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SARS-CoV-2 seroprevalence and associated factors in Manaus, Brazil: baseline results from the DETECTCoV-19 cohort study

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A B S T R A C T

Background: Manaus, located in the Brazilian rainforest, has experienced two health system collapses due to the coronavirus disease 2019 (COVID-19) pandemic. However, little is known about which groups among the general population have been most affected.

Methods: A convenience sampling strategy via online advertising recruited 3046 adults between 19 August 2020 and 2 October 2020. Sociodemographic characteristics, COVID-19–related symptoms, COVID-19 testing, self-medication and prescribed medications were recorded. Serum anti-severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) nucleocapsid immunoglobulin G antibodies were measured with an enzyme-linked immunosorbent assay. Prevalence ratios (PR) were obtained using cluster-corrected and adjusted Poisson’s regression models.

Results: A crude positivity rate among asymptomatic and symptomatic individuals was estimated at 29.10%, with maximum possible seroprevalence of 44.82% corrected by test characteristics and an antibody decay rate of 32.31%. Regression models demonstrated a strong association towards marginalized low-income and vulnerable residents with limited access to healthcare. The presence of a COVID-19 case (PR 1.39, 95% confidence interval (CI) 1.24–1.57) or death (PR 2.14, 95% CI 1.74–2.62) in a household greatly increased the risk of other household members acquiring infection. The seroprevalence of SARS-CoV-2 was higher among those who self-medicated to prevent infection (PR 1.36, 95% CI 1.27–1.46).

Conclusions: Disproportionate socio-economic disparity was observed among the study participants. The syndemic nature of COVID-19 in the Amazon region needs differential policies and urgent solutions to control the ongoing pandemic.

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Introduction

Infectious diseases have a profound impact on humans, particularly vulnerable populations (Fauci and Morens, 2012). The emergence of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and a lack of effective treatment and non-pharmaceutical interventions to curb transmission have led to an exponential increase in the burden of coronavirus disease 2019 (COVID-19) worldwide (Hsiang et al., 2020; Kraemer et al., 2020; Bo et al., 2021).

In April 2020, the Mayor of Manaus declared that the health system had collapsed due to the high volume of severe and critical patients; moreover, the failure in Manaus also meant a state-wide collapse. Since early 2021, Manaus has experienced a reprise of the healthcare collapse of April 2020, with an astounding increase in numbers of reported COVID-19 cases and deaths. As of 25 January 2021, Brazil has reported >8.9 million confirmed cases with a 2.5% death rate, Amazonas state has reported >205,000 cases with a death rate of 2.88% (Ministério da Saúde 2021), and Manaus has registered 110,689 cases and 4911 deaths ( Fundação de Vigilância em Saúde do Amazonas, 2021; Ministério da Saúde, 2021). Overall, adjusted seroprevalence rates of 14% among the general population in June 2020 (measured by Wondfo rapid serological test) and 44% among blood donors in October 2020 (tested by Abbott chemiluminescence assay) have been estimated in Manaus; however, it is still not clear how different sociodemographic characteristics, healthcare access and reported risk factors influence seroprevalence (Hallal et al., 2020; Buss et al., 2021). Identifying these factors is important to aid in the formulation of targeted public health measures (Aragona et al., 2020). A prospective cohort (DETECTCoV-19) in Manaus, Brazil was designed to improve understanding of the epidemiology of SARS-CoV-2 and associated risk factors. This closely monitored cohort will provide a unique opportunity to determine disease attack rates; and to monitor the serological status of residents of Manaus, and the relevance of demographic and socio-economic factors and their association with the prevalence of infection in a setting of high transmission and low non-pharmaceutical containment measures. This article reports the overall prevalence of SARS-CoV-2 infection and associated factors found from the DETECTCoV-19 cohort.

Methods

Ethical approval

The Research Ethics Committee of Federal University of Amazonas approved this study (CAAE:34906920.4.0000.5020) in accordance with Brazilian law, and the Declaration of Helsinki. All participants gave oral and written consent prior to enrolment.

Study design and participants

A longitudinal study (DETECTCoV-19) was designed to follow-up Manaus citizens for up to 6 months after recruitment with a sample collection every 8–12 weeks. The study included people of both sexes, aged ≥18 years, living in Manaus who agreed to participate. A convenience sampling strategy was adopted for recruitment, and 3046 individuals were recruited between 19 August 2020 and 2 October 2020. DETECTCoV-19 was advertised on social media and a university website. All participants registered online and presented at the blood collection centre (Nursing School, Federal University of Amazonas), where they signed the consent form, filled out an electronic questionnaire and donated a blood sample for testing (Figure S1, see online supplementary material).

Data and sample collection

First, sociodemographic data, including age, sex, occupation and residential address, were collected for each participant. Second, information related to COVID-19, symptoms since the start of the pandemic, prior diagnosis, preventive self-medication and prescribed medication used to treat symptoms were recorded. Finally, an independent form was used to record the laboratory results. Participant data were recorded using the Research Electronic Data Capture (REDCap) software, and all data were stored on the local ILMD/Fiocruz Amazonía server. Details of blood collection, sample
processing and storage are described in the online supplementary material.

