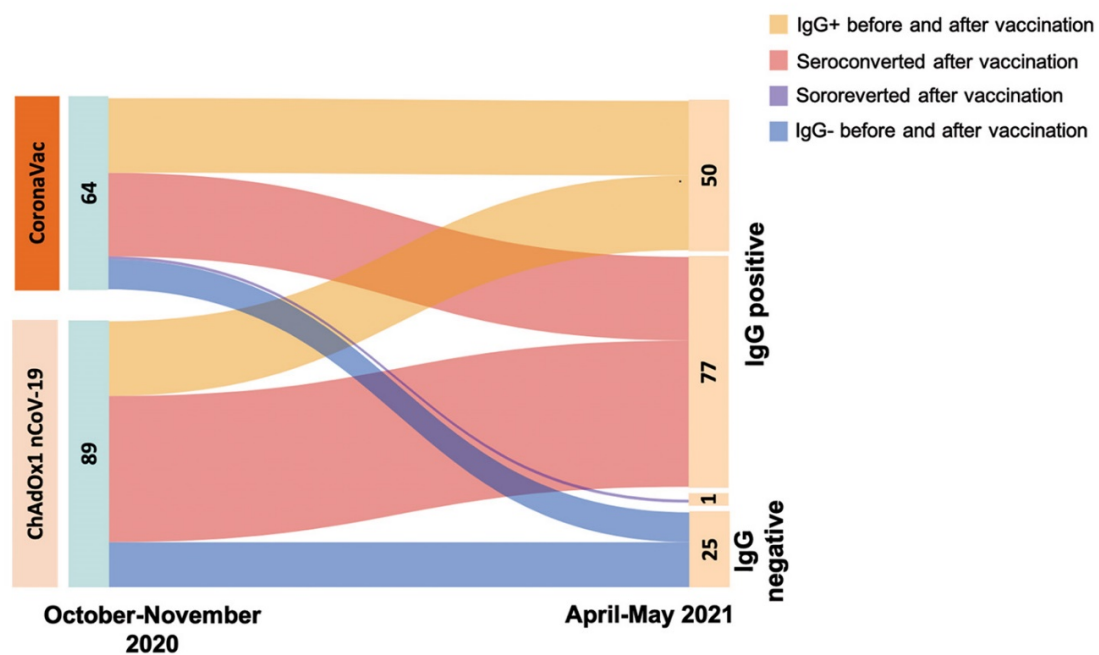
Appendix Figure 2. IgG antibody responses to SARS-CoV-2 in vaccinated participants.
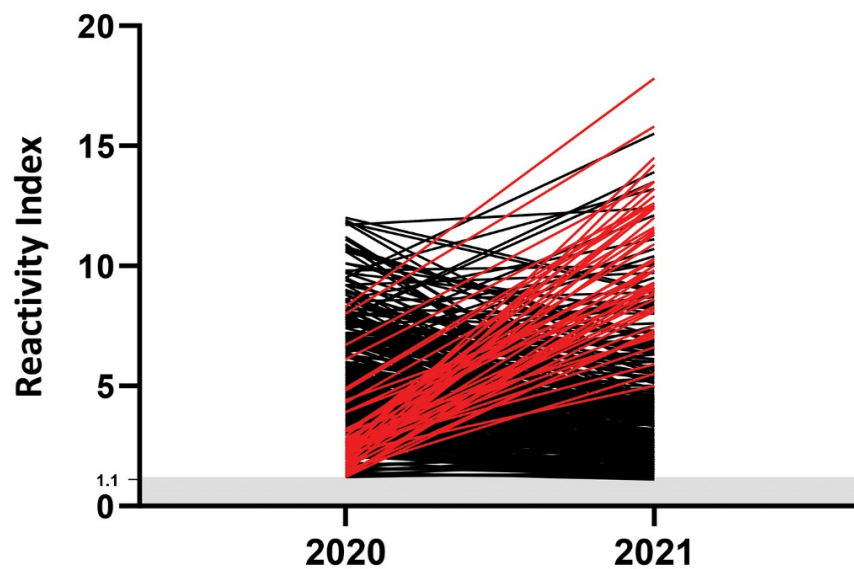
Appendix Figure 3. Paired IgG antibody reactivity indices to SARS-CoV-2 in 347 study participants who remained seropositive from October-November 2020 to April-May 2021. Results for 46 study participants who had a >2-fold increase in reactivity indices are shown in red.
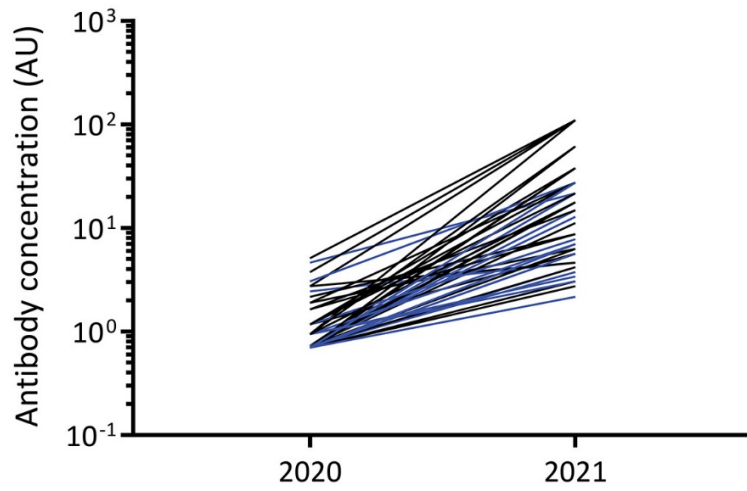
Appendix Figure 4. Paired SARS-CoV-2 IgG concentrations (in arbitrary units, AU) in 46 study participants who had a >2-fold increase in reactivity indices between October-November 2020 and April-May 2021. Results for 18 unvaccinated study participants are shown in blue.