Effectiveness of CoronaVac in the setting of high SARS-CoV-2 P.1 variant transmission in Brazil: A test-negative case-control study

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Abstract

Background

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variant P.1 emerged in the city of Manaus in late 2020 during a large resurgence of coronavirus disease (COVID-19), and has spread throughout Brazil. The effectiveness of vaccines in settings with widespread P.1 transmission has not been reported.

Methods

We performed a matched test-negative case-control study to estimate the effectiveness of an inactivated vaccine, CoronaVac, in healthcare workers (HCWs) in Manaus, where P.1 accounted for 75%

of genotyped SARS-CoV-2 samples at the peak of its epidemic. Information from electronic surveillance databases was used to select cases of RT-PCR-confirmed SARS-CoV-2 infection and matched test-negative controls from 19 January, 2021 to 25 March, 2021. We used conditional logistic regression to estimate the effectiveness in reducing the odds of primary and secondary outcomes of, respectively, symptomatic and any SARS-CoV-2 infection.

Findings

Among 53,176 HCWs, 46,884 (88%) received at least one dose of CoronaVac and 2,656 (5%) underwent RT-PCR testing from 19 January, 2021 to 25 March, 2021. Of 2,797 RT-PCR tests, 776 (28%) were positive. 393 and 135 case-control pairs with and without, respectively, symptomatic illness were selected for the matched analyses. Vaccination with at least one dose was associated with a 0.50-fold reduction (adjusted vaccine effectiveness, 49.6%; 95% CI, 11.3 - 71.4) in the odds of symptomatic SARS-CoV-2 infection during the period 14 days or more after receiving the first dose. Estimated vaccine effectiveness of at least one dose against any SARS-CoV-2 infection was 35.1% (95% CI, -6.6 - 60.5) in the same time period.

Interpretation

Administration of at least one dose of CoronaVac showed effectiveness against symptomatic SARS-CoV-2 infection in the setting of epidemic P.1 transmission, underscoring the need to increase vaccination efforts in response to the spread of this variant in Brazil and globally.

Funding

Pan American Health Organization; Fundação Oswaldo Cruz (Fiocruz); Municipal Health Secretary of Manaus

Keywords

COVID-19; CoronaVac; P.1 variant; test-negative study; case-control study; Brazil

Introduction

The P.1 variant of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in Manaus, Brazil, in December 2020[1–3] and has since spread globally.[4] The World Health Organisation declared P.1 as a *Variant of Concern*[5] given the evidence for its increased transmissibility[2] and potential to cause reinfection.[6] The spread of P.1 has been suggested to be a contributing factor to Brazil's recent COVID-19 resurgence, during which 6,453,057 cases and 151,467 deaths have been reported between 1 December 2020 and 31 March 2021.[7] A critical question is whether available vaccines in Brazil and South America are effective against COVID-19 in the context of P.1 transmission.

Concerns have been raised that available vaccines have reduced immunogenicity against P.1. The variant has three mutations, K417N, E484K and N501Y, in the ACE2 binding site of the SARS-CoV-2 S1 protein which have been speculated to promote immune escape.[2] P.1 emerged in the setting of presumably high selective immune pressure, since Manaus experienced a large epidemic and had estimates of seroprevalence as high as 76%[8,9] prior to emergence of the variant. *In vitro* studies have found decreased sero-neutralisation of P.1 in individuals infected with non-P.1 strains and vaccinated individuals.[10–14] However, evidence is lacking on whether available vaccines are effective against clinical and infection outcomes associated with P.1 and in settings of P.1 transmission in Brazil and beyond.

As part of its vaccination campaign, Brazil has administered CoronaVac, an inactivated vaccine.[15,16] CoronaVac was found to have an efficacy of 50% and 84% against, respectively, mild and moderate COVID-19 in a randomised controlled trial (RCT) conducted in Brazil prior to the emergence of P.1.[17] However, the effectiveness of CoronaVac in the real-world setting and in regions of P.1 transmission is unknown. We performed a test-negative case-control study[18,19] on the effectiveness of CoronaVac in healthcare workers (HCWs) from Manaus, which was among the first cities in Brazil to aggressively implement vaccination. Herein, we report early findings on the effectiveness of administering at least one dose of the two-dose schedule, in response to the need to evaluate current vaccination efforts as they are being widely implemented.

Methods

Study setting

Manaus is a city with 2.2 million inhabitants in the Amazon Basin of Brazil.[20] As of 2 April, 2021, 160,803 cases (cumulative incidence, 7,367 per 100,000 population) and 8,432 COVID-19 associated deaths (cumulative mortality, 386 per 100,000 population) were reported in Manaus during the course of an initial epidemic in March 2020 and a second larger epidemic in late November 2020 (appendix p 13, Figure 1).[21] The second epidemic was associated with the emergence and spread of P.1,[1] which accounted for 75% (657 of 877) of SARS-CoV-2 samples genotyped as part of surveillance during the peak of the epidemic in January 2021.[3] The Municipal Secretary of Health of Manaus (SEMSA) initiated vaccination with CoronaVac and ChAdOx1 on 19 January 2021; CoronaVac has been used in >97% of the vaccinations of HCWs (Figure 1).

Study design

We conducted a retrospective, test negative, matched case-control study to estimate the effectiveness of CoronaVac in reducing the odds of primary and secondary outcomes of, respectively, symptomatic and all RT-PCR-confirmed SARS-CoV-2 infections. The study population was HCWs who had a residential address in Manaus, aged ≥ 18 years on 19 January, 2021, and with complete information, which was

consistent between data sources, on age, sex, neighbourhood (*bairro*) of residence, and vaccination status and dates (Figure 2). For this study, we selected cases and matched controls who had a positive and negative SARS-CoV-2 RT-PCR test result, respectively, during the study period of 19 January to 25 March, 2021.

The study design and statistical analysis plan were specified in advance of extracting information from data sources and are described in a publicly available protocol (https://github.com/juliocroda/VebraCOVID-19) and the appendix (pp 1-12). The study was approved by the Ethical Committee for Research of Federal University of Mato Grosso do Sul (CAAE: 43289221.5.0000.0021).

Data sources

We identified the study population from the SEMSA registry of employed HCWs in Manaus (Figure 2). For the purpose of extracting information for study population eligibility, case and control section, matching criteria, secondary outcomes and covariates, we integrated data from the following sources: the national laboratory testing registry; the national registry of users of the universal health system; the national surveillance system of suspected COVID-19 cases; the national surveillance database of severe acute respiratory illnesses; and the SEMSA COVID-19 vaccination registry. The study was implemented with data that were accessed on 1 April, 2021 and censored after 25 March, 2021 to account for reporting delays.

Selection of cases and matched controls

Cases were selected from the study population who had a SARS-CoV-2 infection, defined as a positive SARS-CoV-2 RT-PCR test result from a respiratory sample that was collected during the study period and the absence of a positive test in the preceding 90 day period; and who did not receive a dose of ChAdOx1 vaccine before sample collection. Controls were selected from the study population who did not have a SARS-CoV-2 infection, defined as a negative SARS-CoV-2 test result from a respiratory sample that was collected during the study period and the absence of a positive test in the subsequent 14-day period or previously in the study period; and did not receive a dose of ChAdOx1 vaccine before sample collection.

We matched one test-negative control to each case according to symptomatic illness status at time of testing; a time window of ±3 days between the case sample collection date; age category defined as <30, ≥30 and <60, and ≥60 years; and neighbourhood of residence. Symptomatic illness was defined as the presence of one or more reported COVID-19 related symptom[22] with an onset within 0-10 days before the date of sample collection.

Statistical analysis

To ensure timely communication of results with potential public health benefit, we planned an early analysis with exposure defined as having received at least one dose of the two-dose CoronaVac schedule, with the first dose administered ≥14 days before the sample collection date for their RT-PCR test. We pre-specified this analysis in the study protocol and used the O'Brien Fleming alpha-spending method to calculate an adjusted critical p-value of 0.0492.[23] We also evaluated the exposure of receiving the first vaccine dose from 0 to 13 days before the sample collection date, as a non-null association during this period, where the vaccine likely has no or limited effectiveness,[15,16] may serve as an indicator of unmeasured confounding or bias. The reference group for vaccination status was individuals who had not received a first vaccine dose by the date of sample collection.

Analyses of the primary outcome of symptomatic SARS-CoV-2 infection included case-control pairs who had symptomatic illness before or at the time of testing. Analyses of the secondary outcome of any SARS-CoV-2 infection included additional case-control pairs who did not have symptomatic illness before or at the time of testing.

