PLOS ONE

RESEARCH ARTICLE

Monitoring SARS-CoV-2 seroprevalence over time among pregnant women admitted to delivery units: Suitability for surveillance

Mariana Yumi Miyadahira[1], Maria de Lourdes Brizot[1], Neal Alexander[2], Ester Cerdeira Sabino[3], Lea Campos de Oliveira da Silva[4], Mara Sandra Hoshida[1,5], Ana Maria da Silva Sousa Oliveira[1], Ana Claudia Silva Farche[1], Rossana Pulcineli Vieira Francisco[1,6], Philippe Mayaud[7] *

1 Disciplina de Obstetrícia, Departamento de Obstetrícia e Ginecologia da Faculdade de Medicina da Universidade de São Paulo (FMUSP), São Paulo, Brazil, 2 Faculty of Epidemiology & Population Health, London School of Hygiene & Tropical Medicine, London, United Kingdom, 3 Departamento de Moléstias Infecciosas e Parasitárias, Instituto de Medicina Tropical da FMUSP, São Paulo, Brazil, 4 Laboratório de Medicina Laboratorial—LIM 03, Hospital das Clínicas da FMUSP, São Paulo, Brazil, 5 Laboratório de Investigação Médica- LIM 57, Hospital das Clínicas da FMUSP, São Paulo, Brazil, 6 Hospital Universitário da Universidade de São Paulo (USP), São Paulo, Brazil, 7 Faculty of Infectious & Tropical Diseases, London School of Hygiene & Tropical Medicine, London, United Kingdom

* Philippe.Mayaud@lshtm.ac.uk

Abstract

Objectives

To determine SARS-CoV-2 seroprevalence over time and risk factors among pregnant women at delivery in São Paulo, Brazil; and to evaluate the suitability of pregnant women as a sentinel population for SARS-CoV-2 serosurveillance.

Methods

Unselected consecutive pregnant women presenting at the labor ward of a single large hospital between July 20th 2020 to February 21st 2021 were enrolled and tested for SARS-CoV-2 serology using two assays: the rapid chromatic Wondfo One Step (for total IgA and IgG detection) and Roche Elecsys assay (detecting anti-nucleoprotein [N] IgG). SARS-CoV-2 seroprevalence was computed as smooth spline function over time with 95% confidence intervals (CI). Risk factors were evaluated for positivity by each assay. We compared time-point seroprevalence by the two assays with four concomitant community household surveys (HHS), in which the Roche assay was used, to determine the sensitivity and relevance of the pregnant women population as sentinel population.

Results

Overall SARS-CoV-2 seroprevalence was 28.9% (221/763) by Roche and 17.9% (137/763) by Wondfo. Reported symptoms experienced during pregnancy were all significantly correlated with being SARS-CoV-2 seropositive at delivery with any assay (with odds-ratios ranging from 3.0 [95% CI: 2.1–4.3] for coryza to 22.8 [95% CI: 12.3–46.6] for ageusia). Seropositivity by either assay was high in women at delivery in the early period of the

pandemic (June 2020), compared with seropositivity in women from the concomitant HHS: 44.1% (95% CI: 21.8–66.4) for Roche, 54.1% (30.9–78.5) for Wondfo, versus 11.4% (95% CI: 9.2–13.6) for HHS. For later periods (October 2020 and January 2021), the seropositivity in women at delivery measured by Roche corresponded well with the prevalence found among women in the HHS using the same assay, whilst prevalence measured by Wondfo dropped.

Conclusions

Women at delivery represent a highly exposed and readily accessible population for sentinel surveillance of emerging infections such as SARS-CoV-2.

Introduction

COVID-19 (coronavirus disease 2019) caused by SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2), a virus completely unknown to the world before the end of 2019, was declared a global pandemic by the World Health Organization (WHO) in March 2020. By May 31st 2022, there had been over 6.0 million deaths worldwide [1], including over 665,000 in Brazil [2], and over 42,000 in the city of São Paulo alone [3]. Whilst risk factors for severe COVID-19 are increasingly known and include age, sex, comorbidities and socio-economic factors [4–8], those for acquiring and transmitting SARS-CoV-2 infection are less well identified.

Physiological changes in the respiratory and immune systems may make pregnant women more susceptible to infection [9]. Recent data suggest that pregnant women might be at increased risk of severe COVID-19. Studies have shown that compared to non-pregnant women, pregnant women are significantly more likely to be hospitalized, admitted to intensive care units (ICU), receive invasive ventilation or extracorporeal membrane oxygenation (ECMO), and die [10–16]. In addition, results from a multinational cohort study pointed out a higher rate of pregnancy related complications, such as hypertensive disorders of pregnancy and fetal distress [17]. There is evidence of significantly elevated risk of preterm birth [13–17] and lower neonatal weight [17] among SARS-CoV-2 affected pregnancies when compared with pregnant women without SARS-CoV-2 infection, even in milder disease. Also, the neonates who tested positive (born to COVID-19 positive mothers) had worse outcomes, with higher rates of ICU admission, fever, gastrointestinal and respiratory symptoms and death, even after adjusting for prematurity [17]. Therefore, this complicated disease course seems to compromise the development and survival of the fetus and neonate, although robust, organized, homogeneous information on the effect of COVID-19 on perinatal, neonatal and long-term outcomes remain insufficient [18–21].

Universal testing for SARS-CoV-2 infection in all pregnant women admitted at labor and delivery (L&D) units has been implemented in a range of settings [22–27]. Screening can be done by virus antigen detection, mostly relying on molecular methods such as reverse-transcriptase PCR, or by serological testing. The first approach will verify the immediate risk of infection in the mother and potential risk of perinatal transmission. Additionally, antigen-detection screening can be useful to organize hospital beds and guide services' isolation practices, neonatal care and the use of personal protective equipment [28], but will likely under-evaluate the risk associated with virus acquisition during pregnancy, since this test detects only transient acute infection [29].

Conversely, in a previously unexposed (and unvaccinated) population of pregnant women, serological status provides information on cumulative exposure to the virus over the duration of pregnancy (assuming antibody decay following natural infection is not much inferior to pregnancy duration) and its possible effects on pregnancy outcome, frequency of asymptomatic and pauci-symptomatic infections [30–33].

Besides verifying exposure of the mother (and consequently of the fetus), and therefore, guiding management, this strategy represents a great opportunity to obtain estimates of SARS-CoV-2 circulation in the community [34]. Also, pregnant women, particularly at the time of delivery, might be a good sentinel population for surveillance of the infection during restrictive quarantine times, when these women must still interact with the health system, whilst most others can avoid or defer doing so.

In the present study we sought to determine the seroprevalence and risk factors of SARS-CoV-2 among pregnant women admitted in L&D units in a public maternity hospital complex in São Paulo, during the first year of the SARS-CoV-2 pandemic in Brazil; and to evaluate the suitability of this population in serosurveillance, contrasting with data obtained from serial community household surveys conducted concomitantly in the city [35].

