PROTOCOL

Evaluation of Vaccine Effectiveness in Brazil against COVID-19 (VEBRA-COVID)
Sub-Study: A Test-Negative Case-Control Study on the Effectiveness of COVID-19 Vaccines amongst the
General Population of São Paulo State in Brazil

Version: 01.3 / April 30th 2021

Table 1. Protocol Revisions

Changes in Version 1.3	Justification
Addition of ChAdOx1 exposure times	We added the time windows following the first and second doses of ChAdOx1 to be 0-13 days, 14-27 days and ≥28 days
Revised expected vaccine effectiveness	In the VEBRA-COVID analysis of the elderly (≥70 years of age) in São Paulo, we aimed to answer the research question of whether vaccines had a real-world effectiveness of public health value rather than whether they had a real-world effectiveness that was consistent with efficacy estimates from RCTs. Thus, we powered the study for a real world effectiveness above a lower threshold of 40%, below which the value of the vaccination would require reconsideration.
Change of matching criteria from CEP (5 digits) to Municipality and self-reported race	We based this decision on three main reasons: 1 – A great proportion of municipalities in São Paulo State has a unique CEP (zipcode), so everyone in that municipality has the same CEP. For these municipalities, we would lose within municipality socioeconomic information 2 – We observed a larger proportion of invalid CEPs mainly in the e-SUS database compared with the SIVEP-Gripe database, which may introduce potential bias since SIVEP-Gripe has a higher proportion of severe COVID-19 cases 3 – A significant number of unique CEPs were inconsistently placed in more than one municipality.
Addition of outcomes for the cohort analysis of test-positive cases	We added ICU admission and respiratory support, occurring within 21 days of initial SARS-CoV-2 test positivity. We also changed hospitalization from occurring within 14 days to within 21 days of initial SARS-CoV-2 test positivity.

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 13 million reported infections as of the middle of April 2021.[1–3] São Paulo, the most populous state in Brazil (~ 46 million inhabitants), is the state with highest number of cases and deaths: 2,827,833 cases and 92,548 deaths as by April 24th 2021.[4] Variants of Concern (VOC) also had a key role on the recent several surges in Brazil and São Paulo State. The P.1 VOC, which was first detected in Manaus on Jan 12, 2021, [5–7] and now consists the majority of new infections, being dominant in several states in Brazil. P1. has accrued mutations associated with decreased neutralization,[8,9] and has since spread throughout Brazil, synchronizing the epidemic in country in a scenario of relaxed non-pharmacological interventions.

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.[10–12] Phase III trials were conducted in Turkey, Chile, Singapore and Brazil. The Brazilian trial was conducted among a study population of healthcare professionals, and reported that the effectiveness of CoronaVac after 14 days following completion of a two dose schedule was 50.7% (95% CI 36.0-62.0) for all symptomatic cases of COVID-19, 83.7% (95% CI 58.0-93.7) for cases requiring medical attention, and 100% (95% CI 56.4-100) for hospitalized, severe, and fatal cases.[12] CoronaVac was approved for emergency use on 17 January in Brazil, and used to vaccinate healthcare workers and the general population. AstraZeneca-Oxford's ChAdOx1 vaccine[13,14] was approved on the same day and was administered beginning on 23 January 2021. In Brazil, ChAdOx1 schedule is for 12 weeks between first and second dose.

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.[15] 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[16,17] of the general population from the São Paulo State to evaluate the effectiveness of COVID-19 vaccines in preventing symptomatic disease in a setting of widespread P.1 VOC transmission.[6] The study will initially evaluate the effectiveness of COVID-19 vaccines, CoronaVac and ChAdOx1 amongst the population with age ≥70 years, since the vaccination campaign prioritized this age group in its first months. 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

To estimate the effectiveness of COVID-19 vaccines against symptomatic SARS-CoV-2 infection amongst the general population from the São Paulo State. Our initial analyses will focus on estimating vaccine effectiveness in the age group of ≥70 years.

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 the general population (Section 3) 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. 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, one minus the odds ratio (1-OR) of vaccination comparing cases and controls estimates the direct effect of vaccination on the disease outcome. 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.

Study Details

Study Site: The State of São Paulo (23°3′S, 46°4'W) is the most populous state in Brazil: an estimated population of 46,289,333 in 2020. São Paulo State has 645 municipalities and its capital, São Paulo city, has 12 million inhabitants. São Paulo State reported 2,827,833 COVID-19 cases (cumulative incidence rate: 6,109 per 100,000 population) and 92,548 deaths (cumulative mortality: 200 per 100,000 population), by 24/04/2021. The State Secretary of Health of Sao Paulo (SES-SP) initiated its COVID-19 vaccination campaign on 17 January 2021 and is administering two vaccines, CoronaVac and ChAdOx1. As of 24 April 2021, 10.7 million doses (6.9 million first doses and 3.8 million second doses) have been administered in the State.

