High anti-SARS-CoV-2 antibody seroconversion rates before the second wave in Manaus, Brazil, and the protective effect of social behavior measures: Results from the DETECTCoV-19 cohort.

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Summary 30 words
We report an intense infection transmission period that preceded the second wave of COVID-19 in Manaus, and identified several modifiable behaviors that increased the risk of seroconversion.
Abstract
We report anti-SARS-CoV-2 nucleocapsid IgG seroconversion rates from a population-based cohort in Manaus, between August and November 2020, before the COVID-19 second wave in Brazil. Among seronegative and uninfected participants, we observed an overall incidence of 13.06% (95%CI, 11.52-14.79) and 6.78% (95%CI, 5.61-8.10) for symptomatic seroconversion. Risk factors for seroconversion were: having a COVID-19 case in the household, not wearing a mask during contact, relaxation of protective measures, and non-remote work. We observed an intense infection transmission period that preceded the second wave of COVID-19 in Manaus, and identified several modifiable behaviors that increased the risk of seroconversion.

Main
Brazil ranks second in worldwide COVID-19 cases and deaths since the start of the pandemic ¹. The healthcare system in Manaus has collapsed twice in less than eight months, and the current second wave has surpassed the number of deaths and cases of the first wave (Figure 1a). The current high toll in morbidity and mortality is aggravating the precarious state in which the first wave left the region, with important consequences for the families and communities affected. In September 2020, several alerts were made regarding a possible second wave of infection if social distancing measures were relaxed ². However, social distancing rules were further relaxed, and their effects reflected on population mobility (Figure 1a); additionally, a research report on achieving herd immunity in Manaus could have further contributed to this behavior ³. In this report, we estimated the incidence and risk factors associated with SARS-CoV-2 seroconversion from the DETECTCoV-19 cohort before the start of the second wave of infections in Manaus.
In our previous cross-sectional baseline analysis of the DETECTCoV-19 cohort, we reported a crude seroprevalence of 29.10% in August 2020. From our initial recruitment cohort (n=3057), a total of 2496 participants (81.64%) returned for a second follow-up visit between 19/10/2020 and 27/11/2020 (Figure 1b). The crude anti-SARS-CoV-2 nucleocapsid IgG seropositivity at baseline, among the 2496 individuals that returned for follow-up was 27.72% (95% CI, 25.98-29.53), which increased to 34.33% (95%CI, 32.47-36.24) at the second visit. Test sensitivity- and specificity-adjusted antibody prevalence increased from 28.70% (95% CI, 26.70-30.80) to 36.40% (95% CI, 34.30-38.60) after follow-up (Figure 1b). A paired analysis of anti-SARS-CoV-2 nucleocapsid-specific IgG Reactivity Index (RI) showed a significant increase at the second visit among the participants that returned for follow-up (Figure 1c). These findings indicate that the number of seropositive individuals and their IgG anti-nucleocapsid antibody RI levels were higher at follow-up (Figure 1c and 1e).

Next, we calculated the incidence of SARS-CoV-2 seroconversion. From the total participants evaluated at follow-up, 204 reported having COVID-19 infection prior to the first visit, and 24 had no data regarding this. From the remaining 2268, 1709 were IgG seronegative at first visit. Of them, 1424 had a RI ≤1.5 at the second visit and deemed still negative. Meanwhile, 214 had a RI >1.5 with a RI ratio between the second and first visit >2.0, and were considered as seroconversion. Seventy-one participants had a second visit positive IgG, but did not meet the criteria of doubling the RI, thus were considered indeterminate and were not included in the analysis (Supplementary Figure 1). We found a high incidence of seroconversion at 13.06%.
(95% CI, 11.52-14.79) with a median follow-up duration of 57 days (IQR, 54-61 days); in other words, 1% of the sample seroconverted every 4.5 days. Of these cases, 48.1% were asymptomatic; therefore, the incidence of symptomatic seroconversion was 6.78% (95% CI 5.61-8.10, Figure 1d, Supplementary Table 1).

To our knowledge, this is the first study that reports SARS-CoV-2 seroconversion rates in a general population prospective cohort. Between the 1st and 2nd visit; only 2.93% of the sample had a positive RT-PCR test, and 5.43% developed symptoms and were diagnosed as COVID-19 (Supplementary Table 1). Between the start of the 1st round collection and 2nd round ending (August 19th to November 27th), Manaus city Fundação de Vigilância em Saúde do Amazonas (FVS Amazonas) reported 30,278 RT-PCR positive cases (http://www.fvs.am.gov.br, date accessed 28 February, 2021), which correspond to a reporting rate of 1.36% (95% CI, 1.35-1.38). These results highlight the variations obtained using different case definition criteria; whereas it is seroconversion, symptomatic seroconversion, clinical diagnosis, diagnosis by PCR, or reporting to health agencies (Supplementary Table 1). Overall, it is important to timely identify the ratio between these divergent incidences and their dynamics during the pandemic in order to do adequate planning of resources and mitigation strategies. Our serology-based incidence rate, 5-10-fold higher than the official reports, indicates that the bulk of transmission prior to the second wave occurred mostly undetected by the current official survey methods.