Materials and methods

Settings and populations

From April 12th 2020, soon after the introduction of SARS-CoV-2 in São Paulo [36], we have been conducting a prospective evaluation of SARS-CoV-2 in all pregnant women presenting for delivery in labor and delivery (L&D) units of a large public hospital complex. Details of the study have been described elsewhere [21]. In brief, the hospital complex consists of two separate L&D units in Hospital das Clínicas and in Hospital Universitário, which are interconnected via cross-referral systems for complex pregnancy cases in usual times. Under newly established COVID-19 management structures, Hospital das Clínicas was the referral hospital for known COVID-19 cases (for all cases, including pregnant women) and Hospital Universitário was a non-COVID-19 hospital.

For the present investigation, consecutive pregnant women admitted for delivery at the L&D unit at Hospital Universitário from July 20th 2020 to February 21st 2021, were invited to participate. The research team was present for 8 hours each weekday. Those pregnant women of all ages who provided signed written informed consent to participate in the study were eligible and included.

Data on participants' socio-demographics, clinical and obstetrical history, details of previous treatments, exam results (including serologies for HIV, syphilis, hepatitis B, hepatitis C, toxoplasmosis, rubella, cytomegalovirus), hospital admissions during pregnancy, experience of COVID-19 or flu-like symptoms during pregnancy were obtained and recorded in a REDCap platform database (Research Electronic Data Capture Consortium, supported by the National Institutes of Health, USA). The following symptoms were considered indicative of COVID-19 according to the WHO case definition at the time: fever, cough, sore throat, coryza, dyspnea, headache, myalgia, asthenia, fatigue, diarrhea, anosmia and ageusia [37]. Delivery and neonatal data were retrieved from patients and their newborns' medical records, immediately after delivery. Medical records were reassessed to get patients´ dates of hospital release and outcome. These data were also recorded in a REDCap database. Maternal venous blood samples were collected at admission for serological testing.

Seroprevalence among pregnant women over time was compared to four consecutive rounds of household surveys (HHS) (each phase comprised 9 to 10 days) that were conducted by the Research and Development Sector of Grupo Fleury (a large private laboratory in São

Paulo), which took place over the same study period. The HHS was conducted to monitor the seroprevalence of SARS-CoV-2 infection in adults in the city of São Paulo during the pandemic development [35]. In brief, the population was sampled using a probabilistic two-stage method, census sector and household. All residents in the selected households over the age of 18 years old were invited to participate. Two specific survey strata were created, city districts with the highest and lowest average household income [35], each corresponding to about half of the surveyed population, and data was stratified by sex. Following administration of a face-to-face questionnaire, a venipuncture blood sample was drawn from each participant. We excluded data from the first (pilot) phase of the HHS conducted prior to our own survey.

Data are presented according to STROBE (Strengthening the reporting of observational studies in epidemiology) recommendations [38]. The checklist is included as S1 Table.

Laboratory methods

SARS-CoV-2 serology. For serological screening at delivery, a qualitative rapid chromatographic immunoassay (RCI) (measuring IgG and IgM antibodies against the SARS-CoV-2 S1 protein) was performed using the Wondfo One Step COVID-19 test (Guangzhou Wondfo Biotech, China) as previously described [39]. Results were read within 15 minutes by three appropriately trained people and communicated back to the L&D unit within 24 hours of collection.

Subsequently, all stored samples were retested in the same laboratory with the Elecsys anti-SARS-CoV-2 E2G300 (Roche Diagnostics, Rotkreuz, Switzerland), an electrochemiluminescence assay that allows a qualitative detection of specific SARS-CoV-2 antibodies. The assay uses a recombinant protein from the nucleocapsid (NC) antigen of the virus with the double antigen sandwich methodology, which favors the detection of high affinity antibodies against SARS-CoV-2.

For the household surveys, serological initial testing measured the presence of anti-SARS-CoV-2 IgM and IgG antibodies using a chemiluminescence assay in survey phases 1 and 2 (Maglumi chemiluminescence assay, Snibe [Shenzhen New Industries Biomedical Engineering Co., Ltd.], Shenzhen, China) (May and June 2020). In later phases 3 (July 2020), 4 (October 2020) and 5 (January 2021), the Roche Elecsys assay was performed in combination with the Maglumi assay. A sample was considered seropositive if any of the two performed tests had a positive result [35].

Additional SARS-CoV-2 testing. A subgroup of women was tested during pregnancy in their own hospital or in private laboratories either by molecular tests of naso-pharyngeal swabs or by serology, because of their job requirements, presence of symptoms or suspicion of being a contact. The results of these tests were evidenced by the printed copy showed by the patient at the L&D admission.

Statistical analysis

Descriptive statistics (frequencies) were calculated including 95% confidence intervals (CI) for binomial proportions using the Wilson method [40]. Positivity on the Wondfo was compared with that on Roche by McNemar's test and the 95% CI for difference in proportions [41]. Cohen's κ (kappa) was also calculated [42]. Values of κ were categorized following Landis & Koch [43].

Risk factors for seropositive status at delivery, as determined by the Roche assay, were analyzed in terms of odds ratios (OR) with their 95% CI and p values from Wald standard errors in logistic regression. For this regression analysis, each independent variable was analyzed

separately, those with missing values for that variable were excluded, and height and body mass index (BMI) were each included as a continuous variable.

Changes over time were described for each diagnostic test by using polynomial splines with B-spline basis [44], and 5 degrees of freedom, to estimate the seroprevalence as a smooth function of epidemiological week, again with logistic regression. Seroprevalence in phase 2 of the HHS was compared descriptively with delivery seroprevalence in the calendar month of June 2020: for phases 3 to 5, the months were July 2020, October 2020 and January 2021 respectively. For each phase, the fitted value from the spline was taken from the epidemiological week closest to the midpoint of the month in question. Analyses were carried out using R software version 4.1.0 was used, with packages "splines", "binom", "irr" and "exact2x2".

Ethical considerations

The study protocol was approved by the ethics committees of both hospitals, Hospital das Clínicas and Hospital Universitário (CAAE: 30270820.3.0000.0068, approved April 11th, 2020 and CAAE: 30270820.3.3001.0076, approved May 20th 2020), and was registered at Clinical-Trial.gov (NCT04647994). All participants provided signed written informed consent.

Results

Population characteristics

A total of 1,867 unselected consecutive pregnant women were admitted for delivery or curettage at Hospital Universitario in São Paulo and were eligible to participate in the study over an eight-month period (June 20th, 2020–February 21st, 2021) (i.e., about 233 women per month), of whom 997 (53.4%) were admitted during periods when it was not possible to include them in the study for logistical reasons (i.e., night shifts and weekends). Of the remaining 870 women, 98 (11.3%) declined participation, leaving 772 eligible and consenting women participating in the study (Fig 1).