Data Sources and Integration: We will identify eligible cases and controls from the State of São Paulo who test positive and negative, respectively, from the *state laboratory testing registry* of public health laboratory network; 2) Determine vaccination status from *state vaccination registries*; and 3) 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. Registries are not available which enables constructing a cohort of people eligible for vaccination in the general population. Data sources for this study will include:

- State laboratory testing registry (**GAL**) of the network of public health laboratories
- State COVID-19 vaccination registry (**Vacina Já**)
- 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

The databases will be integrated by the São Paulo State Government – PRODESP - using CPF numbers (Brazilian citizens' unique identifier code) and send to the VEBRA-COVID group anonymized. The database will be updated on a bi-weekly basis.

Study Population

Inclusion criteria:
- Has a residential address in the State of São Paulo,
- Eligible to receive a COVID-19 vaccine based on age,
- With complete information, which is consistent between databases, on age, sex, and residential address
- With consistent vaccination status and dates for those who were vaccinated.

Exclusion criteria:
- Does not have a residential address in the State of São Paulo,
- Not eligible to receive a COVID-19 vaccine based on age,
- With missing or inconsistent information on age, sex, or city of residence
- With existing but inconsistent vaccination status or dates.

<u>Case definition and eligibility</u>: We will use information from integrated GAL/SIVEP-Gripe/e-SUS databases to identify cases that 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 17, 2021 and 7 days prior to database extraction of information
- Did not have a positive RT-PCR test in the 90 day period preceding the index positive RT-PCR result
- Have complete and consistent data on SARS-CoV-2 RT-PCR test results

<u>Control definition and eligibility</u>: 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 17, 2021,
- Did not have a positive RT-PCR test in the 90 day period preceding the index positive RT-PCR result
- Did not have a subsequent positive RT-PCR test in the 7-day period following the index positive RT-PCR result
- Have complete and consistent data on SARS-CoV-2 PCR test result

When studying each vaccine, individuals that received another vaccine are eligible for selection as a case and/or control until the day they receive their vaccine, i.e. we will consider test positive and test negative cases for RT-PCR collected before the day of receipt of the other vaccine.

<u>Matching</u>: Test-negative controls will be matched 1:1 to the cases. We chose the matching factors to balance the ability to reduce bias and to enroll sufficient case-control pairs. 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 (see Figures 1-5):

- Age, categorized as 5-years age bands (e.g., 70-74, 75-79 years),
- Sex,
- Municipality,
- Self-reported race,
- 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 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.

<u>Exposure definition</u>:
CoronaVac vaccination:

- 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

ChAdOx1vaccination:

- 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:

- o 0-13 days
- o 14-27 days
- o ≥28 days

- Received the second dose in the following time periods relative to sample collection for their PCR test:
  - o 0-13 days
  - o ≥14 days

Statistical Analyses: We will evaluate the effectiveness of CoronaVac and ChAdOx1for 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:
  - o COVID-19 associated hospitalization within 21 days of the symptom onset
  - o COVID-19 associated ICU admission within 21 days of the symptom onset
  - o COVID-19 associated respiratory support
  - o COVID-19 associated death within 28 days of symptom onset

We will evaluate vaccine effectiveness for the primary outcome according to the test-negative design. Table 1 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 time to event/logistic regression analysis of test positive cases to evaluate the association of vaccination status and the risk for hospitalization, ICU admission, COVID-19 respiratory support, and death after infection.

Our initial analyses will focus on estimating vaccine effectiveness in the population with age ≥70 years of age who were the initial priority group of the COVID-19 vaccination campaign.

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.

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. For the CoronaVac analysis, the reference group will be individuals who have not received a first dose of CoronaVac by the date of respiratory sample collection. For the ChAdOx1 analysis, the reference group will be individuals who have not received a first dose of ChAdOx1by 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: A) 0-13 days and ≥14 days after the 1st dose and 0-13 days and ≥14 days after the 2nd dose of CoronaVac; and B) 0-13 days, 14-27 days and ≥28 after the 1st dose and0-13 days and ≥14 days after the 2nd dose of ChAdOx1.

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
- Comorbidities (None, 1-2, ≥3 comorbidities)
- 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 and age subgroups.

Survival/logistic regression analysis of hospitalization, ICU, respiratory support and death: We will perform additional analyses for hospitalization and death amongst individuals 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.

<u>Sample size calculations and timing of analyses</u>: 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.

<u>Timing of final analyses</u>: We will perform an analysis of the primary outcome upon reaching simulated 80% power to detect vaccine effectiveness of 40% ≥14 days after the second dose for the CoronaVac. For the ChAdOx1, we will perform an analysis of effectiveness of at least one dose upon reaching simulated 80% power to detect vaccine effectiveness of 40% ≥28 days after the first dose. In addition, we will perform an analysis of effectiveness of two doses upon reaching simulated 80% power to detect vaccine effectiveness of 40% ≥14 days after the second dose. We chose a vaccine effectiveness of 40% to address the question of whether vaccination with CoronaVac and ChAdOx achieved a threshold of real-world effectiveness, below which the public health value of vaccination may need to be reconsidered.