ELISA to detect anti-SARS-CoV-2 nucleocapsid IgG antibodies

An indirect enzyme-linked immunosorbent assay (ELISA)-based serological assay was developed and used to measure anti-SARS-CoV-2 nucleocapsid immunoglobulin G (IgG) antibody titres in serum samples using recombinant full-length SARS-CoV-2 nucleocapsid protein (residues 1–419, GenBank QHD43423.2) expressed in Escherichia coli, and purified by affinity and size exclusion chromatography (Figure S7, see online supplementary material). The antigen concentration, sample dilution and secondary antibody concentration were determined using a checkerboard method to achieve the optimal signal to noise ratio. Antigen lot and inter-variation oscillations were assessed throughout the development of the assay, before the analysis of study samples. The sensitivity and specificity of the assay were determined using sera from patients who tested positive for SARS-CoV-2 on reverse transcriptase polymerase chain reaction (RT-PCR) (n=293) and pre-pandemic controls (n=229, Table S1, see online supplementary material). The anti-SARS-CoV-2 nucleocapsid IgG ELISA had sensitivity of 89.07% [95% confidence interval (CI) 84.79–92.30] and 94.28% (95% CI 89.44–97.07) for patients ≥7 days and ≥14 days after the onset of COVID-19 symptoms, respectively. Specificity of 97.03% (95% CI 93.72–98.69) was estimated using the pre-pandemic serum samples. An anti-SARS-CoV-2 nucleocapsid IgG antibody reactivity index (RI) was expressed as the ratio between the optical density of the patient sample and the negative control. All samples with RI ≥1.5 (assay cut-off) were considered positive. Further details of the in-house ELISA protocol and performance evaluation are described in the online supplementary material.

Data analysis

This study evaluated the cohort baseline data, constituting a cross-sectional analysis. Crude seroprevalence was further adjusted by ELISA test characteristics (Diggle, 2011) and antibody decay, defined as the proportion of patients who had a current positive ELISA over the number of participants who had any positive serological test at any point before the study, regardless of symptoms or time elapsed. Chi-squared test and Fisher’s exact test with two-by-two contingency tables were used to examine statistical significance and associations between study variables. The presence or absence of antibodies to SARS-CoV-2 was the primary dependent variable, and characteristics that were identified in the descriptive analysis were the independent variables. Significant variables were used in a Poisson regression model with robust variance to estimate prevalence ratios. Variance was corrected per cluster (administrative area), and models were adjusted for sex, age, family income, presence of household members with COVID-19, use of preventive self-medications, and prior COVID-19 diagnosis.

Results

Using online advertising, 4394 individuals agreed to participate in the study by making an appointment via the study website. Of these, 3600 attended an interview (Figure S1, see online supplementary material). In total, 554 (15.38%) individuals were ineligible or refused to participate in the longitudinal study, and were not included. Table 1 details the demographic characteristics and SARS-CoV-2 seropositivity rate estimated by the anti-SARS-CoV-2 nucleocapsid IgG ELISA. Of the 3046 individuals included in the cohort, 60.80% were women. Figure S2 (see online supplementary material) compares the features of the DETECTCoV-19 study cohort with the census data from Manaus. Figure S3 (see online supplementary material) compares the distribution of patients with previous flu-like symptoms before recruitment with the COVID-19 epidemic curve of confirmed SARS-CoV-2 cases in Manaus.

In total, 886 individuals, symptomatic and asymptomatic, tested positive for SARS-CoV-2-specific IgG antibodies, with an estimated crude IgG seroprevalence of 29.10% (95% CI 27.5–30.7). As study participants presented with flu-like symptoms for at least 1 week before recruitment, crude seropositivity was adjusted by the sensitivity and specificity of an ELISA determined for at least 7 days of symptoms. With sensitivity of 89.07% and specificity of 97.03%, the adjusted seroprevalence was 30.34% (95% CI 28.5–32.3) using the method described by Diggle (2011).

In this study, more than two-thirds of the patients with a prior diagnosis of COVID-19 (either by PCR or serology) were still positive at the cohort baseline, regardless of time since symptom onset. Of the 141 participants who had positive serology before the study, 38 tested negative. Using these data, antibody decay of 26.95% was estimated for the whole cohort over a median of 131.5 days (interquartile range 63–152) between the two tests. Scientific literature suggests that the antibody decay rate is higher among asymptomatic patients (Yang et al., 2021), possibly up to 2.08 times based on Chia et al. (2021). Given that 163 of the 886 IgG-positive participants were asymptomatic (18.4%), the antibody decay proportion could be as high as 32.31%. Extrapolating these values, the actual seroprevalence in the study cohort could oscillate between 41.53% and 44.82%. Anti-SARS-CoV-2 antibody levels estimated using RI were elevated in asymptomatic patients compared with asymptomatic patients (Figure S5, see online supplementary material), and positively correlated with patient age (Figure S6, see online supplementary material). Figure S7 (see online supplementary material) describes the distribution of COVID-19-positive individuals stratified by days since onset of flu-like symptoms, self-declared by the study participants.

Seropositivity of SARS-CoV-2 antibodies was significantly higher in men compared with women (Table 1). The highest and lowest age-stratified seroprevalence rates were observed in individuals aged between 50 and 59 years and ≥60 years of age, respectively. The prevalence rates were inversely correlated with occupation type and family income. Prevalence was higher among the poorest (35.50% vs 24.45% among the wealthiest) (Table 1). In general, individuals living in detached or conjugated houses had higher SARS-CoV-2 antibody prevalence compared with individuals living in condo houses and apartment buildings. Moreover, an increase in the number of adults or children in the family household increased the seropositivity rate significantly. Among the administrative zones of Manaus, Centro-Sul (Centre-South) had the lowest prevalence (28.29%) and Leste (East) had the highest prevalence (39.80%) (Table 1).

