Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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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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ABSTRACT

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 health care. 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 Amazona, 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-pharmacological 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).

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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 Amazônia server. Details of blood collection, sample

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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-operator variations were assessed throughout the development of the assay, before the analysis of study samples. The sensibility 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 Fisherś 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-medication, 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 symptomatic 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-medication 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 χ^2 test	IgG positive and symptoms[§]	Prevalencerow %	*P*-value χ^2 test
Total		3046		886	29.10		725	23.84	
Demographic structure									
Sex	Female	1852	60.80	508	27.43	0.012	427	23.08	0.220
	Male	1194	39.20	378	31.68		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	2694	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	32.28	<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	
	2	345	11.51	108	31.30		86	24.93	
	≥3	114	3.80	49	42.98		33	28.95	
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	30.08		130	24.44	

IgG, immunoglobulin G.
[a]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	Prevalencerow %	*P*-valueχ^2 test	IgG positive and symptoms[b]	Prevalencerow %	*P*-valueχ^2 test
Total		3046		886	29.10		725	23.84	
Private insurance	No	1235	40.68	408	33.04	<0.001	329	26.64	0.003
	Yes	1801	59.32	476	26.44		395	21.94	
Last influenza vaccine	2020	1441	47.53	398	27.62	0.272	326	22.62	0.481
	2019	698	23.02	220	31.52		175	25.07	
	Prior to 2019	586	19.33	170	29.01		142	24.23	
	Never	307	10.13	94	30.72		79	25.82	
Risk factors									
Family members with COVID-19[c]	No	1305	43.01	377	28.91	0.346	299	22.93	0.264
	Yes, alive	1395	45.98	419	30.04		351	25.16	
	Yes, died	334	11.01	87	26.05		73	21.86	
Household members with COVID-19	No	2428	80.37	611	25.18	<0.001	486	20.02	<0.001
	Yes, alive	568	18.80	255	44.89		224	39.44	
	Yes, died	25	0.83	14	56.00		11	44.00	
Pregnancy	No	3030	99.47	884	29.18	0.175[a]	723	23.90	0.387[a]
	Yes	16	0.53	2	12.5		2	12.50	
Comorbidities (any)	No	1995	65.58	593	29.74	0.286	478	23.97	0.815
	Yes	1047	34.42	292	27.89		247	23.59	
Which comorbidities	Asthma	198	18.91	58	29.29	0.896	50	25.25	0.688
	Diabetes	186	17.77	64	34.41	0.184	51	27.42	0.294
	Hypertension	447	42.69	131	29.31	0.856	111	24.83	0.701
	Obesity	201	19.20	56	27.86	0.578	49	24.38	0.898
	Cardiopathy	60	5.73	18	30.00	0.965	16	26.67	0.630
	Cancer	17	1.62	5	29.41	0.977	4	23.53	1.000[a]
	Other	257	24.55	61	23.74	0.046	55	21.40	0.361
Preventive self-medication (any)	No	2293	75.38	596	25.99	<0.001	460	20.06	<0.001
	Yes	749	24.62	289	38.64		265	35.43	
Which medication	Nitazoxanide	28	3.74	7	25.00	0.905	7	25.00	0.517
	Azithromycin	161	21.50	67	41.88	<0.001	65	40.63	<0.001
	Hydroxy/chloroquine	10	1.34	5	50.00	0.085	5	50.00	0.019
	Corticosteroids	33	4.41	14	43.75	0.023	13	40.63	0.004
	Ivermectin	268	35.78	83	31.09	0.074	73	27.34	0.006
	Paracetamol	268	35.78	121	45.15	<0.001	116	43.28	<0.001
	Other	260	34.71	104	40.00	<0.001	96	36.92	<0.001

