Title: Seroprevalence of IgG and IgM anti-SARS-CoV-2 among voluntary blood donors in Rio de Janeiro, Brazil

Running title: Seroprevalence of anti-SARS-CoV-2 among blood donors.

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Abstract

Background: In Brazil, mathematical models for deriving estimates and projections of COVID-19 cases have been developed without data on asymptomatic SARS-CoV-2 infection. We estimated the seroprevalence of antibodies to SARS-CoV-2 among blood donors in the State of Rio de Janeiro.

Methods: Data were collected on 2,857 blood donors from April 14 to 27, 2020. We report the crude prevalence of antibodies to SARS-CoV-2, the weighted prevalence by the total state population, and adjusted prevalence estimates for test sensitivity and specificity. To establish the correlates of SARS-CoV-2 prevalence, we used logistic regression models. The analysis included period and site of blood collection, sociodemographic characteristics, and place of residence.

Results: The proportion of SARS-Cov-2 positive tests without any adjustment was 4.0% (95% CI 3.3-4.7%), and the weighted prevalence was 3.8% (95% CI 3.1-4.5%). Further adjustment by test sensitivity and specificity produced lower estimates, 3.6% (95% CI 2.7-4.4%) and 3.3% (95% CI 2.6-4.1%), respectively. The variable most significantly associated with the crude prevalence was the period of blood collection: the later the period, the higher the prevalence. Regarding socio-demographic characteristics, the younger the blood donors, the higher the prevalence, and the lower the educational level, the higher the odds of a positive SARS-Cov-2 antibody. Similar results were found for the weighted prevalence.

Discussion: Although our findings resulted from a convenience sample, they match some basic premises: the increasing trend over time, since the epidemic curve in the state is still on the rise; the higher prevalence among the youngest who are more likely to circulate; and the higher prevalence among the less educated as they have more difficulties in following the social distancing recommendations. Despite the study limitations, it is possible to infer that protective levels of natural herd immunity to SARS-CoV-2 are far from being reached in Rio de Janeiro.

Key words: COVID-19, Blood donors, Prevalence, Serology, SARS-CoV-2

INTRODUCTION

In December 2019, cases of severe pneumonia of unknown origin were reported in Wuhan, China; since the report of the first case, the outbreak has gradually spread across the country and worldwide in a short period. The causal agent was a betacoronavirus - SARS-CoV-2 - which elicits a severe acute respiratory syndrome / SARS, named COVID-19 [1].

Infection and disease spread rapidly, reaching virtually every country in the world. By the end of the first week of May 2020, more than 3.8 million cases have been confirmed, with around 260,000 deaths worldwide [2]. In Brazil, as of May 6th, more than 125,000 cases were confirmed, with 8,536 deaths and a lethality rate of around 7% [3]. The first case in the State of Rio de Janeiro was identified on March 1st, 2020; as of May 6th, 13,295 cases were notified, with 1,205 deaths and a 9.1% lethality rate [3].

The infection can cause mild symptoms, which usually start with cough, muscle pain, and anosmia, progressing to high fever, pneumonia, with severe lung involvement [4] and, in some cases, to death [5, 6, 7]. However, the majority of the infections can evolve with few or no symptoms, representing a significant challenge to prevent disease dissemination, since asymptomatic people might be a substantial source of transmission [8].

The quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) is the gold standard for virus detection and COVID-19 confirmation [9]. Some studies show a high prevalence of false-negative tests because factors such as type of biological sample, inadequate sample collection, fluctuation of viral load and time between collection and the onset of symptoms can influence the test outcome [10]. In this context, it is essential to perform serological tests, either to investigate the presence of acute-phase antibodies (IgM) or memory antibodies (IgG). Due to the need for quick results, a simple, sensitive and specific test is essential, with immediate and accurate results to promptly identify patients who have been infected with SARS-CoV-2 to facilitate the control of viral transmission and ensure timely public health interventions [11].

Knowing the prevalence of SARS-CoV-2 in asymptomatic people is necessary for two major reasons. First, healthy people in epidemic areas may be infected and show no symptoms but might be significant sources of transmission. Indeed, at the beginning of the epidemic in China, about 86% of infections were not detected, but they were the source of infection for about 79% of cases [8]. Second, herd immunity is an essential indicator of the spread of the infection in a community. Monitoring the level of herd immunity might be important as a reference for guiding the future decisions on when would be safe to start relaxing social distancing recommendation, minimizing the possibility of subsequent epidemic outbreaks [12].