We then analyzed the effect of sociodemographic, health-related, behavioral, case clustering, and COVID-19 testing on seroconversion (Supplementary Tables 2-4). In contrast with our previous report, which evaluated prevalence of seropositivity during
August 2020[^4], this study looked at factors that affected incidence over a median two-month period between August and November 2020. While in our first report, we found that prevalence was strongly associated with sociodemographic characteristics—as male sex, older age, lower income, occupation, and number of household members, these were no longer associated with the emergence of new cases during the study period. In contrast, we found that the main risk factors were related to the social behavior of the participants, such as not keeping social distance before August, relaxing social distancing after that, on-site working, and having contact with COVID-19 patients without a mask (Figure 2, Supplementary Table 5). These results show that, between the first and second epidemic waves in Manaus, behavioral risk factors that increase exposure to SARS-CoV-2 were more important than biological or social characteristics as sex or poverty. Having a person with COVID-19 diagnosis in the household affected both the prevalence in August and the incidence in the following two-three months with similar magnitude. Crucially, independent of the moment of the pandemic, our findings confirm that having a COVID-19 contact in the household remains one of the more robust predictors for acquiring the disease[^5]. Not surprisingly, having symptoms or having been diagnosed during the observation period were strongly associated with seroconversion, more so than in the cross-sectional study, underlying the correlation between these events.

Since the start of the pandemic effective surveillance, availability and ease of access to testing, well implemented non-pharmaceutical interventions (NPI) and the public health response measures linked to testing such as quarantine and isolation have been essential in controlling the virus transmission[^6][^8]. Our findings reveal that COVID-19 index cases may have likely driven seroconversion—a representative proxy for
infection burden—within the household, as observed in other high transmission settings \(^9\). Our data suggests that household close contacts of COVID-19 surviving or deceased patients should be tested regardless of symptoms and advised voluntary isolation \(^{10}\); strict follow-up of cases and contacts is essential to reduce virus transmission \(^6,^{11}\). NPI including social distancing, mask use and hygiene have been the pillar to reduce community SARS-CoV-2 transmission worldwide \(^{12}\). Therefore, lifting NPI abruptly diminishes the gains accumulated by previously implemented policies \(^7,^{13}\). In our study, individuals who relaxed social distancing measures \(^{14}\) or had contact with COVID-19 individuals without mask \(^{15,^{16}}\) had the highest risk of acquiring SARS-CoV-2 infection.

Our work has some limitations. A convenience sampling strategy based on on-line and university website advertising potentially excluded individuals who did not have access to this information and may not completely represent the general population. We also had shortcomings in performing active surveillance to identify symptomatic infections and positive RT-PCR tests results. Additionally, most symptomatic participants had a mild or moderate infection, but our surveillance method cannot exclude that severe illness cases and deaths occurred among the non-returning participants, underestimating total seroconversion events. Additionally, antibody response kinetics and their variability among populations limit our interpretations \(^{17}\). Despite these, our longitudinal serology approach to assess exposure and burden, and the size of our cohort, made possible in-depth statistical analysis to identify the risk factors associated with seroconversion in a setting of high transmission and low NPI containment measures. Unfortunately, we could not evaluate the role of the infecting SARS-CoV-2 strains. The cohort period between August and November 2020
theoretically predates the surge and dominance of the P.1 (B.1.1.248) strain in the region; however, we cannot rule out that the high rate of seroconversion in our cohort could have been influenced partially by the emergence of a more infectious strain, like the P.1 lineage in Manaus\textsuperscript{18,19}. We hypothesize that the role of P.1 could be ascribed to accelerating the transmission rate observed after November, but we consider that the high seroconversion incidence found in our cohort might be explained by case clustering and host-related behavioral factors, as observed in high transmission settings \textsuperscript{5,9,20}.

Rampant spread of SARS-CoV-2 infection in settings with low compliance to behavioral and NPI containment measures is a cause of concern due to its high human costs, elevated burden imposed on healthcare systems, and a possible impact on the emergence of new variants –favored in high transmission settings– which can negatively impact the effectiveness of available and future countermeasures including diagnostics, vaccines and therapeutics. This study provides timely actionable evidence for policymakers to inform next steps to mitigate the pandemic in Manaus and elsewhere.
Figure legends

Figure 1: Anti-SARS-COV-2 nucleocapsid antibody prevalence and seroconversion before the second wave of infection from the DETECTCoV-19 cohort in Manaus.

(a) COVID-19 confirmed deaths (upper plot) and cases (middle plot), obtained from the Fundação de Vigilância em Saúde do Amazonas (http://www.fvs.am.gov.br, date accessed 28 February, 2021). Events following the SARS-CoV-2 pandemic were collated from the Amazonas state government website (http://www.amazonas.am.gov.br, date accessed 28 February, 2021); NPI-related, public health measures, and pandemic-related events depicted (text inserts, middle plot). Publicly available Google mobility data for Manaus city was obtained (https://www.google.com/covid19/mobility/, date accessed 01 March 2021) and plotted as percentage mobility change (lower plot). Crude and test-adjusted anti-SARS-COV-2 nucleocapsid antibody seropositivity prevalence depicted as percentage of seropositive individuals for both study visits (whole cohort seroprevalence, upper plot; bars depict 95% CI). (b) Seroprevalence in individuals that returned to second visit (n=2,496; bars depict 95% CI). (c) IgG Reactivity Index (RI) of paired samples from 1st and 2nd visits. Blue horizontal line denotes median values. Paired samples depicted by grey lines. (d) Seroconversion rates according to case definitions for symptomatic and asymptomatic (red) or asymptomatic-only individuals (black). (e) Paired RI of individuals who seroconverted as per study protocol. Red horizontal line denoted the median values in the violin plots. Dotted line at RI 1.5 denotes assay cut-off. Bars depict 95% CI, dotted lines (c and d) depict IR cutoff (IR=1.5).
Figure 2: Multivariate regression model for SARS-CoV-2 seroconversion in a general population cohort from Manaus, Amazonas state of Brazil (N=1,618). Forest Plot showing Incidence Rate Ratios (IRR) obtained via Poisson Regression considering follow-up time with robust variance corrected by clusters. IRRs are shown unadjusted and adjusted per all model variables (please refer Supplementary Table 5 for details). *Not included in the multivariate model due to collinearity with the Household contact variable.