We used conditional logistic regression to estimate the odds ratio (OR) of vaccination among cases and controls. 1-OR provided an estimate of vaccine effectiveness under the assumptions of a test-negative design.[24] We included as covariates in the adjusted model: age as a continuous variable, sex, occupation category, self-reported race/skin colour, number of previous healthcare interactions from the beginning of the pandemic to the start of the study, and a SARS-CoV-2 infection, defined as a positive RT-PCR or antigen detection test, before the study period. A missing indicator was incorporated to address missing information on occupation category or race. We adjusted for the possible effect of COVID-19-associated comorbidities[25] in a separate sensitivity analysis due differential completeness for this covariate between cases and controls.

Power calculation

After generating matched case-control pairs and before performing the analyses, we simulated the power of the data set to identify a vaccine efficacy of 60% comparing those with at least one dose ≥14 days after the first dose to those who had not received a vaccine (appendix pp 1-12). After extracting the surveillance databases on 1st April, 2021, we determined that the power of the study was 92.7%.

All analyses were done in R, version 4.0.2.

Role of the funding source

All funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The Health Surveillance Foundation of the State of Amazonas and SEMSA reviewed the data from the study, but the academic authors retained editorial control. MDTH, OTR, MSST, SO, and JC had full access to de-identified data in the study and MDTH and OTR verified the data, and all authors approved the final version of the manuscript for publication.

Results

Second COVID-19 epidemic and vaccination campaign among HCWs in Manaus

Among 67,718 HCWs that were employed in the city's healthcare facilities and linked to surveillance databases (Figure 2), 3,429 cases of SARS-CoV-2 infection were reported during the 2nd epidemic from 1 October 2020 to 25 March 2021 (Figure 1). Among the 3,429 cases, 2,512 and 917 were associated with and without, respectively, COVID-19 symptoms. The municipal vaccination campaign was initiated on 19 January, 2021 and as of 25 March, 2021, has administered first and second vaccine doses to 55,240 (82%) and 48,225 (71%), respectively, of the 67,718 HCWs.

Study population

Among the 67,718 HCWs, 53,176 were eligible for inclusion in the study (Figure 2). Of the 53,176 HCWs, 1,752 and 904 received RT-PCR testing during the study period of 19 January to 25 March, 2021 who respectively, did or did not report a symptomatic illness at the time of testing. Among the 1,823 and 974 tests performed for HCWs with and without symptomatic illness, respectively, 564 (31% of 1,823) and 212 (22% of 974), respectively, were positive. Through matching, we selected 780 HCWs with 786 RT-

PCR tests to establish 393 case-control pairs with symptomatic illness and 266 HCWs with 270 RT-PCR tests to establish 135 pairs without symptomatic illness. The timing of pair enrollment during the study period is shown in Appendix p 14.

Table 1 shows the distribution of characteristics between cases and controls. The proportion of females was lower among the cases. The proportion who had a positive SARS-CoV-2 RT-PCR or antigen test prior to the study period was small, but higher among controls than cases in pairs with symptomatic illness and without symptomatic illness (2.8 vs 6.6% and 0.7 vs 11.1%, respectively). Of the 528 cases, 10.6% (56) required hospitalisation for their SARS-CoV-2 infection. P.1 was identified in SARS-CoV-2 samples from four of five cases for which genotyping information was available (data not shown).

Primary outcome

After adjusting, vaccination with at least one CoronaVac dose was associated with a 0.50-fold reduction (adjusted VE, 49.6%; 95% CI, 11.3 - 71.4) in the odds of symptomatic SARS-CoV-2 infection during the period 14 days or more after receiving the 1st dose. Of note, the odds of symptomatic SARS-CoV-2 infection was increased (OR, 1.69; 95% CI, 1.09 - 2.64) amongst vaccinated HCWs in the period 0-13 days after receiving the first vaccine dose when compared with HCWs who did not receive the vaccine. Female sex (OR, 0.50; 95% CI, 0.38 - 0.81) and a positive SARS-CoV-2 RT-PCR or antigen test in the pre-study period (OR, 0.38; 95% CI, 0.17 - 0.87) were associated with a reduced odds of symptomatic SARS-CoV-2 infection.

Secondary outcome

Vaccination with at least one dose of CoronaVac was not associated (adjusted VE, 35.1%; 95% CI, -6.6 - 60.5) with a reduction in the risk of SARS-CoV-2 infection, regardless of presence or absence symptomatic illness, during the period 14 days or more after receiving the 1st dose (Table 2). As observed in analyses of the primary outcome, the risk of any SARS-CoV-2 infection was increased amongst vaccinated HCWs in the preceding period 0-13 days after receiving the 1st vaccine dose (OR, 1.85; 95% CI, 1.26 - 2.71) and reduced amongst female HCWs (OR 0.69, 95% CI, 0.50 - 0.94) and those who had a positive SARS-CoV-2 RT-PCR or antigen test in the pre-study period (OR, 0.27; 95% CI, 0.13 - 0.55). For primary and secondary outcomes, estimates for vaccine effectiveness and significant covariates were similar when adjusting for presence of one or more underlying comorbidities (appendix pp 15-16).

Discussion

Here, we provide evidence for the effectiveness of CoronaVac in the setting of widespread P.1 transmission. Estimated effectiveness after at least one dose of vaccine was 49.6% (95% CI 11.3-71.4) against symptomatic COVID-19, starting 14 days after administration of the first dose, in healthcare workers from Manaus. These findings address a key evidence gap on the real-world effectiveness of CoronaVac and its potential effectiveness against P.1. An RCT of CoronaVac in Brazil reported efficacy of 50.7% (95% CI 35.6 - 62.2), 83.7% (95% CI 58.0-93.7) and 100% (95% CI 56.4-100) against respectively mild, moderate and severe SARS-CoV-2 infection.[17] An RCT from Turkey provide consistent evidence for the efficacy of this vaccine.[17] However, these trials were conducted prior to the emergence of P.1. The evidence from our study informs ongoing vaccination efforts with CoronaVac in Brazil and other countries where the P.1 or other variant lineages are circulating.

We could not directly address whether CoronaVac was effective against P.1 as SARS-CoV-2 samples from HCW cases were not routinely genotyped. However, the study was conducted at the epicentre for P.1 emergence and during an epidemic when surveillance of the general population identified the variant in 75% of genotyped samples. Consistent with this finding, P.1 was identified in 4 of 5 genotyped samples from HCW cases. It seems plausible that vaccination with CoronaVac conferred a level of protection against P.1 and that our estimates reflect VE in the real-world setting of high P.1 transmission.

We addressed multiple potential sources of bias in this observational setting. The use of a test negative design allowed for control of healthcare-seeking behaviour among study participants, albeit with limits. For the analysis of the primary outcome, we restricted cases and controls to patients with evidence of any symptoms proximal to the time of testing. We were unable to perform additional matching with detailed symptom data and can not exclude the possibility of differential test-seeking between vaccinated and unvaccinated individuals with varying symptomology.[26] However, a strength of the surveillance system in Manaus was the large source of individual level data that allowed us to match on a number of variables in order to avoid confounding between vaccination and SARS-CoV-2 outcomes.

Our estimates may be subject to unmeasured and residual confounding. We addressed this possibility by evaluating the risk associated with being vaccinated 0-13 days before testing, when COVID-19 is likely to be ineffective or have reduced effectiveness.[15] The significantly higher risk of SARS-CoV-2 infection observed amongst vaccinees during this period may relate to time varying changes in exposure risk or testing behavior, as suggested in other studies.[27,28] Nevertheless, the positive risk association in the bias indicator suggests that our study provides a conservative estimate of VE in the study population.

The generalisability of our VE estimates to other contexts is limited in two ways. Firstly, individuals having received at least one vaccine dose represent a mixture of those who received one and two doses. The estimated effectiveness of having received at least one dose is dependent on the speed of vaccine roll-out and the change in incidence over the study period. As incidence decreased over the study period, fewer cases and controls were selected later in the study (appendix p 14), when vaccinated individuals were more likely to have received two doses.

Secondly, SARS-CoV-2 seroprevalence in Manaus was likely high prior to the vaccination campaign with estimates reported to be 76%.[8,9] Several studies found that vaccination in individuals with a previous infection elicits a strong and rapid immune response,[29,30] as well as cross-neutralizing antibody response to P.1.[11] Overall immunogenicity following a single dose in this population may thus be higher than in a low-seroprevalence population. Conversely, prior natural infection may have conferred protection in unvaccinated individuals, which in turn may lead to downward bias in the VE estimate. We did not have sufficient power to explore the effect modification of vaccine effectiveness by previous infection as access to RT-PCR and antigen detection testing was significantly limited during COVID-19 epidemics in the city.