Table 2 describes COVID-19-related risk factors in the study population. COVID-19 in a distant family member living locally or in another town had no influence on the serological status of the study participant. On the other hand, COVID-19 in a household member increased the prevalence significantly from 25.18% to 44.89% (Table 2). Moreover, a death in the family household due to COVID-19 further increased the SARS-CoV-2 antibody prevalence to 56% among the study participants (Table 2). Individuals with or without comorbidities had similar antibody prevalence. Overall, one-quarter of the study population self-medicated to prevent against SARS-CoV-2 infection. However, prevalence among individuals taking self-medications of over-the-counter drugs or controlled drugs to prevent SARS-CoV-2 infection was 38.64%, compared with 25.99% among individuals who did not self-medicate (Table 2).

Table 3 summarizes individual symptoms reported by study participants since the start of the pandemic, and access to COVID-19 testing in Manaus. Overall, 77% of the study cohort reported at
### Table 1
Study population and health access according to severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) seroprevalence and symptoms in Manaus, Amazonas, Brazil.

| Characteristics                | Categories                                      | Total  | Column % | IgG positive | Prevalencerow % | P-value $\chi^2$ test | IgG positive and symptoms | Prevalencerow | P-value $\chi^2$ test |
|--------------------------------|-------------------------------------------------|--------|----------|--------------|-----------------|------------------------|--------------------------|---------------|------------------------|
| Demographic structure          | Sex                                             |        |          |              |                 |                        |                          |               |                        |
|                                | Female                                          | 1852   | 60.80    | 508          | 27.43           | 0.012                  | 427                      | 23.08         | 0.220                  |
|                                | Male                                            | 1194   | 39.20    | 378          | 31.68           | 0.29                   | 298                      | 25.02         |                        |
|                                | Age (years)                                     |        |          |              |                 |                        |                          |               |                        |
|                                | 18–29                                           | 703    | 23.08    | 197          | 28.06           | 0.025                  | 167                      | 23.82         | 0.015                  |
|                                | 30–39                                           | 758    | 24.89    | 205          | 27.04           |                        | 165                      | 21.83         |                        |
|                                | 40–49                                           | 692    | 22.72    | 207          | 29.91           |                        | 171                      | 24.71         |                        |
|                                | 50–59                                           | 558    | 18.32    | 191          | 34.23           |                        | 158                      | 28.37         |                        |
|                                | >60                                             | 335    | 11.00    | 86           | 25.67           |                        | 64                       | 19.10         |                        |
| Marital status                 | Married/stable union                            | 1424   | 46.98    | 431          | 30.27           | 0.382                  | 353                      | 24.79         | 0.512                  |
|                                | Divorced/widowed                                 | 275    | 9.07     | 81           | 29.45           |                        | 65                       | 23.64         |                        |
|                                | Single                                          | 1332   | 43.95    | 371          | 27.87           |                        | 305                      | 22.92         |                        |
| Sexual orientation             | Heterosexual                                    | 2604   | 90.92    | 789          | 29.30           | 0.057                  | 647                      | 24.03         | 0.188                  |
|                                | Homo-/bi-/trans-sexual                          | 269    | 9.08     | 64           | 23.79           |                        | 55                       | 20.45         |                        |
| Occupation                     | Professional higher                             | 1655   | 54.91    | 437          | 26.42           | 0.004                  | 365                      | 22.08         | 0.090                  |
|                                | Professional middle                             | 353    | 11.71    | 114          | 32.29           |                        | 91                       | 25.93         |                        |
|                                | Worker/informal                                 | 663    | 22.00    | 221          | 33.33           |                        | 171                      | 25.79         |                        |
|                                | Unemployed                                      | 343    | 11.38    | 104          | 30.32           |                        | 91                       | 26.61         |                        |
| Family income                  | 0–3 minimum wages                               | 1080   | 36.27    | 383          | 35.50           | <0.001                 | 310                      | 28.73         | <0.001                 |
|                                | 4–6 minimum wages                               | 712    | 23.91    | 197          | 27.67           |                        | 164                      | 23.03         |                        |
|                                | >6 minimum wages                                | 1186   | 39.83    | 290          | 24.45           |                        | 239                      | 20.15         |                        |
| Housing                        | Detached house                                  | 1524   | 50.33    | 492          | 32.18           | <0.001                 | 399                      | 26.18         | <0.001                 |
|                                | Conjugated house                                | 285    | 9.41     | 98           | 34.39           |                        | 85                       | 29.82         |                        |
|                                | Condo house                                     | 293    | 9.68     | 79           | 26.96           |                        | 61                       | 20.82         |                        |
|                                | Apartment                                       | 926    | 30.58    | 215          | 23.24           |                        | 179                      | 19.35         |                        |
| Number of adults in residence  | 1                                               | 282    | 9.35     | 71           | 25.18           | <0.001                 | 57                       | 20.21         | 0.001                  |
|                                | 2                                               | 1119   | 37.11    | 294          | 26.30           |                        | 237                      | 21.20         |                        |
|                                | 3                                               | 756    | 25.07    | 215          | 28.44           |                        | 181                      | 23.94         |                        |
|                                | ≥4                                              | 858    | 28.46    | 296          | 34.50           |                        | 243                      | 28.32         |                        |
| Number of children in residence| 0                                               | 1758   | 58.64    | 482          | 27.43           | 0.003                  | 403                      | 22.94         | 0.380                  |
|                                | 1                                               | 781    | 26.05    | 235          | 30.09           |                        | 194                      | 24.84         |                        |
|                                | ≥3                                              | 345    | 11.51    | 108          | 31.30           |                        | 86                       | 24.93         |                        |
| Administrative zones           | Centro-Sul (Centre-South)                       | 960    | 31.52    | 230          | 23.98           | <0.001                 | 183                      | 19.10         | <0.001                 |
|                                | Centro-Oeste (Centre-West)                      | 258    | 8.47     | 73           | 28.29           |                        | 64                       | 24.81         |                        |
|                                | Leste (East)                                    | 384    | 12.61    | 149          | 38.80           |                        | 118                      | 30.81         |                        |
|                                | Norte (North)                                   | 568    | 18.65    | 175          | 30.81           |                        | 145                      | 25.57         |                        |
|                                | Oeste (West)                                    | 344    | 11.29    | 99           | 28.78           |                        | 85                       | 24.78         |                        |
|                                | Sul (South)                                     | 532    | 17.47    | 160          | 30.80           |                        | 130                      | 24.44         |                        |

IgG, immunoglobulin G.