Despite this necessity, there are few studies on the seroprevalence of SARS-CoV-2 in asymptomatic populations. A major relevant study about SARS-COV-2 in asymptomatic people is the report from the Diamond Prince cruise ship. After an outbreak during the cruise, Japanese health authorities tested 3,063 passengers by RT-PCR and estimated at 17.9% the asymptomatic proportion among all infected cases [13]. A study in the county of Santa Clara, California, USA, found a 2.8% seroprevalence of SARS-CoV-2, after adjusting for sensitivity and specificity of the test and population demographics [14].

Assessing the prevalence trends of viral infections among blood donors is not only essential to estimate the effectiveness of blood safety strategies, but also to improve current strategies to increase transfusion safety, minimizing the potential risk of Coronavirus virus by blood transfusion [15]. Besides, determining the SARS-CoV-2 prevalence in blood donors can be a way of monitoring the circulation of the virus in healthy people, thus helping to implement strategies to minimize the risk of transmission, especially in the absence of seroprevalence surveys. Nevertheless, there exist few studies on the prevalence among blood donors. Two yet unpublished studies found seroprevalence among blood donors of 1.7% and 2.7% in Denmark and the Netherlands, respectively [16, 17].

In the last two weeks of April 2020, we conducted a seroprevalence survey in voluntary blood donors of Hemorio, the main blood center in Rio de Janeiro State, Brazil. In this manuscript we report the prevalence of antibodies to SARS-CoV-2 based on a sample of 2,857 voluntary blood donors, adjusting for sex and age group to supply information to health authorities for estimates, extrapolations, and health interventions. To date, this is the first study in Latin America addressing the SARS-CoV-2 seroprevalence in asymptomatic blood donors.

METHODS

Study design

Cross-sectional study, consisting of serological testing in voluntary blood donors, with analysis of sociodemographic data - age, sex, donation site (fixed site or mobile vans) - education level and place of residence.

Demographic data were obtained from the blood center´s donor management software (SACS), through a code, without donor identification. The study population corresponds to the total number of people who donated blood in the Hemorio from April 14^{th} through April 27^{th}.

Study subjects

In Brazil, before donating blood, candidates should fill in a written questionnaire and go through a brief medical screening. To be accepted as blood donors in Hemorio, candidates should comply with the Brazilian Ministry of Health and American Association of Blood Banks criteria for donor eligibility [18]. Among these, some have been recently included, all of them related to COVID-19: prospective donors could not have had flu-like symptoms in the 30 days prior to donation; they must not have had close contact with suspected or confirmed COVID-19 cases in the 30 days before donation; they must not have traveled abroad in the last 30 days. Candidates with fever – forehead temperature >37.8^{o}C - on the day of donation are also refused. Therefore, everyone accepted to donate blood had no symptoms of COVID-19 and no known epidemiological history of the disease.

Once they are accepted and donate blood, they are automatically included in the study, provided they agree to sign the informed consent for blood donation and for testing for other pathogens not included in the mandatory list of infectious diseases markers to be tested in all blood donations in Brazil. Blood donation and sample collections were performed at a fixed site – Hemorio central facility – or through mobile collections, in churches and private condominiums, in the Rio de Janeiro area.

The study was approved by the research ethics committee of the Hemorio – (approval number: 4.008.095).

Inclusion criteria: All donors classified as eligible for donation during the study period participated in the survey.

Exclusion Criteria: Refusal to sign the Informed Consent Form for blood donation and testing.

Sample collection and testing methods

Sample collection

Serum obtained from the samples used for infectious disease markers tests were also used for SARS-Cov-2 antibody tests. We collected and barcoded those samples for each blood donor at the beginning of the blood donation process.