VE estimates from this observational study may not be directly translatable to estimates obtained from RCTs. Vaccine effectiveness is dependent on the severity of disease used as outcomes. As our surveillance relied upon individuals seeking testing, our estimate may be most comparable to VE (78%;

95% CI 46.2-90.4) reported from the Brazil trial for moderate COVID-19, for which cases required medical treatment. However, incomplete data on symptoms in our study leaves uncertainty about the severity of illness against which we are estimating vaccine effectiveness

Further investigation is needed to delineate the effectiveness of administering the first dose of CoronaVac alone versus completion of a two dose schedule, the effectiveness against severe COVID-19 outcomes and whether the vaccine is indeed effective against P.1 in a formal sieve analysis in real-world settings. Nevertheless, as the vaccination campaign continues across Brazil and other countries with widespread P.1 circulation, our findings will be important to reduce vaccine hesitancy among the public and reassure policy makers of the effectiveness of mass vaccination with CoronaVac. Furthermore, the evidence generated by this study provides the rationale for rapidly securing vaccine supply and deploying the vaccine, in addition to implementing other effective non-medical countermeasures, as part of the emergency response to the public health crisis in Brazil and potential impending outbreaks in regions where P.1 has spread.

Contributors

MDTH and OTR share co-first authorship. MSST and SBO were responsible for linkage, cleaning and de-identifying the databases. MDTH, OTR, DATC, JRA, AIK and JC participated in the design and concept of the study and designed the data analysis. MDTH and OTR wrote the first version of the manuscript. WNA, MA, RS, AMS, BCA, SHHF, CFC supervised the study. All authors participated in data interpretation, revised the manuscript, and approved the final version of the manuscript. MSST and DB have verified the underlying data.

Declaration of interests

We declare no competing interests.

Data sharing

Deidentified databases as well as the R codes will be deposited in the repository https://github.com/juliocroda/VebraCOVID-19

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Figure Legends

Figure 1. SARS-CoV-2 infections and vaccination coverage amongst 67,718 healthcare workers (HCW) in Manaus, Brazil between 1 October 2020 to 25 March 2021. Daily RT-PCR confirmed SARS-CoV-2 infections with and without COVID-19 symptoms are shown as red and blue bars, respectively. Green and blue lines depict the daily cumulative proportion of the 67,718 HCWs who received respectively, a first and second dose of a COVID-19 vaccine. The grey shade denotes the study period, which began with the initiation of the vaccine campaign on 19 January, 2021 and ended on 25 March, 2021.

Figure 2. Flowchart for case and control selection.

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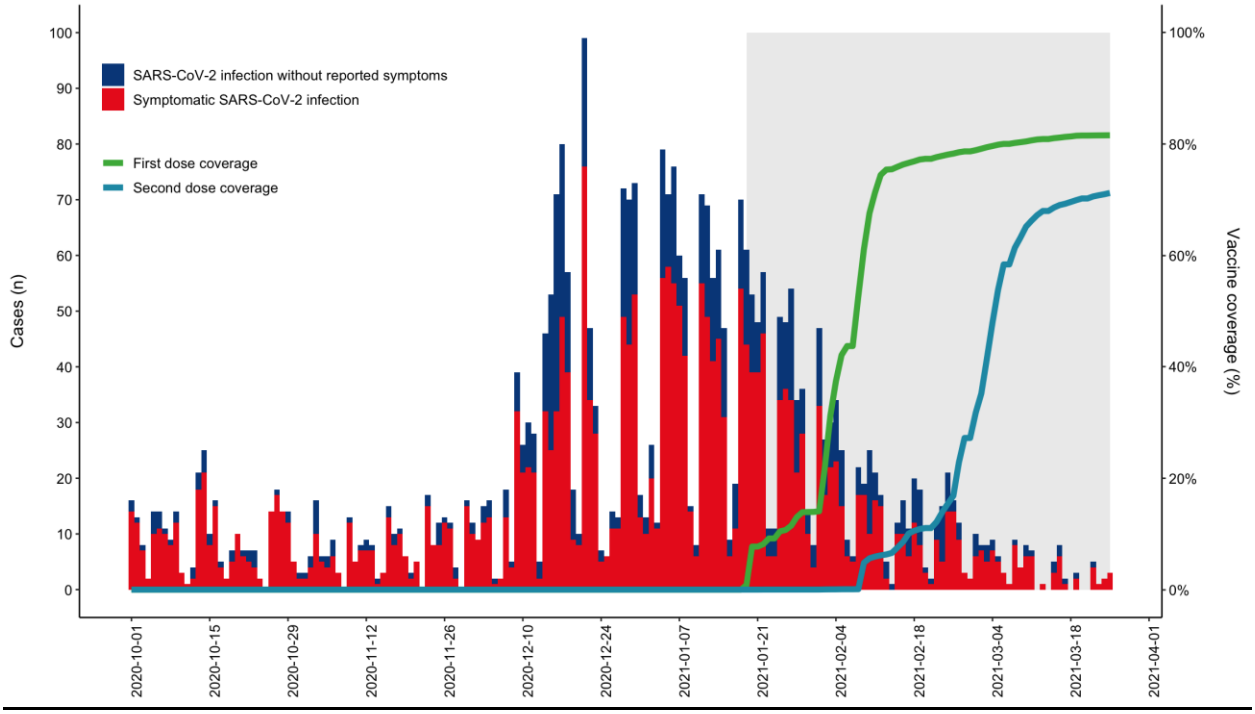