Supplementary Figure legend

Supplementary Figure 1: Flowchart for calculating incidence of SARS-CoV-2 seroconversion.
Methods

Ethics and Study design

The research ethics committee of Federal University of Amazonas (UFAM) approved this study (CAAE:34906920.4.0000.5020) in accordance with Brazilian law, and the Helsinki declaration. All the participants gave oral and written consent prior to enrolment. This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline for cohort studies. A longitudinal study (DETECTCoV-19) in Manaus was designed to follow-up adults of both sexes ≥18 years for up to 6 months after recruitment with a sample collection every 8-12 weeks as previously described. A sample size of 2399 individuals was calculated to estimate seroprevalence with a 95% CI and 2% error.

Recruitment and follow-up

Among the 3,057 individuals recruited between 19/08/2020 and 02/10/2020, 2,496 individuals returned in person for the 2nd visit between 19/10/2020 and 27/11/2020. All participants filled out an electronic questionnaire and donated a blood sample for the SARS-CoV-2 testing (Supplementary Figure 1).

At the second visit, we collected information related to social distancing, information related to COVID-19 disease, symptoms since the start of the pandemic, prior diagnosis, auto-medication and prescribed medication used for treatment of symptoms was recorded. Study participants presented oropharyngeal SARS-CoV-2 RT-PCR or antigen test reports performed by local government or private laboratories when available. We also recorded information about the COVID-19 cases in family and individual's residence. Finally, an independent form was used to record the SARS-CoV-2 serological assay results. Trained interviewers collected participant data using
the Research Electronic Data Capture (REDCap) software, an online web application with integrated quality checks (e.g., range and valid values, date formats, skip logic). The study used an online appointment system to reduce unnecessary gathering and followed all state and federal COVID-19-related regulations. The collection center was equipped with state-compliant measures for appropriate social distancing, and all members of the DETECTCoV-19 team used appropriate PPE. All state and federal COVID-19-related regulations were followed during the course of the study. All test results were communicated by email or via phone messaging to the study participants.

Serological testing
An indirect Enzyme Linked Immunosorbent Assay (ELISA)-based serological assay was used to measure anti-SARS-CoV-2 nucleocapsid IgG antibody levels in serum samples as previously described. The in-house indirect anti-SARS-CoV-2 nucleocapsid IgG ELISA had a sensitivity of 94.28% (95% CI, 89.44 – 97.07), specificity of 97.03% (95% CI, 93.72 – 98.69) for patient with ≥14 days after onset of COVID-19 symptoms. An anti-SARS-CoV-2 N IgG antibody reactivity index (RI) was expressed as the ratio between optical density of patient sample and negative control. All samples with a RI value above 1.5 (assay cut-off) were considered positive.

Cohort definitions for calculating incidence of seroconversion
Inclusion criteria: Patients of both sexes, 18 years old or above, who accepted to participate in the study and have at least two evaluations separated por at least four weeks.

Exclusion criteria: Patients who reported having a COVID-19 diagnosis prior to the first visit, either by PCR, serology, radiology, or clinical diagnosis. Patients who have no
data or did not answer questions regarding COVID-19 diagnosis prior to the first visit.

Patients who had IgG ELISA reactivity index >1.5 at the first visit.

Seroconversion Definition: Patients meeting the selection criteria who had IgG ELISA reactivity index ≤1.5 at first visit, IgG ELISA reactivity index >1.5 at the second visit, and a reactivity index ratio (visit 2/visit 1) >2.

Data analysis

Statistical analysis was performed using Stata version 14.0 (College Station, Texas). Participants who had an indeterminate seroconversion status were not included in the statistical analysis. Number of people in each category were listed in the first table column to show incidence denominators and account for variations due to missing data. Frequency distribution, cumulative incidence and incidence density (considering follow-up time) for seroconversion and for symptomatic seroconversion were calculated for the whole group and according to the study variables. Missing values were excluded from calculations, therefore were not listed as a separate category or into the incidence denominators. Chi-square or Fisher’s exact tests were used to evaluate association between seroconversion incidences and independent variables. Also, Poisson regression models with robust variance corrected per clusters (administrative areas) was used to estimate crude Relative Risk (RR) and crude Incidence Rate Ratios (IRR) that considered follow-up time for each variable. A multivariate model estimating adjusted IRRs was constructed including age, sex, and all the variables that had significative association in the crude models. P values ≤0.05 were considered statistically significant.
Declaration of interests

The authors declare no conflict of interest.

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Figure 1

A. Seroprevalence over time in Manaus, Amazonas state. Data points represent the percentage of seropositive individuals in the community. The X-axis represents the months from January 2020 to February 2021, with shaded areas indicating significant events such as lockdowns and easing of social distancing. The Y-axis shows the number of COVID-19 deaths per day.