Antibody testing

For the detection of IgG and IgM anti-SARS-CoV-2 antibodies, we used the rapid test MedTest Coronavirus 2019-nCoV IgG/IgM, from MedLevensohn manufacturer (Yuhang District, China), which is an immunochromatographic assay and uses a combination of particles coated with SARS-COV-2 antigen for the qualitative detection of IgG and IgM antibodies. The MedTest Coronavirus (COVID-19) IgG / IgM was licensed by the National Agency of Sanitary Vigilance/Anvisa in March 2020 (https://consultas.anvisa.gov.br/#/saude/q/?numeroRegistro=80560310056), and it can detect SARS-CoV-2 antibodies in whole blood, capillary blood, serum, and plasma samples. We performed the tests in serum according to the manufacturer's instructions.

Real Time Polymerase Chain Reaction (RT-PCR tests)

Serum or plasma from antibody-positive samples (IgM, IgG, or IgG + IgM) was tested for the detection of SARS-CoV-2 by qRT-PCR - (Molecular IDT IntegratedDNA TechnologiesSARS-CoV-2 – N1/N2/P, Promega, Madison, USA).

We performed the tests according to the manufacturer´s instructions, using the MDX Instrument from Qiagen (Hilden, Germany) for RNA extraction and Applied Biosystem MDX thermocycler instrument, from Thermo-Fisher (Waltham, USA).

Specificity Estimates: For estimating the MedTest specificity, we tested 100 plasma samples from Hemorio´s blood donor repository, collected in the year 2018, long before the new Coronavirus pandemic. All the donors tested negative for infectious disease markers required for blood donation. We also tested 20 donor samples from 2018; those samples were all positive for one marker of infection (anti-HIV, anti-HCV, HBsAg).

Statistical analysis

The data were stored in an Excel® spreadsheet with demographic and donors code so that it is not possible to identify each donor individually.

Our estimation of the population prevalence of COVID-19 proceeded in three steps. We first reported the crude proportion of positive tests without any adjustment. Second, we estimated the weighted prevalence using the 2020 Rio de Janeiro State population. This adjustment is necessary to balance our sample according to the state population distribution of sex and age. Third, we adjusted the prevalence for test sensitivity and specificity using the manufacturer's estimates of 85% and 99%, respectively. The true or adjusted prevalence and its 95% confidence interval were estimated using a previously published approach [19].

For the statistical analysis, we considered two outcomes: the unadjusted and weighted prevalence of the SARS-Cov-2 antibody test. Also, the following variables were included in the analysis: sex, age group (18-29; 30-49; 50+), blood donation site (Hemorio, churches, condominiums), education level (no college education; college education) and place of residence (capital; other cities in the State of Rio de Janeiro). To investigate a possible increasing trend, the test dates were aggregated in three time periods (April 14th to 18th; April 19th to 23rd; April 24th to 27th).

To establish the correlates of SARS-CoV-2 infection, we used logistic regression models and the odds ratio (OR) as the measure of association. Statistical tests at the 5% significance level were used to test associations of SARS-Cov-2 antibody (IgG, IgM or IgG+IgM) prevalences with the blood donors' characteristics (sex, age group, educational level, place of residence, donation site and period of donation).

Statistical analysis was performed using STATA version 13 (STATA Corp., College Station, Texas, USA).

RESULTS

Test specificity

One out of 120 true negative samples tested positive for IgM; this positive sample came from a confirmed anti-HCV+ donor. Based on these results, our estimates of specificity were 99.16%, which is in accordance with the manufacturer´s estimate specificity (95 CI 95.0% - 99.8%). All samples tested negative for IgG.

Antibody testing

There were 2,857 blood donors included in the study. All of them were tested for IgG and IgM anti-SARS-CoV-2 rapid test. The overall antibody prevalence was 4%; the tables 1, 2 and 3 show these results in detail.

Regarding the type of detected antibody, IgM-only represented 23.7% of positive cases; IgG-only represented 11.4% and IgM+IgG was detected in 64.9%. The figure 1 shows the specific prevalence rates according to time period (April 14-18th, April 19-23rd, and April 24-27th).