Fig 1. Study flowchart.

https://doi.org/10.1371/journal.pone.0280109.g001
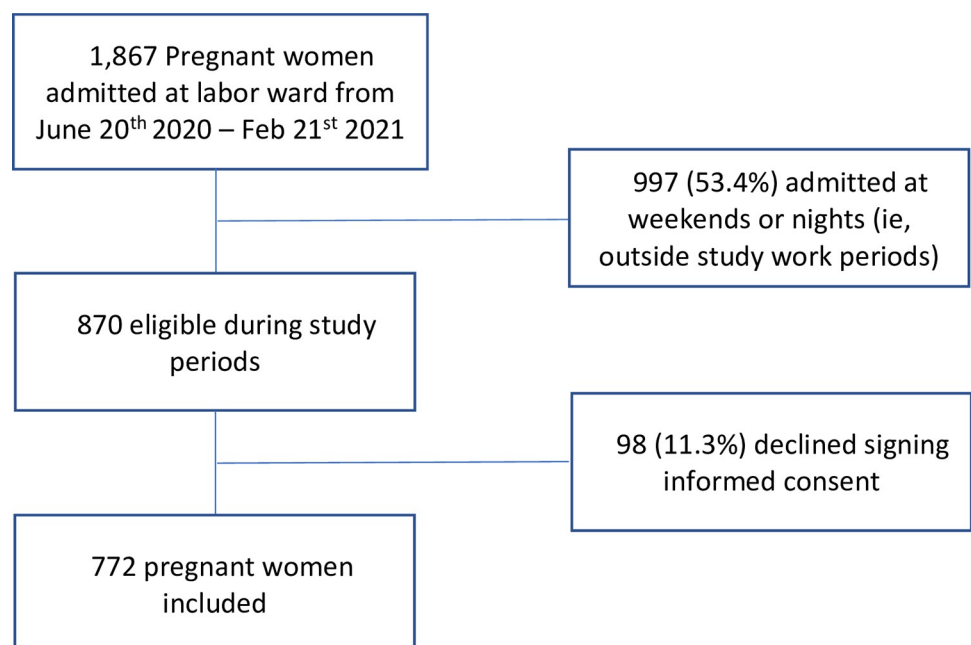
Table 1. Population characteristics. (N = 772 participants).

	Frequency n/N (%) or mean (±SD) or median (IQR)
Maternal age (years, N = 770)	29.1 (± 6.6)
Ethnicity	
White	287/769 (37.3)
Non-white	482/769 (62.7)
BMI group	
< 30 kg/m^2	345/746 (46.2)
≥ 30 kg/m^2	401/746 (53.8)
BMI (kg/m^2, N = 746)	31.3 (± 5.9)
Height (cm, N = 762)	160.8 (± 6.5)
HDI (scaled to max. value 100; N = 746), median (IQR)	77.6 (67.8–80.5)
Years of schooling (N = 766)	11.80 (± 2.73)
Occupation	
Healthcare workers	52/758 (6.9)
Essential non-healthcare workers[a]	92/758 (12.1)
Other (non-essential) workers	614/758 (81.0)
Smoking	72/770 (9.4)
Alcohol use	56/759 (7.4)
Comorbidities	
Diabetes	21/767 (2.7)
Hypertension	41/767 (5.3)
Cardiac	19/768 (2.5)
Lung	49/768 (6.4)
Rheumatic	19/768 (2.5)
Cancer	6/772 (0.8)
Thromboembolic	10/772 (1.3)
Any one of the above comorbidities	147/767 (19.2)
Nulliparous	315/771 (40.9)
HIV seropositive	8/736 (1.1)
Syphilis seropositive[b]	35/722 (4.8)
Hepatitis C seropositive	3/428 (0.7)
Hepatitis B (anti-HBs) seropositive	105/646 (16.3)

BMI = body mass index; HDI = human development index

Where the denominator of a proportion differs from the overall sample size of 772, this is because of missing data.

[a] Essential workers defined as those working with food supply or hospital services such as cleaning and hospitality

[b] Either positive by treponemal assay (e.g., Treponema pallidum particle assay [TPPA]) in those who had never tested positive for syphilis) and/or by non-treponemal assay (e.g., Venereal Diseases Research Laboratory [VDRL]) with a positivity cut-off titer of ≥1:4

All participants were initially screened with the rapid SARS-CoV-2 serological assay (Wondfo) to identify potentially exposed women. There was sufficient leftover sample to test 763 (99%) participants with the Roche assay allowing for the comparative study of seroprevalence over time with the community household surveys.

Table 1 shows participants' characteristics. The mean maternal age was 29.1 years old; 41% were nulliparous; over half had a body mass index (BMI) ≥30 kg/m^2 (defined as obese); at least one chronic comorbidity was observed in 19% of participants, with lung disease the most

prevalent condition (6.4%); 4.9% had reactive syphilis serology (either positive by treponemal assay [e.g., TPPA, *Treponema pallidum* particle agglutination assay] in those who had never tested positive for syphilis and/or positive by a non-treponemal assay [e.g, Venereal Diseases Research Laboratory, VDRL] at a cut-off titer of $\geq$1:4), which was detected and treated during antenatal care; and 1% were HIV seropositive, all of whom were on antiretroviral therapy (ART) at the time of delivery.

Overall, 32 (4%) women attended for curettage following incomplete miscarriage. Among 738 (96%) women attending for delivery, the median gestational age was 39 weeks, there were 16 (2%) twin and 2 (0.2%) triplet pregnancies, and over half of the women (55.7%) were delivered by cesarean section. Nearly all pregnancies resulted in livebirths (99.8%) with a median birthweight of live neonates of 3,230 g (interquartile range, 2,840–3,575). Overall, 78 neonates (10.5%) were born with neonatal malformations, as high-risk pregnancies from Hospital das Clínicas were referred to Hospital Universitario for delivery in the absence of acute COVID-19 symptoms, according to the institutional COVID-19 protocol.

SARS-CoV-2 seroprevalence and risk factors

The overall seroprevalence was 17.9% (137/763) by Wondfo and 28.9% (221/763) by Roche, and 30.3% (231/763) by either assay. Relative to Roche, the Wondfo test therefore categorized 11.0% fewer positive (95% CI: 8.3–13.7, p<0.001). The results of serological testing by the two assays showed an overlap of 127 cases detected by both assays (ie, 55% of all 231 positive by either assay). There were 10 Wondfo positive results (7.3%, 10/137) which were not positive by Roche. Overall, 532/763 (69.7%) samples were negative by both assays. Assay agreement was "substantial" at κ = 0.63 [44].

Over a third of women reported symptoms indicative of possible SARS-CoV-2 infection/ COVID-19 (35.7%, 273/763) during their pregnancy. For each assay, about 60% of positive results were found among symptomatic women. The Roche assay identified the greatest proportion of SARS-CoV-2 seropositive women among both symptomatic (46.9%, 128/273) and asymptomatic women (19.0%, 93/490), compared to Wondfo (28.6%, 78/273; and 12.0%, 59/490, both p<0.001). In addition, 50 (asymptomatic and symptomatic) women with positive SARS-CoV-2 nasopharyngeal swab during pregnancy had results from both the Roche and Wondfo assays. Among these, the Roche assay identified more than the Wondfo assay: 46/50 (92.0%) as opposed to 31/50 (62.0%), a difference of 30.0% (95% confidence interval 13.5– 44.6%, p<0.001).