B. Comparison of crude and test-adjusted seropositive rates over visits 1 and 2. The X-axis represents the visits, and the Y-axis shows the percentage of seropositive individuals.

C. Distribution of IgG Reactivity Index for both visits. The X-axis represents the index values, and the Y-axis represents the number of samples.

D. Seroconversion rates based on different diagnostic tests. The X-axis shows the seroconversion rate (%), and the Y-axis represents the number of samples.

E. IgG Reactivity Index for both visits, with a significant p-value of <0.0001.
Social distancing (before August): Sometimes
Frequently

Social distancing flexibilization (since August): Yes

Work (before August): Remote only

Family members with COVID-19 (until 2nd visit)*: Yes, alive
Yes, deaths

Household members with COVID-19 (until 2nd visit): Yes, alive
Yes, deaths

COVID-19 Contacts (since August): Yes, with mask
Yes, without mask

Symptoms (since August): Flu-like
Other

COVID-19 Diagnosis (since August): Yes

Poisson regression models
- Corrected by follow-up time and clusters
- Corrected as above and adjusted by all model variables

Incidence Rate Ratio (95% CI)

Figure 2
Seroconversion: 214/1638=13.06% (95%CI 11.52-14.79)
Seroconversion with symptoms: 111/1638=6.78% (95%CI 5.61-8.10)
Follow-up time: median 57 (Min-Max: 28-95; IQR 54-61) Mean 58.42 (SD 8.15) Not Normal
Incidence: 0.0022355 cases per patient-day
Equivalent to 1% of the sample seroconverted every 4.5 days
Supplementary Table 1: Comparison of SARS-CoV-2 seroconversion rates using different Case Definitions in a general population cohort from Manaus, Amazonas state of Brazil (N=1,638).

| Case Definition between 1st and 2nd Visit N=1638 | Symptomatic Only | Symptomatic or Asymptomatic |
|-----------------------------------------------|------------------|-----------------------------|
|                                               | n   | (%)  | n   | (%)  |
| PCR only                                      | 48  | (2.93)| 48  | (2.93)|
| PCR and/or Antigen Test                       | 50  | (3.05)| 50  | (3.05)|
| PCR and/or Antigen and/or Serological Test    | 80  | (4.88)| 91  | (5.56)|
| Any Lab result and/or Clinical/Radiology Dx   | 89  | (5.43)| 99  | (6.04)|
| Seroconversion as per Study                   | 111 | (6.78)| 214 | (13.06)|
Supplementary Table 2: SARS-CoV-2 seroconversion according to Demographics and Health access characteristics of a general population cohort from Manaus, Amazonas state of Brazil (N=1,638).