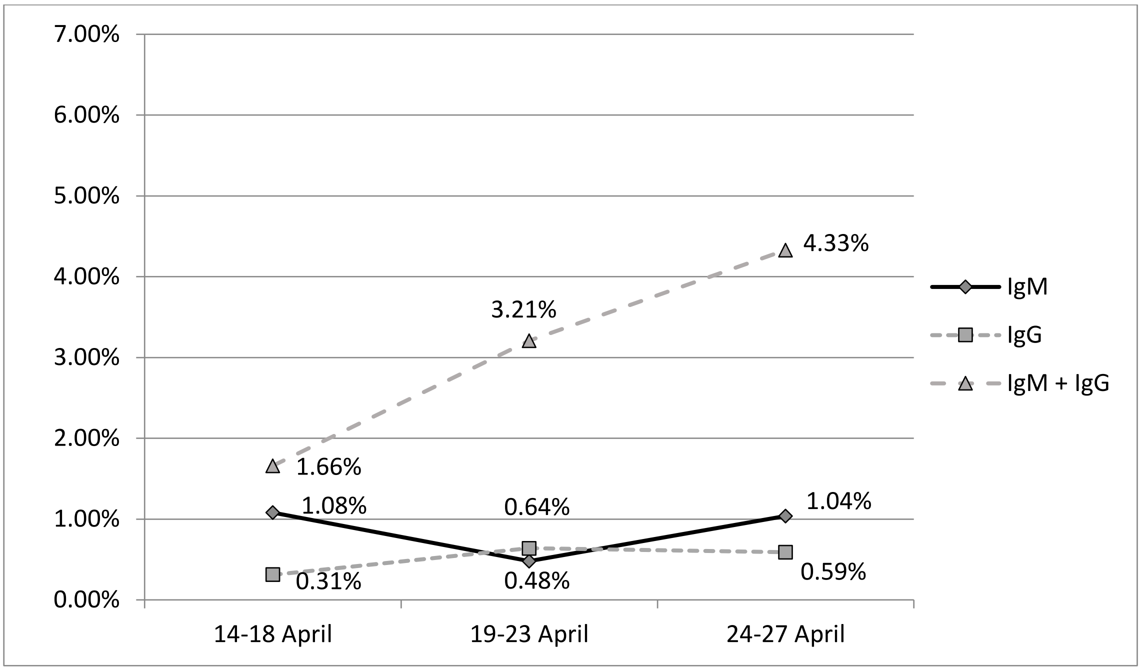

Figure 1: Prevalence by period according to the type of antibody detected

Table 1 shows four prevalence estimates. The proportion of SARS-Cov-2 positive tests without any adjustment (crude prevalence) was 4.0% (95% CI 3.3-4.7%). The weighted prevalence by the Rio de Janeiro State population was slightly lower (3.8%; 95% CI 3.1-4.5%). Further adjustment by the test sensitivity and specificity produced even lower estimates when compared to the crude prevalence: 3.6% (95% CI 2.7-4.4%) for the non-weighted prevalence and 3.3 (95% CI 2.6-4.1%) for the weighted prevalence.

In the logistic regression analyses (Table 2), some of the covariates were significantly associated with the crude SARS-Cov-2 antibody prevalence. The variable most significantly associated to the crude prevalence was the period of blood collection: the later the period, the higher the prevalence. The odds of a positive SARS-Cov-2 antibody in the third period was 2 times greater than in the first period (OR=2.05; 95% CI 1.33-3.16). Regarding socio-demographic characteristics, the younger the blood donors the higher the prevalence; furthermore, the lower the educational level the higher the odds of a positive SARS-Cov-2 antibody. No statistically significant difference was found for sex nor for municipality of residence (capital or elsewhere). The site of the blood collection also showed a significant association with the crude prevalence: the blood donors from condominiums show a significantly lower prevalence when compared to blood donors from Hemorio.

Similar results were found for the weighted SARS-Cov-2 antibody prevalence. The same variables found to be significantly associated to the crude prevalence were also significantly associated with the weighted prevalence. However, weighting of the sample resulted in more accentuated statistical significance for the 18-29 age group (OR=1.86; 95% CI 1.12-3.08%); for lower education level (OR=2.11; 95% CI 1.35-3.28); and for condominium donors (OR=0.45; 95% CI 0.23-0.86%) as compared to Hemorio blood donor. Time period was also significantly associated to the weighted prevalence ($p<0.005$), nevertheless the OR was a little higher for the crude prevalence.