*Participants who had both a SARS-CoV-2 IgG positive test and reported symptoms prior to recruitment since March 2020.*
Table 2
Risk factors for coronavirus disease 2019 (COVID-19) and preventive self-medication according to severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) seroprevalence and symptoms in Manaus, Amazonas, Brazil.

| Characteristics               | Variables | Total   | Column % | IgG positive | Prevalencercov % | P-value $\chi^2$ test | IgG positive and symptoms$^c$ | Prevalencercov % | P-value $\chi^2$ test |
|-------------------------------|-----------|---------|----------|--------------|------------------|----------------------|-------------------------------|------------------|----------------------|
| Total                         |           | 3046    | 886      | 29.10        | 725              |                      |                               | 23.84            |                      |
| Private insurance             | No        | 1235    | 408      | 33.04        | <0.001           | 329                  |                               | 26.64            | 0.003                |
|                               | Yes       | 1801    | 476      | 26.44        |                  | 395                  |                               | 21.94            |                      |
| Last influenza vaccine        | 2020      | 1441    | 398      | 27.62        | 0.272            | 326                  |                               | 22.62            | 0.481                |
|                               | 2019      | 698     | 220      | 31.52        |                  | 175                  |                               | 25.07            |                      |
|                               | Prior to 2019 | 586   | 170      | 29.01        |                  | 142                  |                               | 24.23            |                      |
|                               | Never     | 307     | 94       | 30.72        |                  | 79                   |                               | 25.82            |                      |
| Risk factors                  |           |         |          |              |                  |                      |                               |                  |                      |
| Family members with COVID-19$^b$ | No        | 1305    | 377      | 28.91        | 0.346            | 299                  |                               | 22.93            | 0.264                |
|                               | Yes, alive| 1395    | 419      | 30.04        |                  | 351                  |                               | 25.16            |                      |
|                               | Yes, died | 334     | 87       | 26.05        |                  | 73                   |                               | 21.86            |                      |
| Household members             | No        | 2428    | 611      | 25.18        | <0.001           | 486                  |                               | 20.02            | <0.001               |
|                               | Yes, alive| 568     | 255      | 44.89        |                  | 224                  |                               | 39.44            |                      |
|                               | Yes, died | 25      | 14       | 56.00        |                  | 11                   |                               | 44.00            |                      |
| Pregnancy                     | No        | 3030    | 884      | 29.18        | 0.175$^a$        | 723                  |                               | 23.90            | 0.387$^a$            |
|                               | Yes       | 16      | 2        | 12.5         |                  | 2                    |                               | 12.50            |                      |
| Comorbidities (any)           | No        | 1995    | 593      | 29.74        | 0.286            | 478                  |                               | 23.97            | 0.815                |
|                               | Yes       | 1047    | 292      | 27.89        |                  | 247                  |                               | 23.59            |                      |
| Which comorbidities           | Asthma    | 198     | 58       | 29.29        | 0.896            | 50                   |                               | 25.25            | 0.688                |
|                               | Diabetes  | 186     | 64       | 34.41        | 0.184            | 51                   |                               | 27.42            | 0.294                |
|                               | Hypertension | 447   | 131      | 29.31        | 0.856            | 111                  |                               | 24.83            | 0.701                |
|                               | Obesity   | 201     | 56       | 27.86        | 0.578            | 49                   |                               | 24.38            | 0.898                |
|                               | Cardiopathy | 60   | 18       | 30.00        | 0.965            | 16                   |                               | 26.67            | 0.630                |
|                               | Cancer    | 17      | 5        | 29.41        | 0.977            | 4                    |                               | 23.53            | 1.000$^a$            |
|                               | Other     | 257     | 61       | 23.74        | 0.046            | 55                   |                               | 21.40            | 0.361                |
| Preventive self-medication (any) | No    | 2293    | 596      | 25.99        | <0.001           | 460                  |                               | 20.06            | <0.001               |
|                               | Yes       | 749     | 289      | 38.64        |                  | 265                  |                               | 35.43            |                      |
| Which medication              | Nitazoxanide | 28   | 7        | 25.00        | 0.905            | 7                    |                               | 25.00            | 0.517                |
|                               | Azithromycin | 161  | 67       | 41.88        | <0.001           | 65                   |                               | 40.63            | <0.001               |
|                               | Hydroxy/chloroquine | 10 | 5 | 50.00 | 0.085 | 5 | 50.00 | 0.019 |
|                               | Corticosteroids | 33  | 14      | 43.75 | 0.023 | 13 | 40.63 | 0.004 |
|                               | Ivermectin | 268  | 83       | 31.09        | 0.074            | 73                   |                               | 27.34            | 0.006                |
|                               | Paracetamol | 268  | 121      | 45.15        | <0.001           | 116                  |                               | 43.28            | <0.001               |
|                               | Other      | 260    | 104      | 40.00        | <0.001           | 96                   |                               | 36.92            | <0.001               |

IgG, immunoglobulin G.