| Characteristics¹ | Categories                  | Total | column % | IgG ELISA (-) -> (+) | Incidence row % | p value | Chi2 test | RR⁺ | 95%CI | p value | IRR⁺⁺ | 95%CI | p value |
|------------------|-----------------------------|-------|----------|----------------------|-----------------|---------|-----------|------|-------|---------|-------|-------|---------|
| Total            | 1,638                       | 214   | 13.06    |                      |                 |         |           |      |       |         |       |       |         |
| Demographics     |                             |       |          |                      |                 |         |           |      |       |         |       |       |         |
| Age              |                             |       |          |                      |                 |         |           |      |       |         |       |       |         |
| 18-29            | 372                         | 48    | 12.90    | 0.915                | Ref             | -----   | -----     |     |       |         |       |       |         |
| 30-39            | 420                         | 56    | 13.33    | 1.03                 | 0.68-1.57       | 0.87    |          | 1.00 | 0.66-1.51 | 0.990 |       |         |
| 40-49            | 354                         | 48    | 13.56    | 1.05                 | 0.81-1.36       | 0.703   | 1.02     | 1.02 | 0.80-1.31 | 0.865 |       |         |
| 50-59            | 292                         | 40    | 13.70    | 1.06                 | 0.69-1.63       | 0.786   | 1.02     | 1.02 | 0.66-1.57 | 0.924 |       |         |
| >=60             | 200                         | 22    | 11.00    | 0.85                 | 0.37-1.99       | 0.712   | 0.82     | 0.82 | 0.36-1.90 | 0.648 |       |         |
| Sex              |                             |       |          |                      |                 |         |           |      |       |         |       |       |         |
| M                | 607                         | 84    | 13.84    | 1.10                 | 0.75-1.60       | 0.627   | 1.08     | 1.08 | 0.75-1.56 | 0.663 |       |         |
| F                | 1,031                       | 130   | 12.61    | 0.476                | Ref             | -----   | -----     |     |       |         |       |       |         |
| Race             |                             |       |          |                      |                 |         |           |      |       |         |       |       |         |
| White            | 602                         | 74    | 12.29    | 0.87                 | 0.57-1.32       | 0.515   | 0.89     | 0.89 | 0.58-1.35 | 0.570 |       |         |
| Black            | 109                         | 9     | 8.26     | 0.58                 | 0.12-2.81       | 0.503   | 0.59     | 0.59 | 0.12-2.81 | 0.509 |       |         |
| Indigenous       | 7                           | 0     | 0.00     | NA                  | NA              | NA      | NA       |     |       |         |       |       |         |
| East-Asian       | 35                           | 6     | 17.14    | 1.21                 | 0.51-2.90       | 0.663   | 1.25     | 1.25 | 0.52-3.02 | 0.622 |       |         |
| Marital status   |                             |       |          |                      |                 |         |           |      |       |         |       |       |         |
| Married/Stable Union | 748                   | 97    | 12.97    | 0.593                | Ref             | -----   | -----     |     |       |         |       |       |         |
| Divorced/Widow   | 151                         | 16    | 10.61    | 0.82                 | 0.52-1.28       | 0.378   | 0.82     | 0.82 | 0.54-1.26 | 0.368 |       |         |
| Single           | 732                         | 100   | 13.66    | 1.05                 | 0.80-1.38       | 0.708   | 1.08     | 1.08 | 0.83-1.41 | 0.568 |       |         |
| Sexual Orientation|                             |       |          |                      |                 |         |           |      |       |         |       |       |         |
| Heterosexual     | 1,441                       | 182   | 12.63    | 0.167                | Ref             | -----   | -----     |     |       |         |       |       |         |
| Homo-/Bi-/Transsexual | 164   | 27    | 16.46    | 1.30                 | 0.84-2.02       | 0.234   | 1.33     | 1.33 | 0.86-2.04 | 0.195 |       |         |