Table 1: Seroprevalence of antibodies to SARS-Cov-2 among blood donors estimates according to the adjustment method. Rio de Janeiro State, Brazil, April 14-27, 2020

Estimates	**Sample size**	**Prevalence (%)**	**95%CI**
Unadjusted	2,857	4.0	3.3-4.7
Weighted* by Rio de Janeiro State Population	2,857	3.8	3.1-4.5
Adjusted by sensitivity and specificity	2,857	3.6	2.7-4.4
Weighted* estimate adjusted by sensitivity and specificity	2,857	3.3	2.6-4.1

*Weighted according to Rio de Janeiro State Population aged 18-69 years by age group and sex.

Table 2: Unadjusted seroprevalence of antibodies to SARS-Cov-2 among blood donors according to donor characteristics. Rio de Janeiro State, Brazil, April 14-27, 2020

Variables		**Sample size**	**Prevalence (%)**	**OR**	**95%CI**	**p-value**
Sex	M	1,450	4.2	1.12	0.77-1.63	NS
	F	1,407	3.8	1.00	-	-
Age group	18-29	870	5.2	1.80	1.01-3.22	0.047
	30-49	1,443	3.7	1.26	0.71-2.22	NS
	50-69	544	2.9	1.00	-	-
Education Level	No college education	1,753	4.7	1.72	1.13-2.62	0.011
	College education	1,104	2.8	1.00	-	-
Time Period	April, 14-18	1,565	3.0	1.00	-	-
	April 19-23	623	4.3	1.46	0.90-2.37	NS
	April, 24-27	669	6.0	2.05	1.33-3.16	0.001
Place of Residence	Capital	2,090	3.8	0.86	0.57-1.29	NS
	Other municipalities	767	4.4	1.00	-	-
Blood donation site	Churches	820	3.8	0.81	0.53-1.24	NS
	Condominiums	466	2.1	0.45	0.23-0.88	0.019
	HEMORIO	1,571	4.6	1.00	-	-

Table 3: Weighted* seroprevalence of antibodies to SARS-Cov-2 among blood donors according to donor characteristics. Rio de Janeiro State, Brazil, April 14-27, 2020

Variables		**Sample size**	**Prevalence (%)**	**OR**	**95%CI**	**p-value**
Sex	M	1,387	4.1	1.20	0.82-1.76	NS
	F	1,470	3.5	1.00	-	-
Age group	18-29	718	5.3	1.86	1.12-3.08	0.015
	30-49	1,199	3.6	1.26	0.77-2.04	NS
	50-69	940	2.9	1.00	-	-
Education Level	No college education	1,722	4.8	2.11	1.35-3.28	0.001
	College education	1,135	2.3	1.00	-	-
Time Period	April, 14-18	1,549	2.8	1.00	-	-
	April 19-23	624	4.5	1.60	0.98-2.58	NS
	April, 24-27	684	5.3	1.91	1.22-2.99	0.005
Place of Residence	Capital	2,110	3.7	0.92	0.60-1.41	NS
	Other municipalities	747	4.0	1.00	-	-
Blood donation site	Churches	800	3.6	0.80	0.51-1.24	NS
	Condominiums	515	2.1	0.45	0.23-0.86	0.016
	HEMORIO	1,542	4.5	1.00	-	-

*Weighted according to Rio de Janeiro State Population aged 18-69 years by age group and sex.

qRT-PCR tests

We tested all the antibody-positive samples - IgG and/or IgM – by PCR, and we did not find any PCR-positive test among those samples.

DISCUSSION

In this survey of SARS-CoV-2 antibodies among Brazilian blood donors, we found a seroprevalence of 3.3% (CI: 2.6-4.1) adjusted for sensitivity and specificity of the test and weighted according to Rio de Janeiro State population aged 18-69 years by age group and sex. This estimate is higher than that observed in two seroprevalence surveys among blood donors performed in Denmark and the Netherlands (1.7% and 2.7%, respectively) [16, 17]. We also found a substantial variation of prevalence among subgroups, with significantly higher values among the youngest, and those with no college education. It is worth noting that we found an increasing linear trend in the prevalence along the three weeks of the study (2.8%, 4.5%, and 5.3%, respectively, $p<0.01$), mainly due to the increase in IgG + IgM antibodies.