IgG, immunoglobulin G.
[a] Fisherś 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	Prevalencerow %	*P*-value χ^2 test	IgG positive and symptoms[b]	Prevalencerow %	*P*-value χ^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	–	–	
	Yes	522	17.39	159	30.46				
Flu-like symptoms since March (any)	No	685	22.52	163	23.80	0.001	–	–	
	Yes	2357	77.48	722	30.65				
Which symptoms	Anosmia	525	22.27	341	64.95	<0.001	–	–	
	Body aches	971	41.20	372	38.35	<0.001	–	–	
	Chest pain	337	14.30	138	41.07	<0.001	–	–	
	Chills	343	14.55	156	45.48	<0.001	–	–	
	Conjunctivitis	61	2.59	19	31.15	0.200	–	–	
	Cough	842	35.72	304	36.10	<0.001	–	–	
	Diarrhoea	655	27.79	206	31.45	0.002	–	–	
	Fever	717	30.42	318	44.41	<0.001	–	–	
	Headache	1633	69.28	508	31.13	<0.001	–	–	
	Lack of appetite	339	14.38	178	52.66	<0.001	–	–	
	Palpitations	239	10.14	76	31.80	0.015	–	–	
	Shortness of breath	487	20.66	175	35.93	<0.001	–	–	
	Side chest pain	612	25.97	224	36.66	<0.001	–	–	
	Skin rashes	130	5.52	45	34.62	0.009	–	–	
	Sore throat	1049	44.51	315	30.06	0.004	–	–	
	Vomiting	140	5.94	44	31.43	<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	71.19		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[c]	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	Nitazoxanide	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] Fisherś 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.

Table 4
Crude and adjusted regression models for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) immunoglobulin G positivity in Manaus, Amazonas, Brazil.

Variables		Crude model[a]			Adjusted model[b]		
		cPR	95% CI	*P*-value	aPR	95% CI	*P*-value
Sex	Female	Ref			Ref		
	Male	1.15	1.00–1.33	0.049	1.22	1.08–1.38	0.001
Age (years)	18–29	Ref			Ref		
	30–39	0.96	0.84–1.11	0.610	1.04	0.97–1.12	0.256
	40–49	1.07	0.87–1.31	0.543	1.16	0.97–1.39	0.106
	50–59	1.22	1.02–1.45	0.026	1.35	1.18–1.55	<0.001
	≥60	0.91	0.68–1.24	0.566	1.01	0.81–1.26	0.926
Occupation	Professional higher	Ref					
	Professional mid	1.22	0.97–1.54	0.086	N/A		
	Worker/informal	1.26	1.12–1.42	<0.001			
	Unemployed	1.15	0.89–1.49	0.297			
Family income	0–3 minimum wages	Ref			Ref		
	4–6 minimum wages	0.78	0.65–0.93	0.006	0.79	0.66–0.94	0.009
	>6 minimum wages	0.69	0.55–0.86	0.001	0.66	0.53–0.82	<0.001
Housing	Detached house	Ref					
	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	1	Ref			Ref		
	2	1.04	0.87–1.25	0.633	1.00	0.80–1.26	0.981
	3	1.13	0.94–1.35	0.182	1.09	0.88–1.34	0.448
	4+	1.37	1.19–1.58	<0.001	1.24	1.02–1.51	0.032
Number of children in residence	0	Ref			Ref		
	1	1.10	0.99–1.22	0.081	1.08	0.96–1.20	0.194
	2	1.14	1.00–1.30	0.053	1.11	0.95–1.31	0.194
	3+	1.57	1.30–1.89	<0.001	1.28	1.06–1.54	0.009
Private insurance	No	Ref					
	Yes	0.80	0.63–1.02	0.071	N/A		
Household members with COVID-19	No	Ref			Ref		
	Yes, alive	1.78	1.60–1.99	<0.001	1.39	1.24–1.57	<0.001
	Yes, died	2.22	1.49–3.32	<0.001	2.14	1.74–2.62	<0.001
Preventive self- medication (any)	No	Ref			Ref		
	Yes	1.49	1.38–1.60	<0.001	1.36	1.27–1.46	<0.001
Flu-like symptoms since March	No	Ref			Ref		
	Yes	1.25	1.13–1.38	<0.001	1.13	1.02–1.25	0.018
Prior COVID-19 diagnosis	No	Ref			Ref		
	Yes	2.34	2.09–2.63	<0.001	1.92	1.71–2.17	<0.001

COVID-19, coronavirus disease 2019; cPR, crude prevalence ratio; aPR, adjusted prevalence ratio; CI, confidence interval; Ref, reference value (1.00).
[a] Poisson regression model with robust variance corrected per cluster (administrative areas).
[b] Poisson regression model with robust variance corrected per cluster (administrative areas) and adjusted for sex, age, family income, number of adults in household, number of children in household, presence of household members with COVID-19, use of preventive self-medication, presence of flu-like symptoms since March, and prior COVID-19 diagnosis.