| Occupation       |                             |       |          |                      |                 |         |           |      |       |         |       |       |         |
| Professional Higher | 974                   | 121   | 12.42    | 0.667                | Ref             | -----   | -----     |     |       |         |       |       |         |
| Professional Middle | 163                   | 22    | 13.50    | 1.08                 | 0.59-2.00       | 0.790   | 1.08     | 1.08 | 0.59-1.95 | 0.807 |       |         |
| Worker/Informal  | 296                         | 45    | 15.20    | 1.22                 | 0.94-1.60       | 0.136   | 1.21     | 1.21 | 0.91-1.60 | 0.198 |       |         |
| Unemployed       | 187                         | 25    | 13.37    | 1.08                 | 0.81-1.44       | 0.618   | 1.12     | 1.12 | 0.84-1.48 | 0.435 |       |         |
| Healthcare Job   |                             |       |          |                      |                 |         |           |      |       |         |       |       |         |
| No               | 1,380                       | 179   | 12.97    | 0.795                | Ref             | -----   | -----     |     |       |         |       |       |         |
| Yes              | 258                         | 35    | 13.57    | 1.05                 | 0.73-1.50       | 0.807   | 1.00     | 1.00 | 0.69-1.45 | 1.000 |       |         |
| Family Income    |                             |       |          |                      |                 |         |           |      |       |         |       |       |         |
| >6 minimum wages | 731                         | 96    | 13.13    | 0.713                | Ref             | -----   | -----     |     |       |         |       |       |         |
| 4-6 minimum wages | 390                        | 55    | 14.10    | 1.07                 | 0.79-1.46       | 0.647   | 1.10     | 1.10 | 0.81-1.48 | 0.537 |       |         |
| 0-3 minimum wages | 483                        | 59    | 12.22    | 0.93                 | 0.77-1.13       | 0.466   | 0.95     | 0.95 | 0.78-1.16 | 0.603 |       |         |
| Housing          |                             |       |          |                      |                 |         |           |      |       |         |       |       |         |
| Apartment        | 550                         | 71    | 12.91    | 0.438                | Ref             | -----   | -----     |     |       |         |       |       |         |
| Condo/Conjugated | 303                         | 46    | 15.18    | 1.18                 | 0.98-1.42       | 0.089   | 1.17     | 1.17 | 0.97-1.40 | 0.098 |       |         |
| Detached house   | 775                         | 95    | 12.26    | 0.95                 | 0.71-1.27       | 0.724   | 0.95     | 0.95 | 0.71-1.27 | 0.717 |       |         |
| Number Adults in residence | 1  | 156 | 9.59 | 22 | 14.10 | 0.883 | Ref | ----- | ----- | Ref | ----- | -----
| 2  | 633 | 38.91 | 86 | 13.59 | 0.96 | 0.65-1.43 | 0.852 | 0.94 | 0.64-1.39 | 0.758 |
| 3  | 419 | 25.75 | 53 | 12.65 | 0.90 | 0.59-1.37 | 0.614 | 0.90 | 0.60-1.36 | 0.623 |
| 4+ | 419 | 25.75 | 51 | 12.17 | 0.86 | 0.64-1.16 | 0.336 | 0.87 | 0.64-1.19 | 0.390 |
| Number Children in residence | 0  | 1,006 | 62.37 | 129 | 12.82 | 0.970 | Ref | ----- | ----- | Ref | ----- | -----
| 1  | 395 | 24.49 | 52 | 13.16 | 1.03 | 0.74-1.42 | 0.873 | 1.01 | 0.72-1.41 | 0.950 |
| 2  | 173 | 10.73 | 23 | 13.29 | 1.04 | 0.59-1.81 | 0.899 | 1.01 | 0.59-1.73 | 0.969 |
| 3+ | 39  | 2.42  | 6  | 15.38 | 1.20 | 0.43-3.35 | 0.728 | 1.20 | 0.42-3.42 | 0.737 |
| Administrative zones | Center-South | 581 | 35.47 | 84 | 10.50 | 0.015 | NA | ----- | ----- | Ref | ----- | -----
| Center-West | 148 | 9.04  | 28 | 16.22 | NA | NA | NA | NA | NA |
| East | 163 | 9.95  | 31 | 13.50 | NA | NA | NA | NA | NA |
| North | 274 | 16.73 | 46 | 10.22 | NA | NA | NA | NA | NA |
| West | 169 | 10.32 | 38 | 19.53 | NA | NA | NA | NA | NA |
| South | 303 | 18.50 | 58 | 15.18 | NA | NA | NA | NA | NA |
| HealthCare Access | Private Insurance | No | 609 | 37.20 | 89 | 14.61 | 0.154 | Ref | ----- | ----- | Ref | ----- |
| Yes | 1,028 | 62.80 | 125 | 12.16 | 0.83 | 0.59-1.18 | 0.302 | 0.83 | 0.59-1.17 | 0.295 |
| Influenza Vaccine | Last time 2020 | 806 | 49.21 | 96 | 11.91 | 0.128 | Ref | ----- | ----- | Ref | ----- |
| Last time 2019 | 371 | 22.65 | 62 | 16.71 | 1.40 | 0.98-2.01 | 0.065 | 1.41 | 0.98-2.01 | 0.064 |
| Prior to 2019 | 306 | 18.68 | 38 | 12.42 | 1.04 | 0.66-1.64 | 0.857 | 1.04 | 0.66-1.66 | 0.861 |
| Never | 155 | 9.46  | 18 | 11.61 | 0.98 | 0.57-1.88 | 0.928 | 0.99 | 0.57-1.69 | 0.958 |