$^a$ Fisher’s exact test.

$^b$ Participants who had both SARS-CoV-2 IgG positive test and reported symptoms prior to recruitment since March 2020.

$^c$ Family members with COVID-19 implies family members outside the household.
Table 3
Symptoms and diagnostic tests according to severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) seroprevalence and symptoms in Manaus, Amazonas, Brazil.

| Characteristics | Variables | Total | Column % | IgG positive | Prevalence% | P-value $\chi^2$ test | IgG positive and symptoms$^a$ | Prevalence% | P-value $\chi^2$ test |
|-----------------|-----------|-------|----------|--------------|------------|-----------------------|-----------------------------|------------|-----------------------|
| Total           | 3046      | 886   | 29.10    | 725          | 23.84      |
| Symptoms        |           |       |          |              |            |                       |                             |            |                       |
| Flu-like symptoms in last 7 days | No       | 2479  | 82.61    | 716          | 28.89      | 0.474                 |                             | 0.474      |
|                 | Yes       | 522   | 17.39    | 159          | 30.46      |                       |                             | 0.474      |
| Flu-like symptoms since March (any) | No       | 685   | 22.52    | 163          | 23.80      | 0.001                 |                             | 0.001      |
|                 | Yes       | 2357  | 77.48    | 722          | 30.65      |                       |                             | 0.001      |
| Which symptoms  | Anosmia   | 525   | 22.27    | 341          | 64.95      | <0.001                |                             | <0.001     |
|                 | Body aches| 971   | 41.20    | 372          | 38.35      | <0.001                |                             | <0.001     |
|                 | Chest pain| 337   | 14.30    | 138          | 41.07      | <0.001                |                             | <0.001     |
|                 | Chills    | 343   | 14.55    | 156          | 45.48      | <0.001                |                             | <0.001     |
|                 | Conjunctivitis | 61   | 2.39     | 19           | 31.15      | 0.200                 |                             | 0.200      |
|                 | Cough     | 842   | 35.72    | 304          | 36.10      | <0.001                |                             | <0.001     |
|                 | Diarrhoea | 655   | 27.79    | 206          | 31.45      | 0.002                 |                             | 0.002      |
|                 | Fever     | 717   | 30.42    | 318          | 44.41      | <0.001                |                             | <0.001     |
|                 | Headache  | 1633  | 69.28    | 508          | 31.13      | <0.001                |                             | <0.001     |
|                 | Lack of appetite | 339 | 14.38   | 178         | 52.66      | <0.001                |                             | <0.001     |
|                 | Palpitations | 239 | 10.14   | 76          | 31.80      | 0.015                 |                             | 0.015      |
|                 | Shortness of breath | 487 | 20.66  | 175         | 35.93      | <0.001                |                             | <0.001     |
|                 | Side chest pain | 612 | 25.97  | 224         | 36.66      | <0.001                |                             | <0.001     |
|                 | Skin rashes | 130 | 5.52    | 45          | 34.62      | 0.009                 |                             | 0.009      |
|                 | Sore throat | 1049 | 44.51   | 315         | 30.26      | 0.004                 |                             | 0.004      |
|                 | Vomiting  | 140   | 5.94     | 44           | 31.43      | <0.001                |                             | <0.001     |
| Prior COVID-19 diagnosis | Prior SARS-CoV-2 RT-PCR | No | 2788 | 92.35 | 798 | 28.63 | <0.001 | 647 | 23.21 | <0.001 |
|                 | Yes, negative | 172 | 5.70 | 40 | 23.26 | 36 | 20.93 |
|                 | Yes, positive | 59 | 1.95 | 42 | 71.97 | 37 | 62.71 |
| Prior serological test | No | 2349 | 77.76 | 660 | 28.11 | <0.001 | 528 | 22.49 | <0.001 |
|                 | Yes, negative | 531 | 17.58 | 116 | 21.85 | 103 | 19.40 |
|                 | Yes, positive | 141 | 4.67 | 103 | 73.05 | 89 | 63.12 |
| Prior COVID-19 diagnosis$^b$ | No | 2771 | 91.72 | 728 | 26.28 | <0.001 | 588 | 21.23 | <0.001 |
|                 | Yes | 250 | 8.28 | 154 | 61.60 | 135 | 54.00 |
| Took medications after COVID-19 diagnosis | No | 109 | 43.60 | 51 | 46.79 | <0.001 | 43 | 39.45 | <0.001 |
|                 | Yes | 141 | 56.40 | 103 | 73.05 | 92 | 65.25 |
| Which medications | Nitroxanide | 11 | 7.80 | 10 | 90.91 | 0.008$^a$ | 10 | 90.91 | 0.002$^a$ |
|                 | Azithromycin | 108 | 76.60 | 78 | 72.22 | <0.001 | 70 | 64.81 | <0.001 |
|                 | Hydroxy/chloroquine | 27 | 19.15 | 19 | 70.37 | 0.028 | 17 | 62.96 | 0.028 |
|                 | Corticosteroids | 36 | 25.53 | 28 | 77.78 | 0.001 | 26 | 72.22 | 0.001 |
|                 | Ivermectin | 77 | 54.61 | 52 | 67.53 | 0.005 | 47 | 61.04 | 0.004 |
|                 | Paracetamol | 59 | 41.84 | 46 | 77.97 | <0.001 | 44 | 74.58 | <0.001 |
|                 | Others | 38 | 26.95 | 28 | 73.68 | 0.004 | 25 | 65.79 | 0.005 |

IgG, immunoglobulin G; COVID-19, coronavirus disease 2019; RT-PCR, reverse transcriptase polymerase chain reaction.