Figure 2. Flowchart for case and control selection

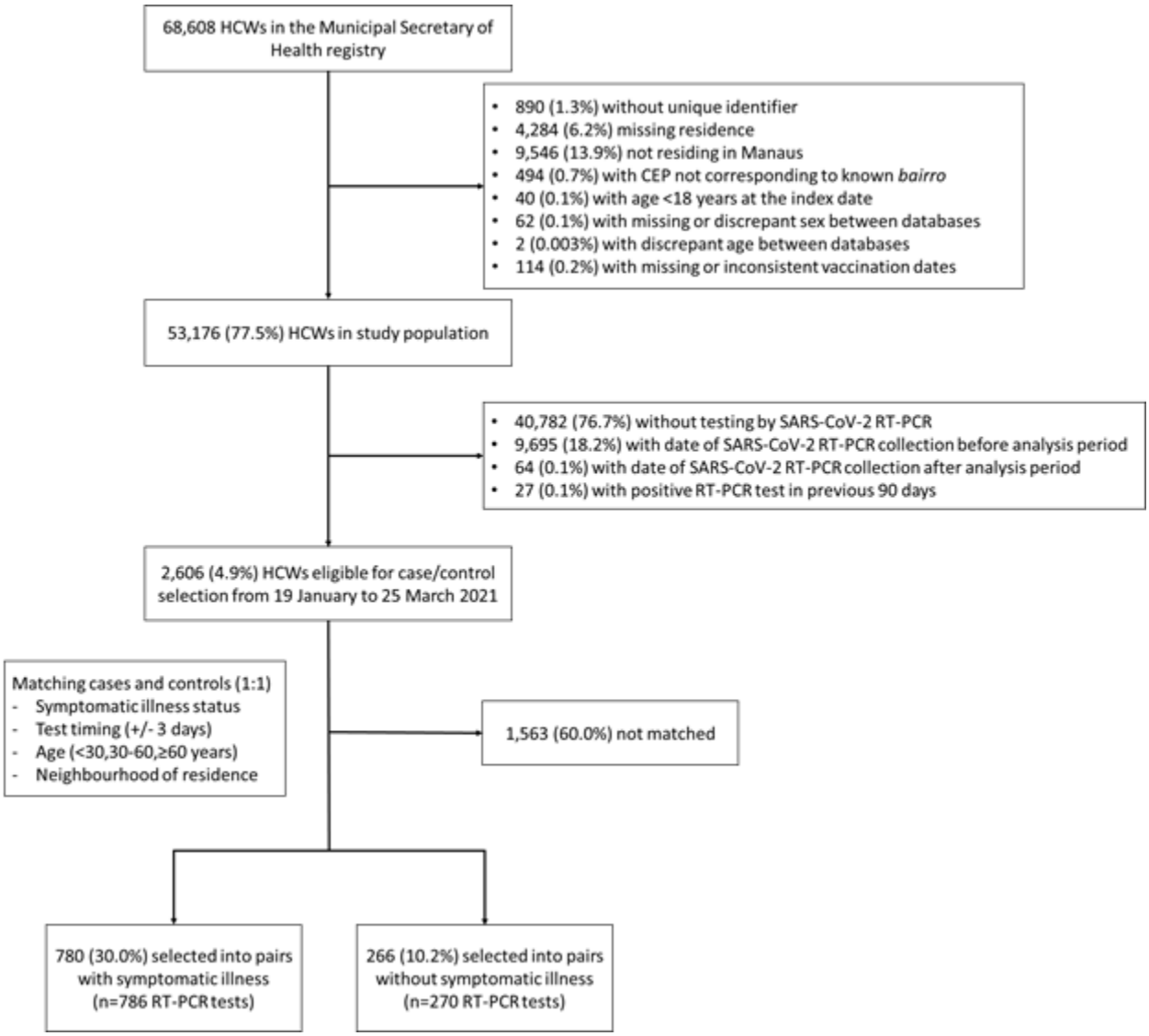

Table 1: Comparison of cases and controls

	With symptomatic illness		Without symptomatic illness	
Characteristics	**Cases (n=393)**	**Controls (n=393)**	**Cases (n=135)**	**Controls (n=135)**
Age	43.3 (9.5)	42.7 (9.4)	43.6 (8.4)	43.4 (9.0)
Female sex	276 (70.2%)	313 (79.6%)	100 (74.1%)	96 (71.1%)
Self-reported race/skin colour*				
Amarela/Yellow	52 (13.2%)	70 (17.8%)	20 (14.8%)	25 (18.5%)
Preta/Black	5 (1.3%)	3 (0.8%)	2 (1.5%)	4 (3.0%)
Pardo/Brown	237 (60.3%)	229 (58.3%)	85 (63.0%)	79 (58.5%)
Branca/White	81 (20.6%)	73 (18.6%)	14 (10.4%)	16 (11.9%)
Missing	18 (4.6%)	18 (4.6%)	14 (10.4%)	11 (8.1%)
Occupation category				
Administrative	108 (27.5%)	90 (22.9%)	43 (31.9%)	39 (28.9%)
Clinician	17 (4.3%)	8 (2.0%)	3 (2.2%)	6 (4.4%)
Nurse/nurse technician	152 (38.7%)	170 (43.3%)	51 (37.8%)	38 (28.1%)
Other health professional	41 (10.4%)	44 (11.2%)	19 (14.1%)	20 (14.8%)
Other health associated	68 (17.3%)	68 (17.3%)	13 (9.6%)	23 (17.0%)
Missing	7 (1.8%)	13 (3.3%)	6 (4.4%)	9 (6.7%)
Number of healthcare encounters[†]	0.81 (1.09)	0.83 (1.14)	0.76 (1.05)	1.09 (1.14)
Prior positive SARS-CoV-2 test[†‡]	11 (2.8%)	26 (6.6%)	1 (0.7%)	17 (12.6%)

*Race/skin colour as defined by the Brazilian national census bureau (Instituto Nacional de Geografia e Estatísticas) https://biblioteca.ibge.gov.br/visualizacao/livros/liv63405.pdf

[†]Prior to the start of the study on 19 January, 2021.

[‡] Defined as SARS-CoV-2 RT-PCR or antigen detection.

Table 2: Vaccine effectiveness against symptomatic SARS-CoV-2 infection, and against any SARS-CoV-2 infection, with at least one dose and at least 14 days after administration of the first dose

	Primary outcome: Symptomatic SARS-CoV-2 infection		**Secondary outcome: All SARS-CoV-2 infection**	
	aOR (95% CI)	**p-value**	**aOR (95% CI)**	**p-value**
Unadjusted Analysis				
0-13 days after 1st vaccine dose vs. unvaccinated*	1.61 (1.07-2.44)	0.02	1.81 (1.26-2.61)	<0.001
≥14 days after 1st vaccine dose vs. unvaccinated*	0.56 (0.32-0.95)	0.03	0.68 (0.43-1.09)	0.11
Adjusted analysis				
0-13 days after 1st vaccine dose vs. unvaccinated*	1.69 (1.09-2.64)	0.02	1.85 (1.26-2.71)	<0.001
≥14 days after 1st vaccine dose vs. unvaccinated*	0.50 (0.29-0.89)	0.02	0.65 (0.40-1.07)	0.09
Age	1.01 (0.99-1.03)	0.26	1.01 (0.99-1.03)	0.24
Female sex	0.55 (0.38-0.81)	<0.001	0.69 (0.50-0.94)	0.02
Self-reported race/skin color†				
Amarela vs. Pardo	0.79 (0.51-1.23)	0.30	0.78 (0.54-1.14)	0.21
Preta vs. Pardo	2.03 (0.43-9.59)	0.37	1.06 (0.33-3.37)	0.93
Branca vs. Pardo	1.19 (0.81-1.77)	0.38	1.11 (0.78-1.59)	0.56
Occupation category				
ADM vs. Nurse	1.09 (0.74-1.62)	0.67	1.03 (0.74-1.44)	0.86
Clinical vs. Nurse	2.13 (0.8-5.63)	0.13	1.44 (0.63-3.25)	0.39
Other Health Associated vs. Nurse	0.92 (0.57-1.47)	0.71	0.78 (0.52-1.17)	0.22
Other Health Professional vs. Nurse	0.90 (0.53-1.54)	0.71	0.79 (0.50-1.25)	0.31
Prior healthcare encounters‡				
1-3 vs. 0	1.01 (0.74-1.39)	0.94	0.85 (0.65-1.11)	0.23
≥4 vs. 0	1.49 (0.58-3.82)	0.41	1.21 (0.54-2.69)	0.65
Prior positive SARS-CoV-2 test‡**	0.38 (0.17-0.87)	0.02	0.27 (0.13-0.55)	<0.001

*At date of index sample collection for cases and controls

†Race/skin color as defined by the Brazilian national census bureau (Instituto Nacional de Geografia e Estatísticas) https://biblioteca.ibge.gov.br/visualizacao/livros/liv63405.pdf

‡Prior to the start of the study on 19 January, 2021

**Defined as SARS-CoV-2 RT-PCR or antigen detection

Effectiveness of an inactivated SARS-Cov-2 vaccine amongst healthcare workers in the setting of high P.1 variant transmission in Brazil

Supplementary Materials

Contents:

Evaluation of Vaccine Effectiveness in Brazil against COVID-19 (VEBRA-COVID):

A Test-Negative Case-Control Study Protocol

Version: 01.1 / April 3rd 2021

I. Background

Since the emergence of severe acute respiratory virus coronavirus 2 (SARS-CoV-2), Brazil has experienced one of the world's highest incidence and mortality rates in the world, with over 10 million reported infections as of the end of February 2021.[1–3] Of grave concern is the resurgence of COVID-19 cases and hospitalizations observed in the city of Manaus, despite high estimated seroprevalence.[4,5] One hypothesis for this resurgence is loss of immunity to the P.1 Variant of Concern (VOC), which was first detected in Manaus on Jan 12, 2021, and now consists the majority of new infections, including all genomes sequenced in this city in February 2021.[6–8] This lineage has accrued mutations associated with decreased neutralization,[9,10] and has since spread throughout Brazil.

The rapid development of novel vaccines against COVID-19 allowed countries to start vaccine distribution programs within a year of the identification of the novel virus. Among the first vaccines to be developed was Sinovac's CoronaVac vaccine.[11,12] Phase III trials were conducted in Turkey and Brazil, and results were announced on February 5, 2021, in which effectiveness after 14 days following vaccination with 2 doses of vaccine was reported to be 50.65% for all symptomatic cases of COVID-19, 84% for cases requiring medical attention, and 100% for hospitalized, severe, and fatal cases.[13] CoronaVac was approved for emergency use on 17 January in Brazil, and used to vaccinate healthcare workers and the general population, beginning with the oldest age groups, on 19 January 2021.

AstraZeneca-Oxford's ChAdOx1 vaccine was approved on the same day and was administered beginning on 23 January 2021.

As vaccine programs continue, there has been much interest in estimation of vaccine effectiveness through observational studies, and specifically in settings where VOC are circulating. Such studies have advantages over clinical trials, including increased size and follow-up time, and reduced cost. However, as vaccinated and unvaccinated individuals are likely different in their SARS-CoV-2 risk and healthcare access, these studies must address bias through design and analysis. Several studies have demonstrated the effectiveness of COVID-19 vaccines against infection caused by the B.1.1.7 variant.[14] However, large-scale real-world investigations on vaccine effectiveness have not been conducted in regions where the P.1 variant is prevalent.

We propose a test-negative case-control study[15,16] of healthcare workers (HCWs) from the city of Manaus to evaluate the effectiveness of vaccines in preventing COVID-19 in a setting of widespread P.1 VOC transmission.[7] Manaus was selected as the site for the HCW study since it was the first city to aggressively vaccinate HCWs in response to the P.1 epidemic. The study will be limited to evaluating the effectiveness of CoronaVac since 97% of vaccinated HCWs in Manaus received this vaccine. We will expand the study population as additional age groups become eligible for vaccination. Furthermore, we expect that additional vaccines will be approved and will evaluate their effectiveness. We will therefore continue to amend the protocol and its objectives accordingly to address these new questions.

II. Objectives

1. To estimate the effectiveness of CoronaVac against symptomatic SARS-CoV-2 infection amongst healthcare workers from the city of Manaus.