1 Variables obtained at 1st Cohort Visit except when indicated.
* Relative Risk (RR) using Poisson Regression with robust variance corrected by clusters.
** Incidence Rate Ratio (IRR) using Poisson Regression considering follow-up time with robust variance corrected by clusters.
Supplementary Table 3: SARS-CoV-2 seroconversion according to COVID-19 Risk Factors and Exposures in a general population cohort from Manaus, Amazonas state of Brazil (N=1,638).

| Characteristics | Variables                      | Total  | Column % | IgG ELISA (-) -> (+) | Incidence % | p value | Chi2 | RR** | 95%CI | p value | IRR*** | 95%CI | p value |
|-----------------|--------------------------------|--------|----------|----------------------|-------------|---------|------|------|-------|---------|--------|-------|---------|
| Risk Factors    |                                |        |          |                      |             |         |      |      |       |         |        |       |         |
| Pregnancy       | No                             | 1,022  | 99.13    | 130                  | 12.72       | 0.612*  | NA   | NA   | NA    | NA      | NA     | NA    | NA      |
|                 | Yes                            | 9      | 0.87     | 0                    | 0.00        |          |      |      |      |          |        |       |         |
| Comorbidities (any) | No                 | 967    | 59.04    | 130                  | 13.44       | 0.585   | Ref  | -----| -----| Ref     | -----  | ----- | -----  |
|                 | Yes                            | 671    | 40.96    | 84                   | 12.52       | 0.93    | 0.66-1.31| 0.683 | 0.94 | 0.66-1.33| 0.723  |       |         |
| Which Comorbidities | Chronic Respiratory | 152    | 9.28     | 25                   | 16.45       | 1.22    | 0.83-1.80| 0.308 | 1.22 | 0.82-1.82| 0.323  |       |         |
|                 | Diabetes                       | 91     | 5.56     | 14                   | 15.38       | 1.14    | 0.75-1.74| 0.528 | 1.13 | 0.73-1.75| 0.589  |       |         |
|                 | Obesity                        | 112    | 6.84     | 19                   | 16.96       | 1.26    | 0.80-2.00| 0.320 | 1.24 | 0.76-2.02| 0.384  |       |         |
|                 | Hypertension                   | 250    | 15.26    | 28                   | 11.20       | 0.83    | 0.59-1.77| 0.297 | 0.84 | 0.59-1.19| 0.331  |       |         |
|                 | Cardiopathy                    | 105    | 6.41     | 11                   | 10.48       | 0.80    | 0.48-1.26| 0.308 | 0.79 | 0.49-1.29| 0.352  |       |         |
|                 | Chronic Renal                  | 30     | 1.83     | 6                    | 20.00       | 1.49    | 0.98-2.26| 0.063 | 1.46 | 0.97-2.22| 0.071  |       |         |
|                 | Immunological                  | 30     | 1.83     | 6                    | 20.00       | 1.49    | 0.70-3.15| 0.299 | 1.53 | 0.74-3.16| 0.256  |       |         |
|                 | Cancer                         | 8      | 0.49     | 0                    | 0.00        |          |      |      |      |          |        |       |         |
| Preventive Self | None/Vit/Homemade medication (any) | 1,359  | 82.97    | 168                  | 12.36       | 0.140   | Ref  | -----| -----| Ref     | -----  | ----- | -----  |
|                 | Over the counter               | 110    | 6.72     | 20                   | 18.18       | 1.47    | 0.90-2.40| 0.122 | 1.47 | 0.89-2.44| 0.132  |       |         |
|                 | Prescription Meds              | 169    | 10.32    | 26                   | 15.38       | 1.24    | 0.91-1.70| 0.166 | 1.26 | 0.92-1.73| 0.153  |       |         |
| Which medication | Nitoxanlide                    | 17     | 1.04     | 2                    | 11.76       |          |      |      |      |          |        |       |         |
|                 | Azithromycin                   | 53     | 3.24     | 10                   | 18.87       |          |      |      |      |          |        |       |         |
|                 | Hydroxy/Chloroquine            | 4      | 0.24     | 1                    | 25.00       |          |      |      |      |          |        |       |         |
|                 | Corticosteroids                | 14     | 0.85     | 2                    | 14.29       |          |      |      |      |          |        |       |         |
|                 | Ivermectin                     | 130    | 7.94     | 28                   | 13.08       |          |      |      |      |          |        |       |         |
| COVID-19 Exposure | Social Distancing Never       | 29     | 1.79     | 7                    | 24.14       | 0.183   | Ref  | -----| -----| Ref     | -----  | ----- | -----  |
|                 | Sometimes                      | 417    | 25.74    | 51                   | 12.23       | 0.51    | 0.36-0.72| <0.001| 0.50 | 0.35-0.72| <0.001 |       |         |
|                 | Frequently                     | 1,174  | 72.47    | 153                  | 13.03       | 0.54    | 0.33-0.88| 0.014 | 0.54 | 0.32-0.91| 0.022  |       |         |
| Flexibilization August | No                       | 257    | 15.86    | 24                   | 9.34        | 0.056   | Ref  | -----| -----| Ref     | -----  | ----- | -----  |
|                 | Yes                            | 1,363  | 84.14    | 187                  | 13.72       | 1.47    | 1.14-1.89| 0.003 | 1.50 | 1.16-1.94| 0.002  |       |         |
| Work before August | On-site                        | 479    | 29.55    | 79                   | 16.49       | 0.014   | Ref  | -----| -----| Ref     | -----  | ----- | -----  |
|                 | Remote Only                    | 780    | 48.12    | 96                   | 12.31       | 0.75    | 0.55-1.01| 0.058 | 0.75 | 0.55-1.01| 0.059  |       |         |
|                 | NA                             | 362    | 22.33    | 36                   | 9.94        |          |      |      |      |          |        |       |         |
| Work since August | On-site | 47.25 | 105 | 13.73 | 0.161 | Ref | ----- | ----- | Ref | ----- | ----- | ----- |
|------------------|---------|-------|-----|-------|-------|-----|-------|-------|-----|-------|-------|-------|
| Only Remote      | 580     | 35.82 | 80  | 13.79 | 1.00  | 0.84-1.20 | 0.957 | 0.98 | 0.82-1.18 | 0.867 |
| NA               | 274     | 16.92 | 26  | 9.49  |       | Ref | ----- | ----- | Ref | ----- | ----- | ----- |
| Family members with COVID-19 (at 2<sup>nd</sup>) | No | 495 | 30.22 | 54 | 10.91 | 0.228 | Ref | ----- | ----- | Ref | ----- | ----- |
| Yes, alive       | 892     | 54.46 | 126 | 14.13 | 1.29  | 1.11-1.51 | 0.001 | 1.29 | 1.11-1.50 | 0.001 |
| Yes, deaths      | 251     | 15.32 | 34  | 13.55 | 1.24  | 0.97-1.59 | 0.087 | 1.22 | 0.95-1.57 | 0.116 |
| Household members | No | 1,261 | 76.98 | 127 | 10.07 | <0.001* | Ref | ----- | ----- | Ref | ----- | ----- |
| COVID-19 (at 2<sup>nd</sup>) | Yes, alive | 371 | 22.65 | 85 | 22.91 | 2.27  | 1.78-2.91 | <0.001 | 2.28 | 1.79-2.90 | <0.001 |
| Yes, deaths      | 6       | 0.37  | 2   | 33.33 | 3.31  | 0.86-12.7 | 0.081 | 3.39 | 0.83-13.8 | 0.089 |
| COVID-19 Contacts | None | 778 | 48.02 | 70 | 9.00  | <0.001 | Ref | ----- | ----- | Ref | ----- | ----- |
| Since August     | Yes, with Mask | 604 | 37.28 | 93 | 15.40 | 1.71  | 1.36-2.16 | <0.001 | 1.72 | 1.35-2.19 | <0.001 |
| Yes, without Mask | 238 | 14.69 | 48  | 20.17 | 2.24  | 1.93-2.61 | <0.001 | 2.28 | 1.93-2.68 | <0.001 |

<sup>1</sup> Variables obtained at 1<sup>st</sup> Cohort Visit except when indicated.

* Fisher’s Exact Test

** Relative Risk (RR) using Poisson Regression with robust variance corrected by clusters.

*** Incidence Rate Ratio (IRR) using Poisson Regression considering follow-up time with robust variance corrected by clusters.
Supplementary Table 4: SARS-CoV-2 seroconversion according to COVID-19 Symptoms and Diagnosis in a general population cohort from Manaus, Amazonas state of Brazil (N=1,638).