$^a$ Fisher’s exact test.

$^b$ Participants who had both SARS-CoV-2 IgG positive test and reported symptoms prior to recruitment since March 2020.

$^c$ Previous COVID-19 diagnosis was defined as individuals positive for SARS-CoV-2 RT-PCR or SARS-CoV-2 serological test or clinical diagnosis as COVID-19.
least one symptom since March 2020, and 17% reported at least one symptom in the 7 days before recruitment. Less than 10% of symptomatic individuals were tested by RT-PCR, and prior COVID-19 diagnosis increased the prevalence of SARS-CoV-2 antibodies to 61.60% among these individuals. Around half of the patients diagnosed with COVID-19 reported not taking any over-the-counter or prescribed medicine. Azithromycin, ivermectin and paracetamol were the main drugs prescribed to patients diagnosed with COVID-19 (Table 3). All the significant variables described above were used in a Poisson regression model (Table 4). Data were corrected and adjusted for variables that were not controlled experimentally to identify true risk factors (Table 4 and Table S2, see online supplementary material). First, men had increased risk compared with women, with an estimated adjusted prevalence rate of 1.22 (95% CI 1.08–1.38). Second, individuals with well-paid jobs, good-quality housing and private insurance had a lower prevalence rate, and subsequently had a lower risk of acquiring COVID-19. Moreover, having four or more adults, or three or more children, in the family significantly increased the risk of acquiring COVID-19. Most importantly, SARS-CoV-2 infection of a household member increased the risk of acquiring infection among other household members, and had an adjusted prevalence rate of 1.39 (95% CI 1.24–1.57). Furthermore, the prevalence rate increased to 2.14 (95% CI 1.74–2.62) when a household had a confirmed death associated with COVID-19. Overall, the seroprevalence rate was significantly higher if the individual had experienced flu-like symptoms since March, and prior COVID-19 diagnosis.

### Discussion

The relative frequencies of anti-SARS-CoV-2 nucleocapsid antibodies and associated factors 6 months after the start of the pandemic in Manaus, Brazil were estimated. The results indicate high seropositivity of 29.10% (30.34% if adjusted for sensitivity and specificity of the test), including asymptomatic and symptomatic individuals. Furthermore, the analyses showed that the pandemic disproportionately affected low-income families and those with limited access to health care. The data demonstrate that living in the same household with a suspected/confirmed patient increased the risk of contracting COVID-19 enormously, questioning whether the individuals adopted any home isolation measures. Additionally, preventive self-medication was associated with higher prevalence of SARS-CoV-2 infection, probably because it is used by people at higher risk of contagion.

### Table 4

| Variables                        | Crude model<sup>a</sup> | Adjusted model<sup>b</sup> |
|----------------------------------|-------------------------|-----------------------------|
|                                  | cPR 95% CI | P-value | aPR 95% CI | P-value |
| Sex                              |            |          |            |          |
| Male                             | 1.15       | 1.00–1.33 | 0.049      | 1.22     | 1.08–1.38 |
| Age (years)                      |            |          |            |          |
| 18–29                            | 0.96       | 0.84–1.11 | 0.610      | 1.04     | 0.97–1.12 |
| 30–39                            | 1.07       | 0.87–1.31 | 0.543      | 1.16     | 0.97–1.39 |
| 40–49                            | 1.22       | 1.02–1.45 | 0.026      | 1.35     | 1.18–1.55 |
| ≥60                              | 0.91       | 0.68–1.24 | 0.566      | 1.01     | 0.81–1.26 |
| Occupation                       |            |          |            |          |
| Professional higher              | 1.22       | 0.97–1.54 | 0.086      | N/A      |          |
| Professional mid                 | 1.26       | 1.12–1.42 | <0.001     |          |          |
| Worker/informal                  | 1.15       | 0.89–1.49 | 0.297      |          |          |
| Unemployed                       | 0.78       | 0.65–0.93 | 0.006      | 0.79     | 0.66–0.94 |
| Housing                          |            |          |            |          |
| Detached house                   | 0.69       | 0.55–0.86 | 0.001      | 0.66     | 0.53–0.82 |
| Conjugated house                 | 1.07       | 0.84–1.35 | 0.603      | N/A      |          |
| Condo house                      | 0.84       | 0.71–0.98 | 0.029      |          |          |
| Apartment                        | 0.72       | 0.58–0.89 | 0.002      |          |          |
| Number of adults in residence    |            |          |            |          |
| 0                                | 1.04       | 0.87–1.25 | 0.633      | 1.00     | 0.80–1.26 |
| 1                                | 1.13       | 0.94–1.35 | 0.182      | 1.09     | 0.88–1.34 |
| 2                                | 1.37       | 1.19–1.58 | <0.001     | 1.24     | 1.02–1.51 |
| Number of children in residence  |            |          |            |          |
| 0                                | 1.10       | 0.99–1.22 | 0.081      | 1.08     | 0.96–1.20 |
| 1                                | 1.14       | 1.00–1.30 | 0.053      | 1.11     | 0.95–1.31 |
| 2                                | 1.57       | 1.30–1.89 | <0.001     | 1.28     | 1.06–1.54 |
| Private insurance                |            |          |            |          |
| No                               | 0.80       | 0.63–1.02 | 0.071      | N/A      |          |
| Yes                              | 1.78       | 1.60–1.99 | <0.001     | 1.39     | 1.24–1.57 |
| Preventive self- medication (any)|            |          |            |          |
| No                               | 2.22       | 1.49–3.32 | <0.001     | 2.14     | 1.74–2.62 |
| Yes                              | 1.49       | 1.38–1.60 | <0.001     | 1.36     | 1.27–1.46 |
| Flu-like symptoms since March    |            |          |            |          |
| No                               | 1.25       | 1.13–1.38 | <0.001     | 1.13     | 1.02–1.25 |
| Yes                              | 2.34       | 2.09–2.63 | <0.001     | 1.92     | 1.71–2.17 |