We conducted separate risk factor analyses for seropositivity by each of the assays. Given that Roche seemed to be the most sensitive assay, we present population characteristics and risk factor analyses based on the Roche serological results in Table 2. Similar analyses with the Wondfo assay are presented in S2 Table.

Overall, 21 variables were included in the risk factor analysis. Factors associated with SARS-CoV-2 seropositivity and other selected variables are presented in Table 2.

Seropositive pregnant women had lower height (OR = 0.96 per cm, 95% CI: 0.93–0.98) than seronegative women. Reported COVID-19 symptoms during pregnancy (fever, cough, sore throat, myalgia, asthenia, coryza, anosmia, ageusia, dyspnea, headache and fatigue) were all significantly more prevalent in seropositive pregnant women (with odds ratios ranging from 3.0 for coryza to 22.8 for ageusia), whilst the lack of symptom reporting was inversely correlated with seropositivity (42.1% of asymptomatic pregnant women during pregnancy were SARS-CoV-2 seropositive). Associations were stronger for symptoms now known to be more closely associated with the first wave of COVID-19, such as ageusia, anosmia, dyspnea, myalgia and fatigue.

Table 2. Factors associated with SARS-CoV-2 seropositivity by Roche.

	Total N = 763	SARS-CoV-2 seropositive N = 221 (29%)	SARS-CoV-2 seronegative N = 542	OR (95%CI)	p-value
Age					
<30 years	409 (53.6)	129 (58.4)	280 (51.7)	1	
≥30 years	354 (46.4)	92 (41.6)	262 (48.3)	0.8 (0.6–1.0)	0.092
Ethnicity					
White	283 (37.2)	80 (36.4)	203 (37.6)	1	
Non-white	477 (62.8)	140 (63.6)	337 (62.4)	1.1 (0.8–1.5)	0.751
Occupation					
Healthcare	52 (6.9)	17 (8.0)	35 (6.5)	1	
Essential non-healthcare[a]	90 (12.0)	30 (14.1)	60 (11.2)	1.0 (0.5–2.2)	0.938
Non-essential	607 (81.0)	166 (77.9)	441 (82.3)	0.8 (0.4–1.5)	0.410
Any comorbidity[b]					
No	613 (80.3)	179 (81.0)	434 (80.1)	1	
Yes	150 (19.7)	42 (19.0)	108 (19.9)	0.9 (0.6–1.4)	0.771
BMI (kg/m^2)	31.3 (5.9)	32.1 (6.2)	31 (5.8)	1.03 (1.00–1.06)	0.024
Height (cm)	160.8 (6.5)	159.5 (6.1)	161.3 (6.6)	0.96 (0.93–0.98)	0.001
Potential COVID-19 symptoms during pregnancy					
Fever	78 (10.2)	45 (20.4)	33 (6.1)	3.9 (2.4–6.4)	<0.001
Cough	134 (17.6)	68 (30.8)	66 (12.2)	3.2 (2.2–4.7)	<0.001
Sore throat	80 (10.5)	42 (19.0)	38 (7.0)	3.1 (1.9–5.0)	<0.001
Myalgia	98 (12.8)	64 (29.0)	34 (6.3)	6.1 (3.9–9.7)	<0.001
Asthenia	89 (11.7)	58 (26.2)	31 (5.7)	5.9 (3.7–9.5)	<0.001
Coryza	164 (21.5)	79 (35.7)	85 (15.7)	3.0 (2.1–4.3)	<0.001
Anosmia	86 (11.3)	74 (33.5)	12 (2.2)	22.2 (12.2–44.1)	<0.001
Ageusia	82 (10.7)	71 (32.1)	11 (2.0)	22.8 (12.3–46.6)	<0.001
Dyspnea	72 (9.4)	51 (23.1)	21 (3.9)	7.4 (4.4–13.0)	<0.001
Headache	137 (18.0)	75 (33.9)	62 (11.4)	4.0 (2.7–5.9)	<0.001
Fatigue	82 (10.7)	55 (24.9)	27 (5.0)	6.3 (3.9–10.5)	<0.001
No symptoms	490 (64.2)	93 (42.1)	397 (73.2)	0.3 (0.2–0.4)	<0.001

Data shown as frequency, n/N (%) or mean (±SD)

BMI = body mass index; CI = confidence interval; OR = odds ratio

[a] Essential workers defined as those working with food supply or hospital services such as cleaning and hospitality

[b] Any comorbidity includes diabetes, hypertension, cardiac, lung, rheumatic diseases, cancer and thromboembolic events

https://doi.org/10.1371/journal.pone.0280109.t002

SARS-CoV-2 seroprevalence over time and comparison with household community surveys

Fig 2 shows SARS-CoV-2 seroprevalence over time among women at delivery, by each of the two assays. SARS-CoV-2 seropositivity was high in June 2020 by both assays, with a visible decline for the following months, with subsequent rise at the beginning of 2021, coinciding with the observed case load in the community [3].