| Characteristics¹ | Variables | Total | Column % | IgG ELISA (+) -> (+) | Incidence % | p value | Chi2 test | RR** | 95%CI | p value | iRR*** | 95%CI | p value |
|------------------|-----------|-------|----------|----------------------|-------------|---------|-----------|-------|-------|---------|--------|-------|---------|
| **Total**        |           | 1,638 | 214      | 13.06                |             |         |           |       |       |         |        |       |         |
| **Symptoms**     |           |       |          |                      |             |         |           |       |       |         |        |       |         |
| None             |           | 1,196 | 73.02    | 103                  | 8.61        | <0.001  | Ref       |       |       |         |        |       |         |
| (Between 1st 2nd visit) |        |       |          |                      |             | <0.001  | Ref       |       |       |         |        |       |         |
| Flu-like         |           | 393   | 23.99    | 104                  | 26.46       | 3.07    | 2.25-4.19 | <0.001 | 3.05  | 2.23-4.18 | <0.001 |
| Others           |           | 49    | 2.99     | 7                    | 14.29       | 1.66    | 0.67-4.14 | 0.277 | 1.68  | 0.67-4.23 | 0.271 |
| **COVID-19 Diagnosis** | |       |          |                      |             |         |           |       |       |         |        |       |         |
| PCR (Between 1st 2nd visit) | Not done | 1,487 | 92.13    | 161                  | 10.83       | <0.001  | Ref       |       |       |         |        |       |         |
| Yes, Negative    |           | 79    | 4.89     | 11                   | 13.92       | 1.29    | 0.85-1.96 | 0.240 | 1.27  | 0.83-1.95 | 0.269 |
| Yes, Positive    |           | 48    | 2.97     | 36                   | 75.00       | 6.93    | 5.54-8.66 | <0.001 | 6.70  | 5.28-8.51 | <0.001 |
| Serology (Between 1st 2nd visit) | Not done | 1,030 | 63.74    | 116                  | 11.26       | <0.001  | Ref       |       |       |         |        |       |         |
| Yes, Negative    |           | 531   | 32.86    | 63                   | 11.86       | 1.05    | 0.77-1.44 | 0.743 | 1.08  | 0.80-1.47 | 0.607 |
| Yes, Positive    |           | 55    | 3.40     | 32                   | 58.18       | 5.17    | 3.31-8.06 | <0.001 | 5.02  | 3.25-7.75 | <0.001 |
| Antigenic Test (Between 1st 2nd visit) | Not done | 1,088 | 98.64    | 124                  | 11.40       | <0.001* | Ref       |       |       |         |        |       |         |
| Yes, Negative    |           | 9     | 0.82     | 1                    | 11.11       | 0.97    | 0.15-6.50 | 0.979 | 1.11  | 0.16-7.71 | 0.916 |
| Yes, Positive    |           | 6     | 0.54     | 6                    | 100.00      | 8.77    | 6.61-11.7 | <0.001 | 8.90  | 6.60-12.0 | <0.001 |
| COVID-19 (Between 1st 2nd visit) | No | 1,538 | 93.95    | 151                  | 9.82        | <0.001  | Ref       |       |       |         |        |       |         |
| Yes              |           | 99    | 6.05     | 63                   | 63.64       | 6.48    | 4.24-9.90 | <0.001 | 6.28  | 4.08-9.68 | <0.001 |

¹ Variables obtained at 1st Cohort Visit except when indicated.
* Fisher’s Exact Test
** Relative Risk (RR) using Poisson Regression with robust variance corrected by clusters.
*** Incidence Rate Ratio (IRR) using Poisson Regression considering follow-up time with robust variance corrected by clusters.
Supplementary Table 5: Multivariate Regression Model for SARS-CoV-2 seroconversion in a general population cohort from Manaus, Amazonas state of Brazil (N=1,618).

| Characteristics¹ | Variables                         | IRR*  | 95% CI       | p value |
|------------------|-----------------------------------|-------|--------------|---------|
| Age              | 18-29 Ref                         | ----- | -----        |         |
|                  | 30-39 0.97                        | 0.68-1.37 | 0.854    |         |
|                  | 40-49 1.05                        | 0.81-1.36 | 0.725    |         |
|                  | 50-59 1.13                        | 0.66-1.93 | 0.652    |         |
|                  | >=60 1.17                         | 0.55-2.51 | 0.683    |         |
| Sex              | F Ref                             | ----- | -----        |         |
|                  | M 1.13                            | 0.82-1.56 | 0.458    |         |
| Social Distancing| Never Ref                         | ----- | -----        |         |
|                  | Sometimes 0.80                    | 0.53-1.21 | 0.285    |         |
|                  | Frequently 1.11                   | 0.57-2.18 | 0.758    |         |
| SD Flexibilization| No Ref                           | ----- | -----        |         |
|                  | Yes 1.31                          | 1.05-1.64 | 0.017    |         |
| Work before August| On-site Ref                      | ----- | -----        |         |
|                  | Remote Only 0.74                  | 0.56-0.97 | 0.030    |         |
| Household members| No Ref                            | ----- | -----        |         |
|                  | with COVID-19 (2nd)  Yes, alive 1.49 | 1.21-1.83 | <0.001  |         |
|                  | Yes, deaths 2.22                   | 0.68-7.27 | 0.186    |         |
| COVID-19 Contacts| None Ref                          | ----- | -----        |         |
|                  | Yes, with Mask 1.20               | 0.90-1.60 | 0.219    |         |
|                  | Yes, without Mask 1.25            | 1.09-1.45 | 0.002    |         |
| Symptoms since August| None Ref                  | ----- | -----        |         |
|                  | Flu-like 1.79                     | 1.23-2.59 | 0.002    |         |
|                  | Others 1.20                       | 0.60-2.40 | 0.616    |         |
| COVID-19 Diagnosis| No Ref                           | ----- | -----        |         |
|                  | Yes 3.57                          | 2.27-5.63 | <0.001   |         |

¹ Variables obtained at 1st Cohort Visit except when indicated.

* Incidence Rate Ratio (IRR) using Poisson Regression considering follow-up time with robust variance corrected by clusters, and adjusted per all model variables.