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 household 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 2020 or had a prior diagnosis of COVID-19 (Table 4).

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.

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 sensibility 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 Amazona, 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 overestimated 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 (Addetia 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; Poustchi 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 presential 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 (Bambra 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, IVPF, DSSS, TBNM, 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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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2021.07.017.

References

Addetia A, Crawford KHD, Dingens A, Zhu H, Roychoudhury P, Huang M-L, et al. Neutralizing antibodies correlate with protection from SARS-CoV-2 in humans during a fishery vessel outbreak with a high attack rate. J Clin Microbiol 2020;58:e02107–20.

Amanat F, Stadlbauer D, Strohmeier S, Nguyen THO, Chromikova V, McMahon M, et al. A serological assay to detect SARS-CoV-2 seroconversion in humans. Nat Med 2020;26:1033–6. doi:10.1038/s41591-020-0913-5.

Anderson RM, Vegvari C, Truscott J, Collyer BS. Challenges in creating herd immunity to SARS-CoV-2 infection by mass vaccination. Lancet 2020;396:1614–16.

Aragona M, Barbato A, Cavani A, Costanzo G, Mirisola C. Negative impacts of COVID-19 lockdown on mental health service access and follow-up adherence for immigrants and individuals in socio-economic difficulties. Public Health 2020;186:52–6.

Aschwanden C. The false promise of herd immunity for COVID-19. Nature 2020;587:26–8.

Bambra C, Riordan R, Ford J, Matthews F. The COVID-19 pandemic and health inequalities. J Epidemiol Community Health 2020;74:964–8.

Bo Y, Guo C, Lin C, Zeng Y, Li HB, Zhang Y, et al. Effectiveness of non-pharmaceutical interventions on COVID-19 transmission in 190 countries from 23 January to 13 April 2020. Int J Infect Dis 2021;102:247–53.

Buss LF, Prete Jr CA, Abrahim CMM, Mendrone Jr A, Salomon T, de Almeida-Neto C, et al. Three-quarters attack rate of SARS-CoV-2 in the Brazilian Amazon during a largely unmitigated epidemic. Science 2021;371:288–92.

Chia WN, Zhu F, Ong SWX, Young BE, Fong S-W, Le Bert N, et al. Dynamics of SARS–CoV-2 neutralising antibody responses and duration of immunity: a longitudinal study. Lancet Microbe 2021;2:e240–e2e9.

Cota G, Freire ML, de Souza CS, Pedras MJ, Saliba JW, Faria V, et al. Diagnostic performance of commercially available COVID-19 serology tests in Brazil. Int J Infect Dis 2020;101:382–90.

Deeks JJ, Dinnes J, Takwoingi Y, Davenport C, Spijker R, Taylor-Phillips S, et al. Antibody tests for identification of current and past infection with SARS-CoV-2. Cochrane Database Syst Rev 2020;6.

Diggle PJ. Estimating prevalence using an imperfect test. Epidemiol Res Int 2011;2011.

Elmore R, Schmidt L, Lam J, Howard BE, Tandon A, Norman C, et al. Risk and protective factors in the COVID-19 pandemic: a rapid evidence map. Front Public Health 2020;8.

Fauci AS, Morens DM. The perpetual challenge of infectious diseases. N Engl J Med 2012;366:454–61.

Ferrante L, Steinmetz WA, Almeida ACL, Leão J, Vassão RC, Tupinambás U, et al. Brazil's policies condemn Amazonia to a second wave of COVID-19. Nat Med 2020;26:1315.

Fontanet A, Cauchemez S. COVID-19 herd immunity: where are we? Nat Rev Immunol 2020;20:583–4.

Fundação de Vigilância em Saúde do Amazonas, Manaus, Amazonas, Brazil. Dados epidemiológicos e financeiros das ações de combate à COVID-19. FVS; 2021.

Giannouchos TV, Sussman RA, Mier JM, Poulas K, Farsalinos K. Characteristics and risk factors for COVID-19 diagnosis and adverse outcomes in Mexico: an analysis of 89,756 laboratory-confirmed COVID-19 cases. Eur Respir J 2020. doi:10.1183/13993003.02144-2020.