III. Methods

1. Study Design: We will conduct a retrospective matched case-control study, enrolling cases who test positive for SARS-CoV-2 and controls who test negative for SARS-CoV-2 amongst HCWs (Section 3) and the general population (Section 4) as of the day that the COVID-19 vaccination campaign was initiated at the study sites. The study will evaluate vaccine effectiveness on the primary outcome of symptomatic SARS-CoV-2 infection and secondary outcome of SARS-CoV-2 RT-PCR test positivity regardless of symptoms. We will identify cases and matched controls by extracting information from health surveillance records and ascertain the type and data of vaccination by reviewing the state COVID-19 vaccination registry. In this design, the odds ratio of vaccination comparing cases and controls estimates the direct effect of vaccination on the disease outcome. We will perform interim analyses aimed at evaluating the effectiveness of receiving at least one vaccine dose and a final analysis that will evaluate the effectiveness of completing the approved vaccine series. In a separate analysis, we will assess the association between vaccination and hospitalization and/or death among individuals who have tested positive for SARS-CoV-2.

2. IRB and Ethics Statement: The protocol has been submitted to the Ethical Committee for Research of Federal University of Mato Grosso do Sul (CAAE: 43289221.5.0000.0021). The work of investigators at the University of Florida, Yale University, Stanford University, and Barcelona Institute for Global Health was conducted to inform the public health response and was therefore covered under Public Health Response Authorization under the US Common Rule.

3. Study Details

Study Site: Manaus (3°5'S, 60°W) is the capital of the state of Amazonas, the major urban metropolis in the middle of the Amazon jungle, and a major river port for seafaring vessels. In 2020, Manaus, with an estimated population of 2,219,580 inhabitants, reported 144,767 COVID-19 cases (cumulative incidence: 6,522 per 100,000 population) and 7,605 deaths (cumulative mortality: 342 per 100,000 population). Manaus has 40 Family Health (*Plano Saúde da Familia*) teams, 53 primary health care centers and 15 other health units under the responsibility of the Secretariat of Health of Manaus, and 20 private or public hospitals. The Municipal Secretary of Health of Amazonas initiated its COVID-19 vaccination campaign on 19 January 2021 and is administering two vaccines, CoronaVac and ChAdOx1. CoronaVac has been used in >97% of the vaccinations of HCWs.

Data Sources and Integration: The overall approach will be to: 1) Identify the cohort of all HCW from Manaus from *state HCW registries*; 2) Identify eligible cases and controls from the cohort who test positive and negative, respectively, from the *state laboratory testing registry* of public health laboratory network; 3) Determine vaccination status from *municipal vaccination registries*; and 4) Extract information from *national healthcare and surveillance databases* that will be used to define outcomes, match controls to cases, determine vaccination status, serve as covariates for post-stratification and provide a source for cross-validation of information from databases. Data sources will include:

- SES-AM HCW registry
- National health plan registry of users (**CADSUS**)
- National laboratory testing registry (**GAL**) of the network of public health laboratories
- Municipal COVID-19 vaccination registry
- National surveillance database of severe acute respiratory illnesses (**SIVEP-Gripe**) created by Ministry of Health Brazil in 2009
- National surveillance system of suspected cases of COVID-19 (**e-SUS**) from mild to moderate "influenza like illness", created by the Ministry of Health Brazil in 2020
    - e-SUS includes information from healthcare telemonitoring, whereby teams of healthcare practioners make daily telephone calls, assess symptoms, identify signs of severity and triage patients to healthcare facilities.
    - National mortality registry (**SIM**) from the Ministry of Health

We will build a parent database using the mySQL language and integrated individual datasets using an application programming interface (API), which was developed using ElasticSearch. Table 1 in the Supplementary material lists the variables extracted from datasets and incorporated in the parent database. The database will be updated on a weekly basis.

We will use CPF numbers (Brazilian citizens' unique identifier code) to integrate datasets. For those entries missing CPF in SEMSA, we will perform a probabilistic record linkage between SEMSA and CADSUS (registration database of users of the public universal health system [SUS] in Brazil). For the probabilistic method we will use the Reclink III software,[17] and consider sex, the phonetic code of the first and last name and the phonetic code of the first name of the mother as blocking variables. We will compare the similarity of the name, mother's name with a threshold of >85% similarity, and date of birth a threshold of >65% similarity. All pairs identified by the probabilistic method will be manually reviewed and revised.

Some variables were reported in multiple data sources. To define a single variable, we drew from each database with priority given to databases that were more complete, reliable, and up-to-date. We will choose age from the data sources in the following order: CADSUS, SEMSA, GAL, e-SUS, SIVEP-Gripe. We will define neighborhoods (*bairro*) by extracting information on CEP (Brazilian zipcode) and transforming them to neighborhoods or directly extracting information on neighborhoods from data sources in the following order: CADSUS (CEP), CADSUS (*bairro*), SEMSA (CEP), and e-SUS (CEP).

Study Population

Inclusion criteria:

- Healthcare worker as defined by the SEMSA registry,
- Has a residential address within the city of Manaus,
- Age ≥ 18 years before 19 January 2021,
- With complete information, which is consistent between databases, on age, sex, and residential address defined by CEP (zip code)
- With complete and consistent vaccination status and dates.

Exclusion criteria:

- Not a healthcare worker as defined by the SEMSA/SES-AM registry,
- Does not have a residential address within the city of Manaus,
- Aged < 18 years before 19 January 2021,
- With missing or inconsistent information on age, sex, or residential address defined by CEP (zip code)
- With incomplete or inconsistent vaccination status or dates.

Case definition and eligibility: We will use information from integrated GAL/SIVEP-Gripe/e-SUS databases to identify eligible cases. Cases are defined as eligible members of the study population (as defined above, Study Population) who:

- Had a sample with a positive SARS-CoV-2 RT-PCR, which was collected between January 19, 2021 and 7 days prior to database extraction of information

- Did not have a positive RT-PCR test in the preceding 90 day period,
- Have complete and consistent data on SARS-CoV-2 PCR test result,
- Did not receive a dose of ChAdOx1 vaccine before the date of respiratory sample collection.

Control definition and eligibility: We will use integrated GAL/SIVEP-Gripe/e-SUS databases to identify eligible controls. Controls are defined as eligible members of the study population who:

- Had a sample with a negative SARS-CoV-2 RT-PCR result, which was collected after January 19, 2021,
- Did not have a subsequent positive PCR test in the following 7-day period
- Have complete and consistent data on SARS-CoV-2 PCR test result,
- Did not receive a dose of ChAdOx1 vaccine before the date of respiratory sample collection.

Matching: Test-negative controls will be matched 1:1 to the cases. Matching factors will include variables that are anticipated to be causes of the likelihood of receiving the vaccine, risk of infection and likelihood of receiving PCR testing for SARS-CoV-2 (i.e. healthcare access and utilization) (see Figures 1-3):

- Symptomatic illness status at time of testing, defined as the presence or absence of one or more reported COVID-19 related symptom with onset within 0-10 days before the date of their positive and negative PT-PCR test, respectively, for case and controls,
- Residential address (neighborhood [*bairro*], which is identified based on the first 5 digits of 8 digit CEP),
- Age (categorized as <30, ≥30 and <60, and ≥60 years; Figure 3 shows similar rates of testing positive for individuals aged 30-60),
- Window of ±3 days between collection of RT-PCR positive respiratory sample for cases and collection of RT-PCR negative respiratory sample for controls. If the date of respiratory sample collection is missing, the date of notification of testing result will be used.

We chose the matching factors to balance the ability to reduce bias and to enroll sufficient case-control pairs. We chose to categorize age as <30,30-60, and ≥60 years because the proportion of RT-PCR tests that were positive appeared to be fairly constant in the middle age band, reflecting a similar risk for infection in this group, albeit possibly differential healthcare utilization. We observed a negative correlation between the proportion of positive RT-PCR tests in a neighborhood and the average time until receipt of first dose, possibly implying a positive correlation between access to testing and access to vaccination. Finally, as incidence decreased over the study period as vaccine coverage increased, time was a clear confounder, and matching was considered an efficient way to address this bias.

We will use the standard algorithms to conduct matching which include: 1) setting a seed, 2) locking the database, 4) creating a unique identifier for matching after random ordering, 5) implementing exact matching based on matching variables, sampling controls at random if more than one available per case within strata.

An individual who fulfils the control definition and eligibility and later has a sample tested that fulfils the case definition and eligibility can be included in the study as both a case and a control. An individual who fulfils the control definition for multiple different sample collection dates can be included in the study as a control for each collection date, up to a maximum of three times.

