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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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 ⁴. 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^{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^{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 ⁴. 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 ⁴. 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 ⁴.

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 **B** **C** **D** **E**

Figure 2

Supplementary Figure 1

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 1 st and 2 nd 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).

Characteristics ¹	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	22.71	48	12.90	0.915	Ref	-----	-----	Ref	-----	-----
	30-39	420	25.64	56	13.33		1.03	0.68-1.57	0.878	1.00	0.66-1.51	0.990
	40-49	354	21.61	48	13.56		1.05	0.81-1.36	0.703	1.02	0.80-1.31	0.865
	50-59	292	17.83	40	13.70		1.06	0.69-1.63	0.786	1.02	0.66-1.57	0.924
	>=60	200	12.21	22	11.00		0.85	0.37-1.99	0.712	0.82	0.36-1.90	0.648
Sex	F	1,031	62.94	130	12.61	0.476	Ref	-----	-----	Ref	-----	-----
	M	607	37.06	84	13.84		1.10	0.75-1.60	0.627	1.08	0.75-1.56	0.663
Race	Mestizo	885	54.03	125	14.12	0.290	Ref	-----	-----	Ref	-----	-----
	White	602	36.75	74	12.29		0.87	0.57-1.32	0.515	0.89	0.58-1.35	0.570
	Black	109	6.65	9	8.26		0.58	0.12-2.81	0.503	0.59	0.12-2.81	0.509
	Indigenous	7	0.43	0	0.00		NA			NA		
	East-Asian	35	2.14	6	17.14		1.21	0.51-2.90	0.663	1.25	0.52-3.02	0.622
Marital status	Married/Stable Union	748	45.86	97	12.97	0.593	Ref	-----	-----	Ref	-----	-----
	Divorced/Widow	151	9.26	16	10.61		0.82	0.52-1.28	0.378	0.82	0.54-1.26	0.368
	Single	732	44.88	100	13.66		1.05	0.80-1.38	0.708	1.08	0.83-1.41	0.568
Sexual Orientation	Heterosexual	1,441	89.78	182	12.63	0.167	Ref	-----	-----	Ref	-----	-----
	Homo-/Bi-/Transsexual	164	10.22	27	16.46		1.30	0.84-2.02	0.234	1.33	0.86-2.04	0.195
Occupation	Professional Higher	974	60.12	121	12.42	0.667	Ref	-----	-----	Ref	-----	-----
	Professional Middle	163	10.06	22	13.50		1.08	0.59-2.00	0.790	1.08	0.59-1.95	0.807
	Worker/Informal	296	18.27	45	15.20		1.22	0.94-1.60	0.136	1.21	0.91-1.60	0.198
	Unemployed	187	11.54	25	13.37		1.08	0.81-1.44	0.618	1.12	0.84-1.48	0.435
Healthcare Job	No	1,380	84.25	179	12.97	0.795	Ref	-----	-----	Ref	-----	-----
	Yes	258	15.75	35	13.57		1.05	0.73-1.50	0.807	1.00	0.69-1.45	1.000
Family Income	>6 minimum wages	731	45.57	96	13.13	0.713	Ref	-----	-----	Ref	-----	-----
	4-6 minimum wages	390	24.31	55	14.10		1.07	0.79-1.46	0.647	1.10	0.81-1.48	0.537
	0-3 minimum wages	483	30.11	59	12.22		0.93	0.77-1.13	0.466	0.95	0.78-1.16	0.603
Housing	Apartment	550	33.78	71	12.91	0.438	Ref	-----	-----	Ref	-----	-----
	Condo/Conjugated	303	18.61	46	15.18		1.18	0.98-1.42	0.089	1.17	0.97-1.40	0.098
	Detached house	775	47.60	95	12.26		0.95	0.71-1.27	0.724	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			NA		
	Center-West	148	9.04	28	16.22		NA			NA		
	East	163	9.95	31	13.50		NA			NA		
	North	274	16.73	46	10.22		NA			NA		
	West	169	10.32	38	19.53		NA			NA		
	South	303	18.50	58	15.18		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	0.57-1.68	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 ¹	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							
Risk Factors												
Pregnancy	No	1,022	99.13	130	12.72	0.612*	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	0.59-1.17	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		NA			NA		
Preventive Self medication (any)	None/Vit/Homemade	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	Nitazoxanide	17	1.04	2	11.76		NA			NA		
	Azithromycin	53	3.24	10	18.87		NA			NA		
	Hydroxy/Chloroquine	4	0.24	1	25.00		NA			NA		
	Corticosteroids	14	0.85	2	14.29		NA			NA		
	Ivermectin	130	7.94	28	13.08		NA			NA		
COVID-19 Exposure												
Social Distancing before August	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	765	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							
Family members with	No	495	30.22	54	10.91	0.228	Ref	-----	-----	Ref	-----	-----
COVID-19 (at 2 nd)	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 nd)	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

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 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).

Characteristics ¹	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												
Symptoms	None	1,196	73.02	103	8.61	<0.001	Ref	-----	-----	Ref	-----	-----
(Between 1 st 2 nd visit)	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 1 st 2 nd visit)	Not done	1,487	92.13	161	10.83	<0.001	Ref	-----	-----	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 1 st 2 nd visit)	Not done	1,030	63.74	116	11.26	<0.001	Ref	-----	-----	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 1 st 2 nd visit)	Not done	1,088	98.64	124	11.40	<0.001*	Ref	-----	-----	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 1 st 2 nd visit)	No	1,538	93.95	151	9.82	<0.001	Ref	-----	-----	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).

Characteristics ¹	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 before August	Never	Ref	-----	-----
	Sometimes	0.80	0.53-1.21	0.285
	Frequently	1.11	0.57-2.18	0.758
SD Flexibilization since August	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
	NA			
Household members with COVID-19 (2 nd)	No	Ref	-----	-----
	Yes, alive	1.49	1.21-1.83	<0.001
	Yes, deaths	2.22	0.68-7.27	0.186
COVID-19 Contacts since August	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 since August	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.

Figures

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.