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Anti-SARS-CoV-2 nucleocapsid ELISA

A robust in-house indirect IgG ELISA using nucleocapsid protein was developed and used as a tool to estimate seropositivity. Test performance for the in-house assay was similar to other tests reported in other countries and regions (Cota et al., 2020; Deeks et al., 2020). The performance of the assay was tested using a wide range of samples, including outpatients and patients with mild symptoms, and inpatients or hospitalized patients with severe disease, to determine the sensitivity of the assay. Hence, the approach maximized the range of detection of serum IgG antibody concentrations that can be expected in an exposed heterogeneous population. The performance of the test improved markedly 14 days after symptom onset (Cota et al., 2020). Additionally, the specificity analysis included samples that were tested for other tropical diseases and infections to evaluate cross-reactivity. Pre-pandemic plasma samples positive for dengue, leptospirosis and malaria cross-reacted using the in-house assay (Cota et al., 2020; Masyeni et al., 2021). However, the authors did not have access to other (seasonal) coronavirus-positive samples, and could not evaluate cross-reactivity against the nucleocapsid proteins of other coronaviruses. In-house assays with robust validation and good performance can be a viable alternative to commercial assays in low-income countries.

Seroprevalence in Manaus

The crude seropositivity rate and the rate adjusted by test performance were high. The estimated number of affected people in this study suggests that the disease burden could be several times higher than that estimated using RT-PCR (Fundação de Vigilância em Saúde do Amazonas, 2021). In part, this difference could be due to the inclusion of asymptomatic and oligosymptomatic patients who did not usually seek medical attention or SARS-CoV-2 viral testing, and because of the lower testing rates in Brazil. A population-based household survey in the Maranhão capital region between July and August 2020 reported seroprevalence of 40.4%, which is one of the highest seroprevalence estimates in Brazil (Silva et al., 2020). A repeated cross-sectional survey observed increasing prevalence of SARS-CoV-2 antibodies in the Amazon (North) and North-east region of Brazil between May and June 2020 (Hallal et al., 2020). The high seroprevalence observed by the present authors and others in areas of Brazil characterized by higher poverty rates is in agreement with the recent evidence that COVID-19 has disproportionately affected marginalized populations, in whom the human development index is lowest (Horta et al., 2020). Buss et al. estimated an attack rate of 66% in June 2020 rising to 76% in October 2020 based on mathematical estimates of antibody decline among blood donors in Manaus (Buss et al., 2021); they assumed constant antibody decay rates towards nucleocapsid protein, which may have overstated the attack rate (Prowse et al., 2020; Buss et al., 2021). In the present study, 61.6% (154/250) of patients with a prior COVID diagnosis (either by PCR or serology) were still positive at the cohort baseline, regardless of time since symptom onset. Using patient-reported data, the antibody decay proportion for the whole cohort can be estimated as 32.31%, putting a maximum disease prevalence at 44.82%. The maximum seroprevalence estimate is high, but also suggests that a large proportion of the vulnerable population is still susceptible (Aschwanden, 2020). Therefore, it is proposed that the high proportion of susceptible individuals may explain, in part, the recent resurgence of SARS-CoV-2 infection in Manaus (Ferrante et al., 2020).

To date, the role of antibodies in controlling disease severity during infection, the duration of serological responses, and the extent to which patient antibody responses may be protective against re-exposure remain to be fully elucidated. Antibody measurements do not necessarily reflect protection after infection, nor do they fully indicate the efficacy of active immunization (Adetia et al., 2020; Fontanet and Cauchemez, 2020; McMahan et al., 2021). Therefore, inferring immunity or protection from a single biomarker, at individual or population levels, can be misleading. This limitation is acknowledged, and the serological findings and their implications should be interpreted with caution. Future studies comparing vaccine-induced immune responses with those stimulated by viral infection, and those of individuals who become re-infected, will help to clarify the immunological correlates of protection (Anderson et al., 2020).

Associated factors

It is true that male sex, older age and comorbidities are associated with higher complication rates and mortality; however, their role in acquiring the infection is less clear (Giannouchos et al., 2020; Petrakis et al., 2020). This study found that male sex was associated with higher seropositivity, which is in agreement with the results from other studies (Elmore et al., 2020). However, other population-based studies have failed to identify gender differences in the risk of SARS-CoV-2 infection (Amanat et al., 2020; Hallal et al., 2020; Pousetchi et al., 2021). This study found increasing seroprevalence with age until 50–59 years, with a marked decrease among individuals aged ≥60 years. This could be explained by increased risk of acquiring the infection with age (Elmore et al., 2020; Hallal et al., 2020; Stringhini et al., 2020), which was attenuated in the older group by a state mandate for elderly people to stay at home and stop performing presental work activities.

These data demonstrate a strong association between socioeconomic status and risk of acquiring SARS-CoV-2 infection. Additionally, it was noted that seroprevalence was lower among people living in condo houses and apartment buildings, probably because they live in closed communities with strict COVID-19 rules. Marked differences were found between different geographic areas. Seroprevalence in East Manaus, which is the most crowded and poorest area of the city, was up to 38.8%, which was almost double that in the most affluent area (South-Centre Manaus). When adjusting for all these factors, family income was the strongest factor predicting infection, with a 33% reduction in infection in higher income households compared with lower income households. Access to primary health for economically vulnerable people has always been a limiting factor worldwide; during the ongoing pandemic, this inequality has been even more evident (Bambrä et al., 2020; Hallal et al., 2020; Orellana et al., 2020). All of these studies demonstrated an undemocratic distribution of SARS-CoV-2 virus and its consequences.