Table 3 presents the results of SARS-CoV-2 seropositivity over time comparing results among pregnant women with both assays with results of the community household survey (HHS) at concomitant timepoints. Results of HHS are presented as overall prevalence, stratified by wealthiest and poorest strata (two strata representing 50% of respondents each), and for women only results. In phase 2 (June 2020) seroprevalence among women at delivery was higher than in the HHS population: women at delivery, 44.1% (21.8–66.4) by Roche, and
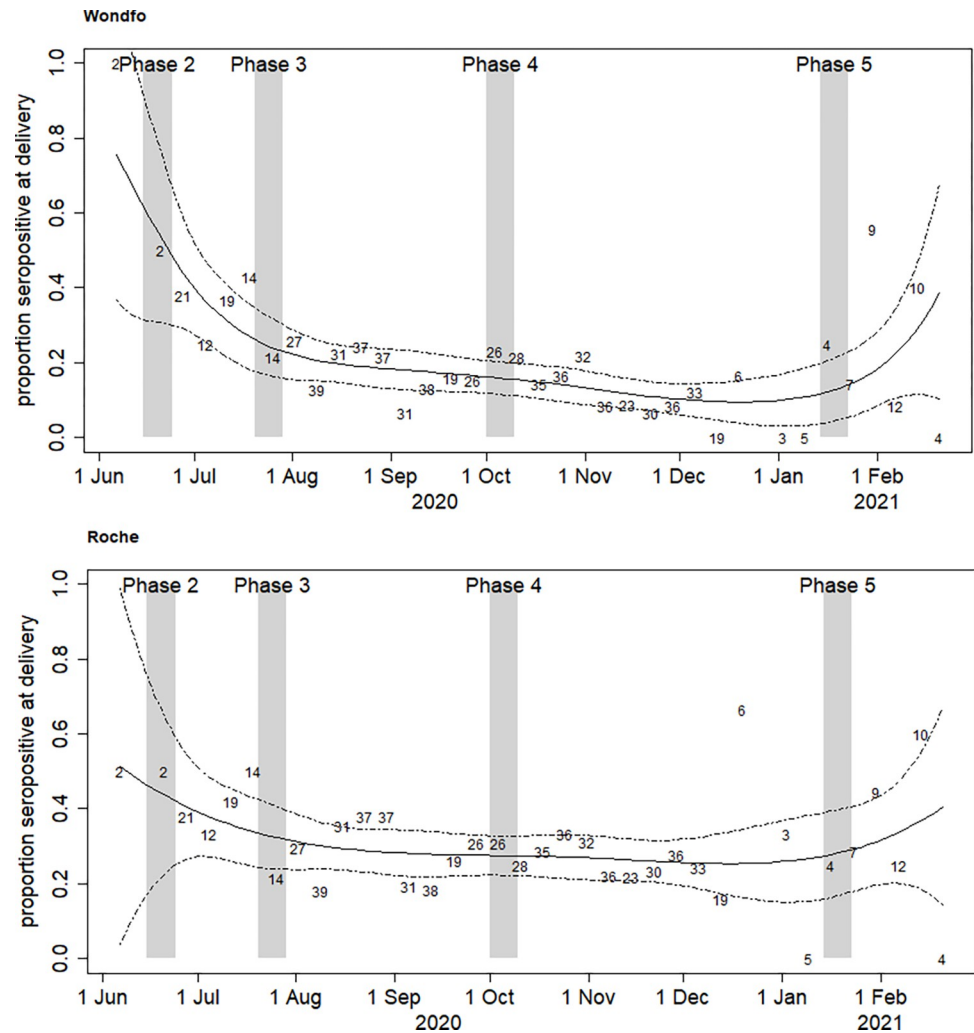
Fig 2. SARS-CoV-2 seroprevalence over time (June 2020-February 2021) among pregnant women at delivery in São Paulo, by Wondfo (Panel A) and Roche Elecsys (Panel B) assays. The grey bars show the dates of the second to fifth household surveys (HHS). The first survey is not shown because it was before the start of the study in pregnant women (see dates in Table 3). The numbers in the body of the figure are the numbers tested in each epidemiological week. The solid line is the estimate of the proportion seropositive from the spline fit, and the two dashed line are the 95% confidence limits.

https://doi.org/10.1371/journal.pone.0280109.g002

54.1% (30.9–78.5) by Wondfo; compared with 11.2% (8.5–13.9) among women of HHS, and 16% (12.2–19.8) for the respondents from the poorest strata, all tested by Maglumi only. Interestingly, at this stage seropositivity by Wondfo was highest. In phase 3 (July 2020), this difference narrowed: women at delivery, 32.7% (24.1–41.4) by Roche; HHS women, 18.4% (15.2–22.1) by a combination of Roche and Maglumi, although by then, the seroprevalence measured by Wondfo was dropping among women at delivery to 24.7% (16.7–32.7). In phase 4 (October 2020) and phase 5 (January 2021), the seroprevalence in women at delivery by Roche (27.5% and 28.0%, respectively) corresponded well with the seroprevalence found among women in the HHS using the Roche and Maglumi combination (25.7% and 30.7% in women, respectively), whilst seroprevalence by Wondfo had dropped substantially (at 15.9% and 12.5% in Phases 4 and 5, respectively).

Table 3. SARS-CoV-2 seroprevalence by different assays at selected periods corresponding to phases of community household surveys (HHS).

	SARS CoV-2 SEROPOSITIVE AT DELIVERY, % (95% CI)				
	PHASE 1	PHASE 2	PHASE 3	PHASE 4	PHASE 5
	May 4th- 12th	June 15th- 24th	July 20th- 29th	October 1st- 10th	January 14th-
	2020	2020	2020	2020	23rd 2021
Household surveys (HHS)[a]					
Tests used	Maglumi	Maglumi	Roche+Maglumi	Roche+Maglumi	Roche+Maglumi
All	4.7	11.4	17.9	26.2	29.9
	(2.7–7.0)	(9.2–13.6)	(15.0–20.9)	(22.5–29.9)	(26.2–33.5)
	(N = 517)	(N = 1183)	(N = 1470)	(N = 1129)	(N = 1194)
Wealthiest (Half)	NA	6.5	9.4	21.6	22.8
		(4.4–8.5)	(6.0–12.8)	(17–26.3)	(18.1–27.6)
Poorest (Half)		16.0	22.0	30.4	36.4
		(12.2–19.8)	(17.2–26.7)	(24.7–36.0)	(31.1–41.8)
Women only	NA	11.2	18.4	25.7	30.7
		(8.5–13.9)	(15.2–22.1)	(21.7–29.6)	(27.1–34.4)
		(N = 632)	(N = 785)	(N = 603)	(N = 638)
Women at delivery[b]					
Wondfo	NA	54.7	24.7	15.9	12.5
		(30.9–78.5)	(16.7–32.7)	(11.6–20.2)	(4.2–20.8)
Roche	NA	44.1	32.7	27.5	28.0
		(21.8–66.4)	(24.1–41.4)	(22.3–32.7)	(16.4–39.5)

[a] Project soroEpi MSP: https://monitoramentocovid19.org

[b] The percentages seropositive are from the spline fit through the whole data, rather than time- specific numerators and denominators.

https://doi.org/10.1371/journal.pone.0280109.t003

Discussion

The overall SARS-CoV-2 seroprevalence among pregnant women presenting at one of the largest university hospitals for delivery in Sao Paulo during the study period ranged from 17.9% to 28.9% depending on assay. The Roche Elecsys assay appeared more sensitive than Wondfo to identify participants with COVID-19 like symptoms or in the subgroup of women who were PCR-positive on nasopharyngeal swabs during pregnancy. Importantly, SARS-CoV-2 seroprevalence varied over time, reaching the highest levels at the beginning of the period (June 2020) and gradually decreasing, mirroring the COVID-19 pandemic's trajectory in São Paulo, when based on case reporting [3].

The presence of COVID-19 symptoms during pregnancy (particularly anosmia and ageusia in this early phase of the COVID-19 pandemic) was associated with SARS-CoV-2 seropositivity by either assay, highlighting the importance of eliciting symptoms through simple questionnaire in the diagnostic cascade. However, our data demonstrate that a relatively high proportion of asymptomatic women were also seropositive by Roche Elecsys (19%) or Wondfo (12%), underscoring the fact that without testing it would be challenging to estimate the actual size of the epidemic.