After two months since the identification of the first COVID-19 case in the State of Rio de Janeiro, more than thirteen thousand confirmed cases and a thousand deaths had been reported [3]. Despite the implementation of a wide-range of measures aiming at restricting the social interaction of people and improving the diagnostic capacity in the early weeks of March [20], the epidemic curve is still on the rise with an imminent collapse of hospital services for COVID-19 care [21].

In this context, issues concerning if and when such suppression measures should be lifted or strengthened have been on the center of the debate among public health researchers and professionals, health authorities, and the community. One of the possible indicators for guiding such a decision is the level of herd immunity that the population could achieve. Levels around 60% have been considered based on the available estimates of the basic reproduction number of the infection [22]. Since there is no available vaccine against COVID-19, such a level of herd immunity would have to be attained by natural infection. However, in settings such as Rio de Janeiro, in which a forthcoming breakdown of the health care system is soon expected, fostering natural herd immunity is not a reasonable option since it would require relaxing the suppression measures in course and, consequently, increase the number of deaths by COVID-19. On the other hand, the effectiveness and duration of suppression measures will decrease the capacity of building herd immunity and will create difficulties in the implementation of exit strategies and increase the possibility of future new epidemic waves [23].

The results of our study indicate that the aim of building an effective level of herd immunity would be challenging to be achieved in the short term. Therefore, any relaxing of social distancing recommendations might be unwise in the immediate horizon and would have to be carefully considered in the future, taking into consideration the degree of availability of hospital infrastructure, in particular ICU beds and ventilators, to provide adequate care for severe COVID-19 patients. Besides, it is not clear whether the antibody response provides the necessary neutralizing effect for avoiding a new infection [24]. If just a fraction of individuals with antibodies shows neutralizing antibodies, then the target herd immunity level would have to be increased. In this situation, it is most likely that the desired level of herd immunity will not be achieved before an effective vaccine becomes available.

To the best of our knowledge, this study is the first large seroprevalence survey for SARS-CoV-2 infection among asymptomatic people in Rio de Janeiro, Brazil. Although the survey was not based on a random population sample, it does cover a demographically and socially heterogeneous healthy population allowing a preliminary outlook of the antibody prevalence in asymptomatic people. The prevalence estimates provided by our study are corrected by the sensitivity and specificity of the test employed and weighted by the population age and sex structure, giving a better view of the antibody prevalence at the population level.

In this sense, it is reassuring that our results match some basic premises. First, the increasing seroprevalence across time was something expected, since the epidemic curve is on the rise for the last two months in Rio de Janeiro, without any clear sign of reduction [21]. Also, the higher prevalence among the youngest was predictable, because they are the core of the workforce and are more likely to circulate and thus being exposed to infection, even under the social distancing restrictions. The higher prevalence of infection among those with less formal education is also expected since those at the lower socioeconomic stratum are probably those who have the most difficulties in following the social distancing recommendation since they have to look for some source of income. In addition, many of them live in crowded households without access to piped water, which makes it difficult to adopt basic hygiene measures. A study in the State of Ceará indicated that subjects with primary education considered themselves at a lower risk for getting COVID-19 and were less engaged in voluntary quarantine as compared to those with a higher level of education [25]. Finally, it was also anticipated that blood donors from “condos” should have lower prevalence, since they are donating blood where they live and are probably at home following the social distancing recommendations. Contrarily, those who donate blood at the Hemorio are more likely to do so while coming to the city center for working or other reasons.

Otherwise, the results of our study should be considered with caution. Although varied in demographic and social terms, the population studied is a convenience sample, and any extrapolation for the whole population of the State of Rio de Janeiro, even only for those aged 18-69 years, might be biased. The study population was also not selected to give estimates for different regions of the State, but the prevalence of infection is expected to show huge variation across geographical areas of the city.

Even considering such limitations, it is possible to infer that protective levels of natural herd immunity to SARS-CoV-2 under the social distancing policies implemented in Rio de Janeiro is far from being built, and should not be considered a target to inform a short-term exit plan. Therefore, the timing for relaxing social distancing strategies in the short-term should rely mostly on the availability of adequate health care infrastructure, until a larger and population-based serological survey could be done. Such a survey should be designed to identify variations in the level of herd immunity within the state and eventually recommend a more-locally oriented strategy based on the levels of natural herd immunity, degree of vulnerability of the population and the availability of adequate resources for testing and treating the severe cases of COVID-19.

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