Households with four or more adults, or with three or more children, had a staggering proportion of positive cases (>40%). In addition, the study data showed that having a household member diagnosed with COVID increased the chance of an individual being positive by 40%, and in the case of death of a household member, the probability of being positive was more than doubled. These findings reveal that SARS-CoV-2-positive index cases may not have followed directives on home isolation. Given that household isolation is voluntary and is not strictly enforced, many newly infected family members could spread the disease, making these houses ‘hotbeds’ for infection. A possible solution is to employ centralized isolation in government-sponsored facilities. Additionally, strict follow-up of diagnosed patients, and their family members and contacts, is recommended to reduce virus transmission.

This study found that people who self-medicated as prophylaxis had higher seroprevalence of COVID-19. Evidence suggests that most of the drugs used as prophylaxis may not be effective (Mega, 2020). Moreover, taking a preventive self-medication could
produce a false sense of safety and security from the disease, leading to the neglect of other well-established preventive measures. Non-pharmaceutical interventions have been a source of debate in Brazil, hampering the SARS-CoV-2 control efforts. Additionally, conflicting stances between the state and federal governments in Brazil on strategies to face the pandemic could have played a role in its course (Ferrante et al., 2020; Hsiang et al., 2020).

Limitations and strengths

A convenience sampling strategy was adopted instead of a population approach due to financial and logistical constraints. Sampling was based on online and university website advertising, which potentially excluded individuals who did not have access to this information; higher education and university employees were oversampled. There was only one collection centre, and this may have excluded individuals who did not have resources to travel to the study centre for recruitment or lived far from the recruitment centre. The recruited cohort may not completely represent the general population of Manaus, and may vary with health seeking and social distancing behaviour, immune response to infection and risk of disease exposure; as such, the prevalence results of the study groups and associated risk factors should be interpreted with caution and not extrapolated to the population of Manaus. Additionally, it is possible that participants enrolled in the study to find out their serological status for COVID-19, meaning they considered themselves to be at higher risk or to have had a higher percentage of flu-like symptoms since March 2020, leading to over-reporting. Therefore, the present seroprevalence estimates should be confirmed and extended by other studies, including serosurveys that use probabilistic sampling to enrol more representative populations. Regarding the in-house assay, it is acknowledged that while the nucleocapsid and spike proteins are expressed abundantly in SARS-CoV-2-infected cells and tissues, and that antibody responses towards both are highly correlated (Jiang et al., 2020; Noval et al., 2021), the in-house approach only included the nucleocapsid protein as an antigen to detect seropositivity, and this may have underestimated the true seroprevalence of SARS-CoV-2.

On the contrary, this study and analysis had numerous strengths. The study cohort was representative of both sexes, all age and economic groups, and included individuals from all administrative zones of Manaus. Additionally, clinical, pharmacological and SARS-CoV-2 testing data from both symptomatic and asymptomatic recruits were available. Data collection was performed using electronic forms with internal checks to improve the quality of the data, and there were few missing values. In addition, a highly specific and sensitive immunoassay was used. To the best of the authors’ knowledge, DETECTCoV-19 is one of the first longitudinal studies in the north of Brazil that aims to understand the epidemiology and associated risk factors of SARS-CoV-2.

Conclusion

The baseline analysis of the DETECTCoV-19 cohort revealed high seroprevalence in Manaus, and demonstrated disproportionate socio-economic disparity among the study participants. Further prospective analyses of the cohort will enable the determination of seroconversion rates over time, behavioural aspects of virus transmission, and the role of declining antibody titres and subsequent re-infection with SARS-CoV-2. In Amazonas and worldwide, socio-economic disparities, as well as inequalities in access to primary health, have amplified the impact of the COVID-19 pandemic. Governmental policies that do not consider the syndemic nature of COVID-19 will have disproportionate long-term economic, social and health consequences for those who are already disadvantaged. Taken together, mass molecular testing and contact tracing, strict enforcement of voluntary isolation rules, and non-pharmaceutical interventions are needed urgently as part of the community control measures to reduce SARS-CoV-2 transmission and its health and social impact.

Declaration of Competing Interest

None declared.

Author contributions

PL and JDBL were the principal investigators of this study and acquired the necessary funding. PL and JDBL conceived the study with input from CFC, PES, BCA, CAG and RVA. Antigen design and protein purification were performed by PL, BBS, IBC, JNSN, ENA and SAF. Sample collection was led by JDBL with assistance from CFC, PES and BCA. The laboratory set-up and sample processing were coordinated by PL and BBS. BBS, IPVF, DSSS, TBNN, MFJ, ARCB, ROS, NOC and WBSS processed blood samples, performed laboratory testing, collected data and approved the test results, supervised by PL. PL, BBS and JDBL coordinated data acquisition and data management. Data were cleaned and prepared by PL, BBS and RVA. Statistical analyses and data visualization were performed by PL and CAG, and RVA led the statistical analyses. PL, JDBL, CAG and RVA wrote the manuscript. All authors revised and approved the final version of this manuscript.

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Ethical approval

The Research Ethics Committee of Federal University of Amazonas approved this study (CAAE:34906920.4.0000.5020) in accordance with Brazilian law, and the Declaration of Helsinki. All participants gave oral and written consent prior to enrolment.

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