The choice of antibody assays is crucial for the quality of epidemiological information obtained. As pointed out by others, assays that detect and quantify long-lasting serological response are important to obtain reliable estimates of exposure. Conversely, immune assays with rapidly waning response may be useful for performing studies of recent infection [46]. We observed that the two assays used in this study behaved slightly differently, providing different profiles for antibody reactivity over time. The anti-SARS-CoV-2 total assay detecting

total antibodies against the spike S1 protein (Wondfo) is predicted to be highly sensitive to detect recent infection [45], and indeed a high prevalence was found in the early months of the epidemic, with good correlation with COVID-19 symptoms, but possibly waning thereafter in parallel with the actual COVID-19 epidemic, meaning it could be used as a diagnostic/confirmatory tool, but would be less accurate to determine the proportion infected up to that time. By contrast, the NC-directed antibody assay (Roche Elecsys) showed more steady signals over time, reflecting exposure during the different preceding periods, resulting in a possibly more accurate measure of cumulative risk [45].

As health authorities need to quickly know the proportion of the population infected or still susceptible to infection to guide public policies, serosurveillance in easily accessible, sensitive and, ideally, representative population groups is of paramount importance. This study shows that women presenting to delivery services, and, by extension, pregnant women could be a good sentinel population to track community infection spread for several reasons. Firstly, this group usually benefits from consecutive medical appointments during antenatal care, allowing for longitudinal monitoring at frequent intervals, but also represents a group that might still be exposed even at times of social isolation, as they need to at least come to delivery units, hence risk of infection can be measured. Secondly, in the early phase of the pandemic and prior to roll-out of a vaccination program, serology probably reflected well recent exposure, given the likely similar duration of pregnancy and half-life of SARS-CoV-2 antibodies [46]. Our study findings support these hypotheses. Three months into the pandemic, by June 2020, SARS-CoV-2 seroprevalence in women at delivery was notably higher than in the household survey population, which could be explained by the sustained exposure of pregnant women who had to travel to medical appointments during that period, whilst the general population had been requested to stay at home. From June 2020 on, with the official easing of social distancing [47], the difference in seroprevalence between the pregnant women and community populations (HHS) started to narrow, and seroprevalence was comparable thereafter. Infectious disease surveillance in pregnant women or women at delivery might thus be more sensitive than that obtained through household surveys because of the former population's greater exposure, more easily implemented and at lower cost. However, pregnant women are not representative of the whole female population because they tend to be younger, and, depending on recruitment sites, infection levels will reflect the particular socio-economic strata from their catchment area.

Other potential sentinel surveillance populations for infectious diseases include blood donors and healthcare workers. However, these two groups are also likely to be less homogeneously exposed than pregnant women. For example, data show that the attendance of blood donors had dropped significantly during lockdown measures [48–50], thus biasing estimates to lower levels. On the other hand, healthcare workers represent both a potentially more exposed population by nature of their work, but also a group more likely to receive and make proper use of personal protective equipment. Extrapolation of serological patterns obtained this group to the community's might be biased [51, 52].

The main limitations of our study include the lack of true longitudinal measurements of serological response among the same individuals to determine recency of infection through seroconversion, the lack of confirmation of true infection through molecular testing, particularly challenging to organize if a large proportion of infections may be pauci-symptomatic, and the relatively small sample size to determine associations with precision. These constraints are applicable to most serosurveillance surveys. Furthermore, an exact comparison of SARS-CoV-2 seropositivity between the HHS population and pregnant women was not possible, because seroprevalence in the former was based on positivity on either of the two assays (Maglumi and Roche). However, knowing that Roche was the most sensitive assay helps in the

interpretation of the HHS results, and we can compare trends over time between HHS and pregnant women.

As for other surveillance programs, the monitoring of infectious diseases trends is largely constrained by the choice of population, the performance and standardization of monitoring tools (ie, serological assays) and the trajectory of the epidemic/pandemic itself including response to it. In the case of SARS-CoV-2, appearance of new variants as well as the introduction of vaccination is prompting for adjustment of serological testing to be able to distinguish recent from past infection or vaccination and reflect the circulation of current variants.

In conclusion, we have shown that it was possible to organize SARS-CoV-2 serological surveillance among women coming to delivery units in Sao Paulo, that seroprevalence was both substantial and varying over time, reflecting the COVID-19 pandemic trajectory during that period. Pregnant women or women coming to delivery constitute a highly exposed and accessible population, suitable for the sentinel surveillance of emerging infections.

Supporting information

S1 Table. The STROBE statement- checklist of items that should be included in reports of cross-sectional studies.
(DOCX)

S2 Table. Factors associated with SARS-CoV-2 seropositivity by Wondfo.
(DOCX)

Acknowledgments

Author Contributions

Conceptualization: Maria de Lourdes Brizot, Rossana Pulcineli Vieira Francisco, Philippe Mayaud.

Formal analysis: Neal Alexander.

Funding acquisition: Maria de Lourdes Brizot, Ester Cerdeira Sabino, Rossana Pulcineli Vieira Francisco, Philippe Mayaud.

Investigation: Mariana Yumi Miyadahira, Mara Sandra Hoshida, Ana Maria da Silva Sousa Oliveira, Ana Claudia Silva Farche.

Methodology: Mariana Yumi Miyadahira, Maria de Lourdes Brizot, Neal Alexander, Philippe Mayaud.

Project administration: Maria de Lourdes Brizot, Rossana Pulcineli Vieira Francisco.

Resources: Ester Cerdeira Sabino, Lea Campos de Oliveira da Silva.

Supervision: Maria de Lourdes Brizot.

Writing – original draft: Mariana Yumi Miyadahira, Maria de Lourdes Brizot, Neal Alexander, Ester Cerdeira Sabino, Philippe Mayaud.

Writing – review & editing: Mariana Yumi Miyadahira, Maria de Lourdes Brizot, Neal Alexander, Rossana Pulcineli Vieira Francisco, Philippe Mayaud.

References

1. WHO Coronavirus (COVID-19) Dashboard. 2020. Available from: https://covid19.who.int.

2. Painel Coronavírus. 2022. Available from: https://covid.saude.gov.br.

3. Boletim diário COVID-19. 2020.Available from: https://www.prefeitura.sp.gov.br/cidade/secretarias/upload/saude/20220531_boletim_covid19_diario.pdf

4. Gao YD, Ding M, Dong X, Zhang JJ, Kursat Azkur A, Azkur D, et al. Risk factors for severe and critically ill COVID-19 patients: A review. Allergy. 2021; 76(2):428–55. https://doi.org/10.1111/all.14657 PMID: 33185910

5. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020; 395(10229):1054–62. https://doi.org/10.1016/S0140-6736(20)30566-3 PMID: 32171076

6. Wolff D, Nee S, Hickey NS, Marschollek M. Risk factors for Covid-19 severity and fatality: a structured literature review. Infection. 2021; 49(1):15–28. https://doi.org/10.1007/s15010-020-01509-1 PMID: 32860214