Hallal PC, Hartwig FP, Horta BL, Silveira MF, Struchiner CJ, Vidaletti LP, et al. SARS–CoV-2 antibody prevalence in Brazil: results from two successive nationwide serological household surveys. Lancet Glob Health 2020;8:e1390–e13e8.

Horta BL, Silveira MF, Barros AJD, Barros FC, Hartwig FP, Dias MS, et al. Prevalence of antibodies against SARS-CoV-2 according to socioeconomic and ethnic status in a nationwide Brazilian survey. Rev Panam Salud Publica 2020;44:e135.

Hsiang S, Allen D, Annan-Phan S, Bell K, Bolliger I, Chong T, et al. The effect of large-scale anti-contagion policies on the COVID-19 pandemic. Nature 2020;584:262–7.

Jiang H-W, Li Y, Zhang H-N, Wang W, Yang X, Qi H, et al. SARS-CoV-2 proteome microarray for global profiling of COVID-19 specific IgG and IgM responses. Nat Commun 2020;11:3581.

Kraemer MUG, Yang C-H, Gutierrez B, Wu C-H, Klein B, Pigott DM, et al. The effect of human mobility and control measures on the COVID-19 epidemic in China. Science 2020;368:493–7.

Masyeni S, Santoso MS, Widyaningsih PD, Asmara DGW, Nainu F, Harapan H, et al. Serological cross-reaction and coinfection of dengue and COVID-19 in Asia: experience from Indonesia. Int J Infect Dis 2021;102:152–4.

McMahan K, Yu J, Mercado NB, Loos C, Tostanoski LH, Chandrashekar A, et al. Correlates of protection against SARS-CoV-2 in rhesus macaques. Nature 2021;590:630–4.

Mega ER. Latin America's embrace of an unproven COVID treatment is hindering drug trials. Nature 2020;586:481–2.

Ministério da Saúde. Painel de casos de doença pelo coronavírus 2019 (COVID-19). Brasil: Ministério da Saúde; 2021 Available at https://covid.saude.gov.br/ .

Noval MG, Kaczmarek ME, Koide A, Rodriguez-Rodriguez BA, Louie P, Tada T, et al. Antibody isotype diversity against SARS-CoV-2 is associated with differential serum neutralization capacities. Sci Rep 2021;11:5538.

Orellana JDY, Cunha GMD, Marrero L, Horta BL, Leite IDC. Explosion in mortality in the Amazonian epicenter of the COVID-19 epidemic 19. Cad Saude Publ 2020;36 e00120020.

Petrakis D, Margină D, Tsarouhas K, Tekos F, Stan M, Nikitovic D, et al. Obesity – a risk factor for increased COVID–19 prevalence, severity and lethality. Mol Med Rep 2020;22:9–19.

Poustchi H, Darvishian M, Mohammadi Z, Shayanrad A, Delavari A, Bahadorimonfared A, et al. SARS-CoV-2 antibody seroprevalence in the general population and high-risk occupational groups across 18 cities in Iran: a population-based cross-sectional study. Lancet Infect Dis 2021;21:473–81.

Prowse TAA, Purcell T, Baía-da-Silva DC, Sampaio V, Monteiro WM, Wood J, et al. Inferred resolution through herd immmunity of first COVID-19 wave in Manaus, Brazilian Amazon; 2020 medRxiv2020.09.25.20201939.

Silva A, Lima-Neto LG, Azevedo C, Costa L, Braganca M, Barros Filho AKD, et al. Population-based seroprevalence of SARS-CoV-2 and the herd immunity threshold in Maranhao. Rev Saude Publ 2020;54:131.

Stringhini S, Wisniak A, Piumatti G, Azman AS, Lauer SA, Baysson H, et al. Seroprevalence of anti-SARS-CoV-2 IgG antibodies in Geneva, Switzerland (SERO-CoV-POP): a population-based study. Lancet 2020;396:313–19.

Yang Y, Wang X, Du R-H, Zhang W, Si H-R, Zhu Y, et al. Serological investigation of asymptomatic cases of SARS-CoV-2 infection reveals weak and declining antibody responses. Emerg Microb Infect 2021;10:905–12.