Exposure definition: CoronaVac vaccination in the following stratifications:

- Received the first vaccine dose, and not having received a second dose, in the following time periods relative to sample collection for their PCR test:
    - 0-13 days
    - ≥14 days
- Received the second dose in the following time periods relative to sample collection for their PCR test:
    - 0-13 days
    - ≥14 days

Statistical Analyses: We will evaluate the effectiveness of CoronaVac for the following SARS-CoV-2 infection outcomes:

- Primary: Symptomatic COVID-19, defined as one or more reported COVID-19 related symptom with onset within 0-10 days before the date of their positive RT-PCR test
- Secondary:
    - SARS-CoV-2 RT-PCR test positivity
    - COVID-19 associated hospitalization within 14 days of the symptom onset
    - COVID-19 associated death within 28 days of symptom onset

We will evaluate vaccine effectiveness for the primary outcome and the secondary outcome of test positivity in case control analyses according to the test-negative design. Table 2 shows a list of all planned analyses in the test-negative design. The test-negative design may introduce bias when evaluating outcomes of hospitalizations and deaths during an epidemic. We will therefore perform survival analyses of HCWs who test positive to evaluate the association of vaccination status and the risk for hospitalization and death after infection.

Case-control analysis: Analyses of the primary outcome will be restricted to case and control pairs who are matched based on the presence of a COVID-19 related symptom before or at the time of testing. Analyses of the secondary outcomes of test positivity will include additional case and control pairs who are matched based on the absence of a COVID-19 related symptom before or at the time of testing.

We will use conditional logistic regression to estimate the odds ratio (OR) of vaccination among cases and controls, accounting for the matched design, where 1-OR provides an estimate of vaccine effectiveness under the standard assumptions of a test-negative design. The reference group will be individuals who have not received a first dose of CoronaVac by the date of respiratory sample collection. Date of notification of the testing result will be used if the date of respiratory sample collection is

missing. To evaluate potential biases and the timing of vaccine effectiveness after administration, we will evaluate the windows of vaccination status corresponding to 0-13 days and ≥14 days after the first dose and 0-13 days and ≥14 days after the 2nd dose.

We will include the following covariates in the adjusted model, which we hypothesize are predictive of vaccination, the risk of SARS-CoV-2 infection and COVID-19 severity and healthcare access and utilization:

- Age as continuous variable
- Sex
- Occupation category
- Self-reported race/skin color
- Number of previous entries in e-SUS or SIVEP-Gripe surveillance databases
- Evidence of prior SARS-CoV-2 infection (defined as positive PCR test, antigen test or rapid antibody test)

Although data on comorbidities is available through e-SUS and SIVEP-Gripe, this data may have different degrees of missingness between databases and between cases and control groups. Adjusting for comorbidities using complete case data will likely introduce bias. We will explore the feasibility of multiple imputation of comorbidity in a sensitivity analysis. Additional sensitivity analyses will evaluate potential effect modification of the vaccine effectiveness by history of a positive RT-PCR, antigen or serological test result prior to the vaccination campaign.

***Survival analysis of h*ospitalization and death**: We will perform proportional survival analyses for hospitalization and death amongst HCWs who test positive and estimate the hazards according to vaccination status at the date of positive test, adjusting for covariates described in the case-control analyses. Sensitivity analyses will be conducted to evaluate the association of influence of a positive RT-PCR, antigen or serological test result prior to the vaccination campaign.

Sample size calculations and timing of analyses: The power of a matched case-control study depends on the assumed odds ratio and the number of discordant pairs (i.e. pairs in which the case is exposed and the control is unexposed, or vice versa), which is a function of the assumed odds ratio and the expected prevalence of exposure among controls. Moreover, the estimate of the odds ratio for one level of a categorical variable compared to baseline is determined by the distribution of all discordant pairs. As vaccine coverage and incidence are changing over time, the latter in ways we cannot predict, and there is no power formula for this analysis, we will simulate power and enroll individuals until we have reached a target power, which we can assess without analyzing the data. In particular, after determining the number of discordant case-control pairs for each combination of exposure categories, we will randomly assign one of each pair to each relevant exposure type according to a Bernoulli distribution, with the probability determined by the assumed odds ratio comparing the two categories. We will run an unadjusted conditional logistic regression on the simulated dataset to determine the p-value, and estimate the power as the proportion of N=1,000 simulations that return $p<0.05$. Code to perform the power calculation can be found at https://github.com/mhitchings/VEBRA_COVID-19.

Timing of interim and final analyses: Interim analyses will be performed to allow for early reporting of significant results for the benefit of public health, specifically the question whether receiving at least one dose of the vaccine is effective. For the primary outcome (symptomatic SARS-CoV-2 infection), we will perform an interim analysis of the effectiveness following at least one dose of the vaccine, as we expect this analysis to be the first to reach desired power. This interim analysis will be triggered upon reaching simulated power of 70% to detect vaccine effectiveness of 60% of at least one dose ≥14 days after the first dose. Once the interim analysis above has been triggered, we will perform one additional analysis when 80% power is achieved. To correct for multiple testing we will use the O'Brian Fleming alpha-spending method, meaning that for two interim analyses, the critical p-values at each analysis will be 0.0054 and 0.0492. We will perform final analyses of the primary outcome upon reaching simulated 80% power to detect vaccine effectiveness of 70% ≥14 days after the second dose.

Privacy: Only SEMSA, SES-AM and OPAS technicians had access to the identified dataset to linkage the datasets by name, date of birth, mother's name and CPF. After the linkage, the CPF was encrypted and the de-identified dataset was sent to the team for analysis.

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Figure 1: PCR testing rate, PCR positive testing rate, test-positive proportion, and vaccine coverage, by age (from data extracted on February 26, 2021)

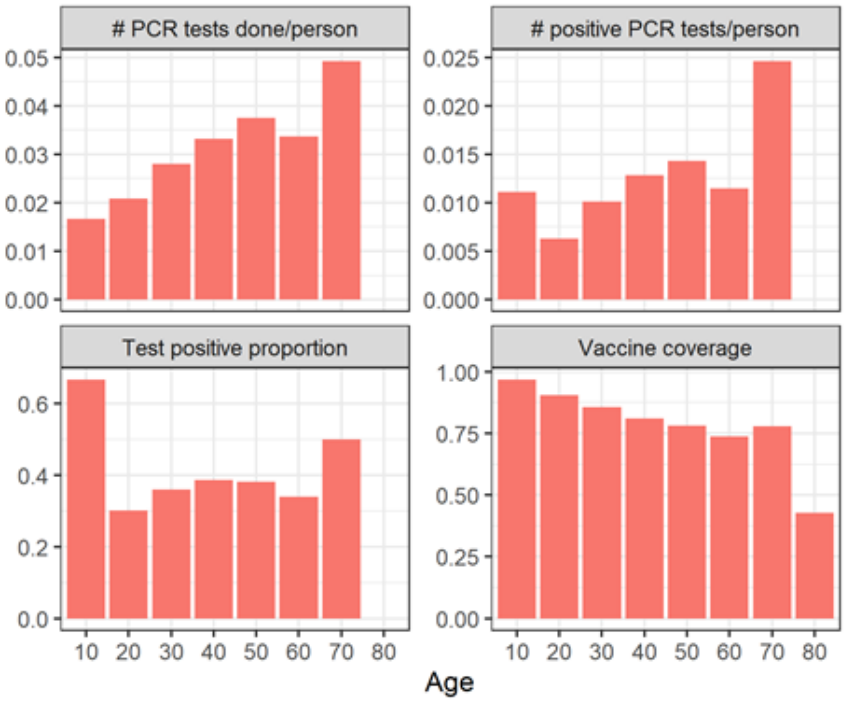


Figure 2: PCR testing rate, PCR positive testing rate, test-positive proportion, and vaccine coverage, by sex (from data extracted on February 26, 2021)

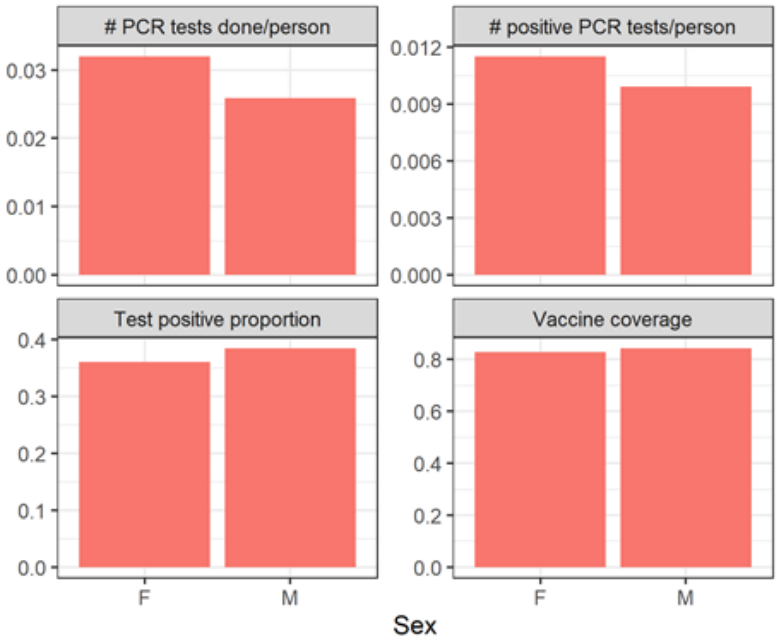


Figure 3: PCR testing rate, and PCR positive testing rate against average time from start of campaign to first dose administration, by *bairro*, (from data extracted on March 16, 2021)