7. Zhang J, Wang X, Jia X, Li J, Hu K, Chen G, et al. Risk factors for disease severity, unimprovement, and mortality in COVID-19 patients in Wuhan, China. Clin Microbiol Infect. 2020; 26(6):767–72. https://doi.org/10.1016/j.cmi.2020.04.012 PMID: 32304745

8. Ebinger JE, Achamallah N, Ji H, Claggett BL, Sun N, Botting P, et al. Pre-existing traits associated with Covid-19 illness severity. PLoS One. 2020; 15(7):e0236240. https://doi.org/10.1371/journal.pone.0236240 PMID: 32702044

9. Dashraath P, Wong JLJ, Lim MXK, Lim LM, Li S, Biswas A, et al. Coronavirus disease 2019 (COVID-19) pandemic and pregnancy. Am J Obstet Gynecol. 2020; 222(6):521–31. https://doi.org/10.1016/j.ajog.2020.03.021 PMID: 32217113

10. Ellington S, Strid P, Tong VT, Woodworth K, Galang RR, Zambrano LD, et al. Characteristics of Women of Reproductive Age with Laboratory-Confirmed SARS-CoV-2 Infection by Pregnancy Status—United States, January 22-June 7, 2020. MMWR Morb Mortal Wkly Rep. 2020; 69(25):769–75. https://doi.org/10.15585/mmwr.mm6925a1 PMID: 32584795

11. Zambrano LD, Ellington S, Strid P, Galang RR, Oduyebo T, Tong VT, et al. Update: Characteristics of Symptomatic Women of Reproductive Age with Laboratory-Confirmed SARS-CoV-2 Infection by Pregnancy Status—United States, January 22-October 3, 2020. MMWR Morb Mortal Wkly Rep. 2020; 69 (44):1641–7. https://doi.org/10.15585/mmwr.mm6944e3 PMID: 33151921

12. Allotey J, Stallings E, Bonet M, Yap M, Chatterjee S, Kew T, et al. Clinical manifestations, risk factors, and maternal and perinatal outcomes of coronavirus disease 2019 in pregnancy: living systematic review and meta-analysis. BMJ. 2020; 370:m3320. https://doi.org/10.1136/bmj.m3320 PMID: 32873575

13. McClymont E, Albert AY, Alton GD, Boucoiran I, Castillo E, Fell DB, et al. Association of SARS-CoV-2 Infection During Pregnancy With Maternal and Perinatal Outcomes. JAMA. 2022. https://doi.org/10.1001/jama.2022.5906 PMID: 35499852

14. Wastnedge EAN, Reynolds RM, van Boeckel SR, Stock SJ, Denison FC, Maybin JA, et al. Pregnancy and COVID-19. Physiol Rev. 2021; 101(1):303–18. https://doi.org/10.1152/physrev.00024.2020 PMID: 32969772

15. Di Toro F, Gjoka M, Di Lorenzo G, De Santo D, De Seta F, Maso G, et al. Impact of COVID-19 on maternal and neonatal outcomes: a systematic review and meta-analysis. Clin Microbiol Infect. 2021; 27 (1):36–46. https://doi.org/10.1016/j.cmi.2020.10.007 PMID: 33148440

16. Birol Ilter P, Prasad S, Mutlu MA, Tekin AB, O'Brien P, von Dadelszen P, et al. Maternal and perinatal outcomes of SARS-CoV-2 infection in unvaccinated pregnancies during Delta and Omicron waves. Ultrasound Obstet Gynecol. 2022. https://doi.org/10.1002/uog.24916 PMID: 35441407

17. Giuliani F, Oros D, Gunier RB, Deantoni S, Rauch S, Casale R, et al. Effects of prenatal exposure to maternal COVID-19 and perinatal care on neonatal outcome: results from the INTERCOVID Multinational Cohort Study. Am J Obstet Gynecol. 2022. https://doi.org/10.1016/j.ajog.2022.04.019 PMID: 35452653

18. Juan J, Gil MM, Rong Z, Zhang Y, Yang H, Poon LC. Effect of coronavirus disease 2019 (COVID-19) on maternal, perinatal and neonatal outcome: systematic review. Ultrasound Obstet Gynecol. 2020; 56 (1):15–27. https://doi.org/10.1002/uog.22088 PMID: 32430957

19. Diriba K, Awulachew E, Getu E. The effect of coronavirus infection (SARS-CoV-2, MERS-CoV, and SARS-CoV) during pregnancy and the possibility of vertical maternal-fetal transmission: a systematic review and meta-analysis. Eur J Med Res. 2020; 25(1):39. https://doi.org/10.1186/s40001-020-00439-w PMID: 32887660

20. Chi H, Chiu NC, Tai YL, Chang HY, Lin CH, Sung YH, et al. Clinical features of neonates born to mothers with coronavirus disease-2019: A systematic review of 105 neonates. J Microbiol Immunol Infect. 2021; 54(1):69–76. https://doi.org/10.1016/j.jmii.2020.07.024 PMID: 32847748

21. Maeda MFY, Brizot ML, Gibelli MABC, Ibidi SM, Carvalho WB, Hoshida MS, et al. Vertical transmission of SARS-CoV2 during pregnancy: A high-risk cohort. Prenat Diagn. 2021; 41(8):998–1008.

22. Prabhu M, Cagino K, Matthews KC, Friedlander RL, Glynn SM, Kubiak JM, et al. Pregnancy and postpartum outcomes in a universally tested population for SARS-CoV-2 in New York City: a prospective cohort study. BJOG. 2020; 127(12):1548–56. https://doi.org/10.1111/1471-0528.16403 PMID: 32633022

23. Reale SC, Lumbreras-Marquez MI, King CH, Burns SL, Fields KG, Diouf K, et al. Patient characteristics associated with SARS-CoV-2 infection in parturients admitted for labour and delivery in Massachusetts during the spring 2020 surge: A prospective cohort study. Paediatr Perinat Epidemiol. 2021; 35(1):24–33. https://doi.org/10.1111/ppe.12743 PMID: 33496995

24. Goldfarb IT, Diouf K, Barth WH, Robinson JN, Katz D, Gregory KE, et al. Universal SARS-CoV-2 testing on admission to the labor and delivery unit: Low prevalence among asymptomatic obstetric patients. Infect Control Hosp Epidemiol. 2020; 41(9):1095–6. https://doi.org/10.1017/ice.2020.255 PMID: 32456729

25. Fassett MJ, Lurvey LD, Yasumura L, Nguyen M, Colli JJ, Volodarskiy M, et al. Universal SARS-Cov-2 Screening in Women Admitted for Delivery in a Large Managed Care Organization. Am J Perinatol. 2020; 37(11):1110–4. https://doi.org/10.1055/s-0040-1714060 PMID: 32620022

26. Cavaliere AF, Carabaneanu AI, Perelli F, Matarrese D, Brunelli T, Casprini P, et al. Universal screening for SARS-CoV-2 in pregnant women admitted for delivery: how to manage antibody testing? J Matern Fetal Neonatal Med. 2020:1–2.