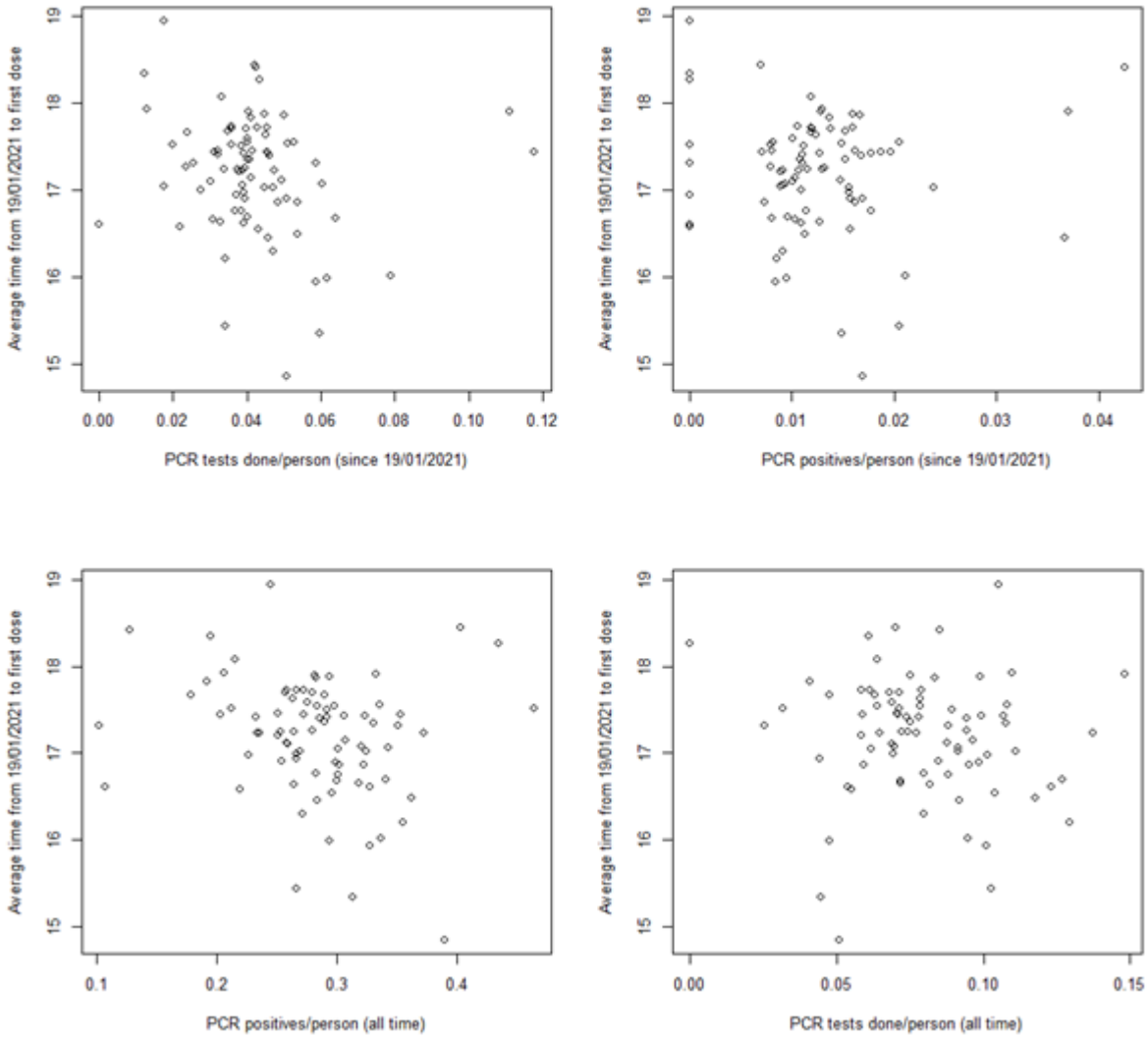
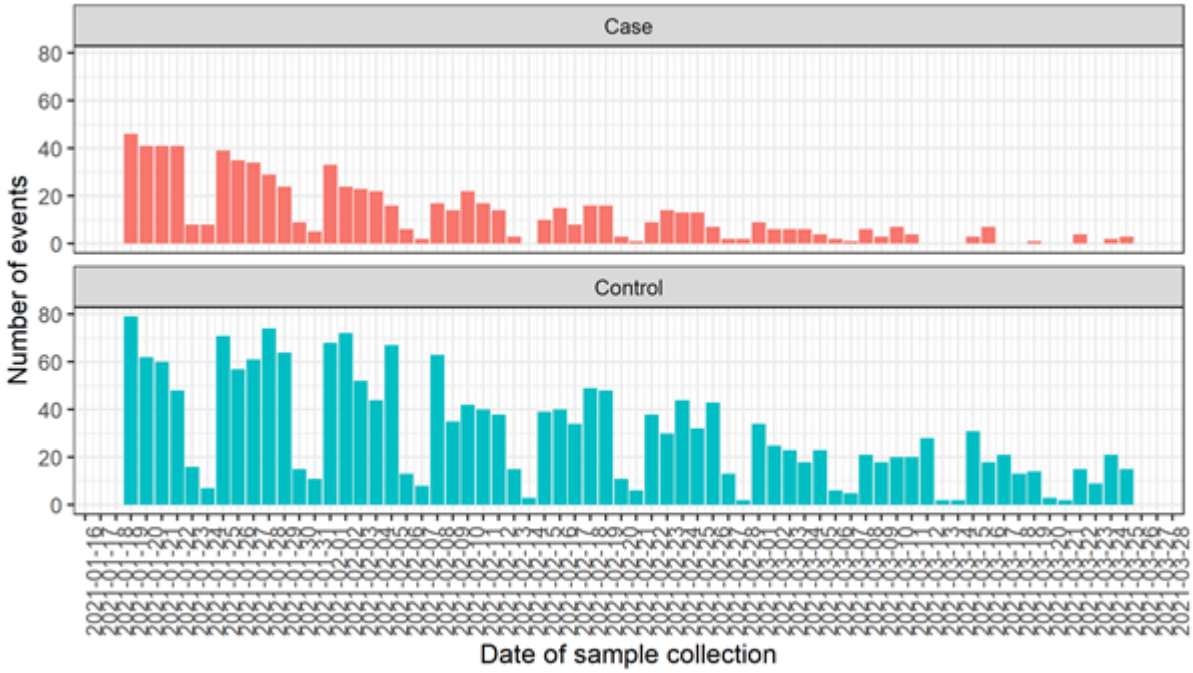
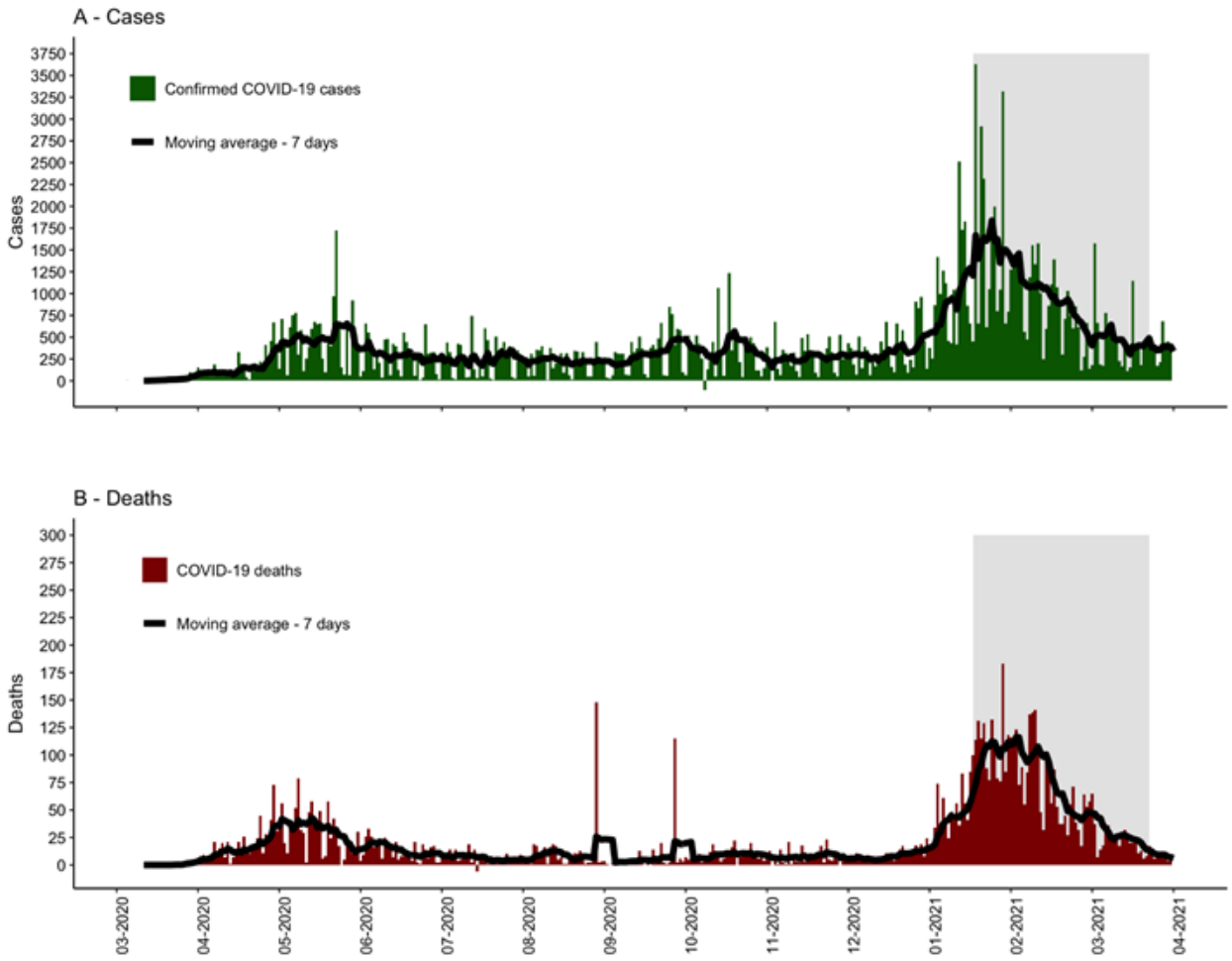


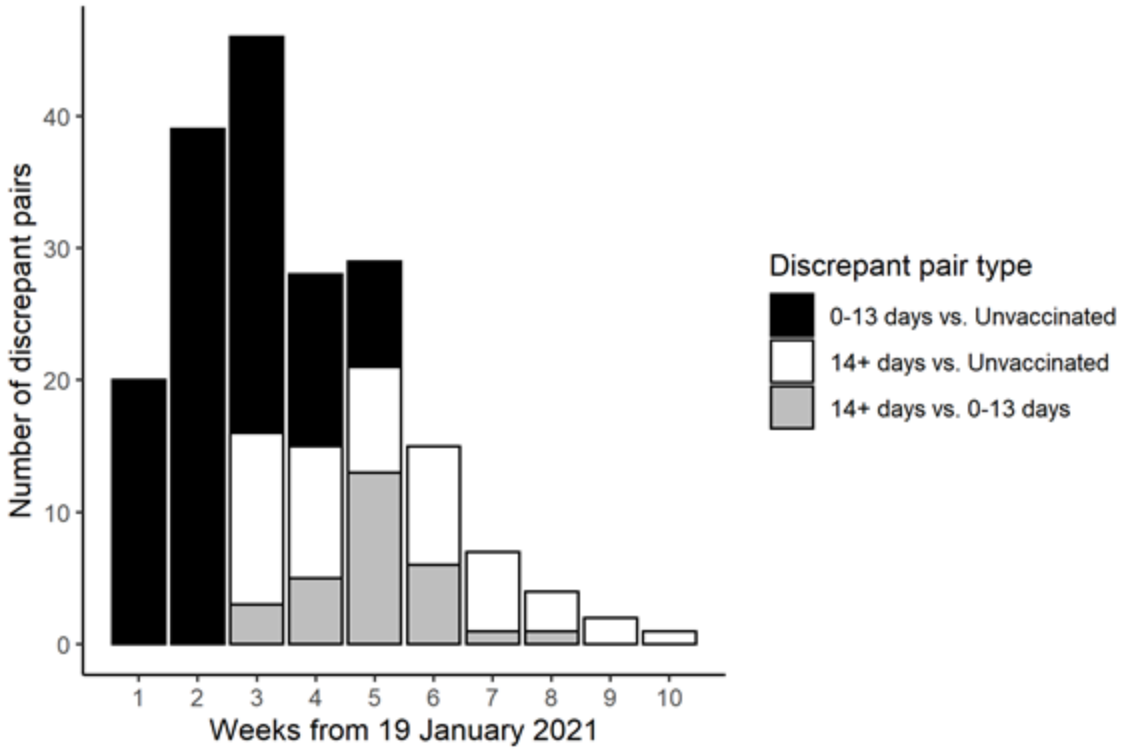
Figure 4: Number of positive and negative PCR tests over time in the study period, in the study population (from data extracted on April 4, 2021)



Supplementary Figure 1. Reported confirmed COVID-19 cases (Panel A) and COVID-19 associated deaths (Panel B) for case (n=160,398) and death (n=8424) counts in the general population of the city of Manaus, Brazil from 13 March 2020 to April 1, 2021. Lines depict moving seven day averages for case and death counts**.** Data was obtained from the State Secretary of Health of Amazonas and collected by brasil.io.



Supplementary Figure 2. Timing of enrollment of discordant case-control pairs by vaccination category



Supplementary Table 1. Vaccine effectiveness against symptomatic SARS-CoV-2 infection, and against all SARS-CoV-2 infection, with at least one dose and at least 14 days after administration of the first dose, including presence of one or more comorbidities as a covariate

	Primary outcome: Symptomatic SARS-CoV-2 infection		**Secondary outcome: All SARS-CoV-2 infection**	
	aOR (95% CI)	**p-value**	**aOR (95% CI)**	**p-value**
Unadjusted Analysis				
0-13 days after 1st vaccine dose vs. unvaccinated*	1.61 (1.07-2.44)	0.02	1.81 (1.26-2.61)	<0.001
≥14 days after 1st vaccine dose vs. unvaccinated*	0.56 (0.32-0.95)	0.03	0.68 (0.43-1.09)	0.11
Adjusted analysis				
0-13 days after 1st vaccine dose vs. unvaccinated*	1.73 (1.11-2.7)	0.02	1.87 (1.27-2.75)	<0.001
≥14 days after 1st vaccine dose vs. unvaccinated*	0.52 (0.29-0.92)	0.02	0.66 (0.40-1.08)	0.1
Age	1.00 (0.98-1.03)	0.64	1.01 (0.99-1.02)	0.55
Female sex	0.53 (0.36-0.78)	<0.001	0.68 (0.5-0.93)	0.02
Self-reported race/skin color**				
Amarela vs. Pardo	0.84 (0.53-1.32)	0.45	0.81 (0.55-1.18)	0.27
Preta vs. Pardo	1.85 (0.39-8.75)	0.44	0.98 (0.31-3.12)	0.97
Branca vs. Pardo	1.22 (0.82-1.81)	0.33	1.12 (0.79-1.61)	0.52
Occupation category				
ADM vs. Nurse	1.11 (0.74-1.64)	0.62	1.04 (0.75-1.45)	0.81
Clinical vs. Nurse	1.93 (0.72-5.17)	0.19	1.35 (0.59-3.08)	0.48
Other Health Associated vs. Nurse	0.96 (0.59-1.56)	0.88	0.81 (0.53-1.23)	0.32
Other Health Professional vs. Nurse	0.93 (0.55-1.58)	0.79	0.80 (0.51-1.26)	0.34
Prior healthcare encounters***				
1-3 vs. 0	0.93 (0.67-1.29)	0.66	0.78 (0.58-1.03)	0.08
≥4 vs. 0	1.31 (0.51-3.37)	0.57	1.10 (0.49-2.47)	0.81
Prior positive SARS-CoV-2 test*** ****	0.38 (0.17-0.87)	0.02	0.26 (0.13-0.54)	<0.001

Reported comorbidities				
Any vs. none	1.44 (0.95-2.18)	0.09	1.36 (0.95-1.95)	0.09

*At date of index sample collection for cases and controls

†Race/skin color as defined by the Brazilian national census bureau (Instituto Nacional de Geografia e Estatísticas) https://biblioteca.ibge.gov.br/visualizacao/livros/liv63405.pdf

‡Prior to the start of the study on 19 January, 2021

**Defined as SARS-CoV-2 RT-PCR or antigen detection