27. Gagliardi L, Danieli R, Suriano G, Vaccaro A, Tripodi G, Rusconi F, et al. Universal severe acute respiratory syndrome coronavirus 2 testing of pregnant women admitted for delivery in 2 Italian regions. Am J Obstet Gynecol. 2020; 223(2):291–2. https://doi.org/10.1016/j.ajog.2020.05.017 PMID: 32407787

28. Sutton D, Fuchs K, D'Alton M, Goffman D. Universal Screening for SARS-CoV-2 in Women Admitted for Delivery. N Engl J Med. 2020; 382(22):2163–4. https://doi.org/10.1056/NEJMc2009316 PMID: 32283004

29. Miller TE, Garcia Beltran WF, Bard AZ, Gogakos T, Anahtar MN, Astudillo MG, et al. Clinical sensitivity and interpretation of PCR and serological COVID-19 diagnostics for patients presenting to the hospital. FASEB J. 2020; 34(10):13877–84. https://doi.org/10.1096/fj.202001700RR PMID: 32856766

30. Crovetto F, Crispi F, Llurba E, Figueras F, Gómez-Roig MD, Gratacós E. Seroprevalence and presentation of SARS-CoV-2 in pregnancy. Lancet. 2020; 396(10250):530–1. https://doi.org/10.1016/S0140-6736(20)31714-1 PMID: 32771084

31. Cosma S, Borella F, Carosso A, Sciarrone A, Cusato J, Corcione S, et al. The "scar" of a pandemic: Cumulative incidence of COVID-19 during the first trimester of pregnancy. J Med Virol. 2021; 93 (1):537–40. https://doi.org/10.1002/jmv.26267 PMID: 32633869

32. Interim Guidelines for COVID-19 Antibody Testing. 2020. Available from: https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-guidelines.html.

33. Haizler-Cohen L, Davidov A, Blitz MJ, Fruhman G. Severe acute respiratory syndrome coronavirus 2 antibodies in pregnant women admitted to labor and delivery units. Am J Obstet Gynecol. 2021; 224 (1):112–4. https://doi.org/10.1016/j.ajog.2020.09.022 PMID: 32971014

34. Flannery DD, Gouma S, Dhudasia MB, Mukhopadhyay S, Pfeifer MR, Woodford EC, et al. SARS-CoV-2 seroprevalence among parturient women in Philadelphia. Sci Immunol. 2020; 5(49). https://doi.org/10.1126/sciimmunol.abd5709 PMID: 32727884

35. SoroEpi MSP. 2020. Available from: https://www.monitoramentocovid19.org.

36. Rodriguez-Morales AJ, Gallego V, Escalera-Antezana JP, Méndez CA, Zambrano LI, Franco-Paredes C, et al. COVID-19 in Latin America: The implications of the first confirmed case in Brazil. Travel Med Infect Dis. 2020; 35:101613. https://doi.org/10.1016/j.tmaid.2020.101613 PMID: 32126292

37. Coronavirus disease (COVID-19)- Symptoms. 2020. Available from: https://www.who.int/health-topics/coronavirus#tab=tab_3.

38. Vandenbroucke JP, von Elm E, Altman DG, Gøtzsche PC, Mulrow CD, Pocock SJ, et al. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. PLoS Med. 2007; 4(10):e297. https://doi.org/10.1371/journal.pmed.0040297 PMID: 17941715

39. Costa SF, Buss L, Espinoza EPS, Vieira JM, de Oliveira da Silva LC, de Souza RM, et al. Performance of a qualitative rapid chromatographic immunoassay to diagnose COVID-19 in patients in a middle-income country. J Clin Virol. 2020; 131:104592. https://doi.org/10.1016/j.jcv.2020.104592 PMID: 32827898

40. Agresti A, Coull B. Approximate is better than "exact" for interval estimation of binomial proportions. The American Statistician. 1998(52):119–26.

41. Siegel S, Castellan N. Nonparametric Statistics for the Behavioral Sciences. New York: MacGraw-Hill International editions; 1988.

42. Cohen J. A coefficient of agreement for nominal scales. Educational and Psychological Measurement 1960; 20:37–46.

43. Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics. 1977; 33(1):159–74. PMID: 843571

44. Hastie T, Tibshirani R. Generalized Additive Models. London: Chapman and Hall; 1990.

45. Di Germanio C, Simmons G, Kelly K, Martinelli R, Darst O, Azimpouran M, et al. SARS-CoV-2 antibody persistence in COVID-19 convalescent plasma donors: Dependency on assay format and applicability to serosurveillance. Transfusion. 2021; 61(9):2677–87. https://doi.org/10.1111/trf.16555 PMID: 34121205

46. Luo YR, Yun C, Chakraborty I, Wu AHB, Lynch KL. A SARS-CoV-2 Label-Free Surrogate Virus Neutralization Test and a Longitudinal Study of Antibody Characteristics in COVID-19 Patients. J Clin Microbiol. 2021; 59(7):e0019321. https://doi.org/10.1128/JCM.00193-21 PMID: 33827900

47. Diário Oficial- Estado de São Paulo. 2020. Available from: http://diariooficial.imprensaoficial.com.br/nav_v5/index.asp?c=4&e=20200529&p=1.

48. Silva-Malta MCF, Rodrigues DOW, Chaves DG, Magalhães NNS, Ribeiro MA, Cioffi JGM, et al. Impact of COVID-19 in the attendance of blood donors and production on a Brazilian Blood Centres. Transfus Med. 2021; 31(3):206–12. https://doi.org/10.1111/tme.12733 PMID: 33118220

49. Elnasser Z, Obeidat H, Amarin Z, Alrabadi N, Jaradat A, Alomarat D, et al. Prevalence of COVID-19 among blood donors: The Jordan University of Science and Technology experience. Medicine (Baltimore). 2021; 100(41):e27537. https://doi.org/10.1097/MD.0000000000027537 PMID: 34731151

50. Dinardo CL, Vieira MJ, Rocha V, Mendrone-Júnior A. Changes in blood donation and utilization secondary to Covid-19 outbreak. Transfus Apher Sci. 2021; 60(3):103102. https://doi.org/10.1016/j.transci.2021.103102 PMID: 33674207

51. Kayı İ, Madran B, Keske Ş, Karanfil Ö, Arribas JR, Pshenihnaya N, et al. The seroprevalence of SARS-CoV-2 antibodies among health care workers before the era of vaccination: a systematic review and meta-analysis. Clin Microbiol Infect. 2021; 27(9):1242–9. https://doi.org/10.1016/j.cmi.2021.05.036 PMID: 34116205

52. Galanis P, Vraka I, Fragkou D, Bilali A, Kaitelidou D. Seroprevalence of SARS-CoV-2 antibodies and associated factors in healthcare workers: a systematic review and meta-analysis. J Hosp Infect. 2021; 108:120–34. https://doi.org/10.1016/j.jhin.2020.11.008 PMID: 33212126