<u>Privacy</u>: Only SES-SP, São Paulo State data management 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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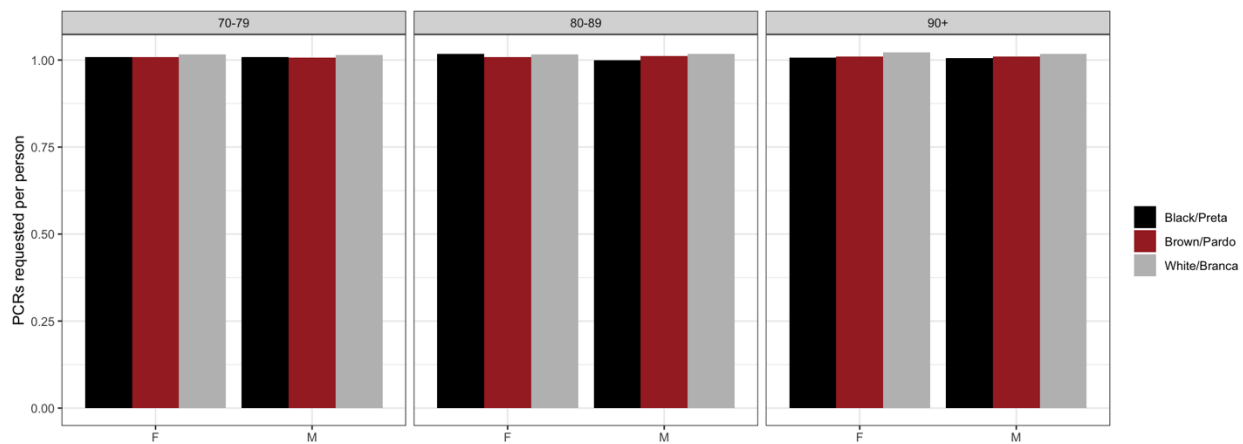
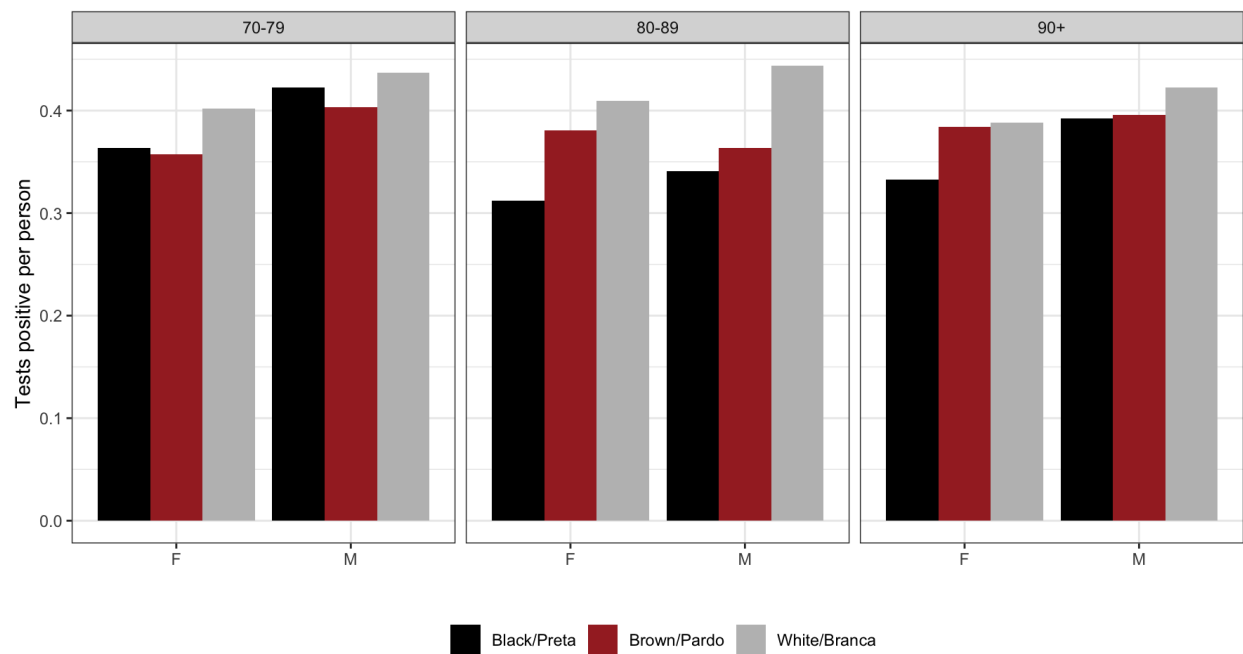Figure 1: PCR testing rate by age, sex and self-reported race (from data extracted on April 07, 2021)

Figure 2: PCR positive testing rate by age, sex and self-reported race (from data extracted on April 07, 2021)
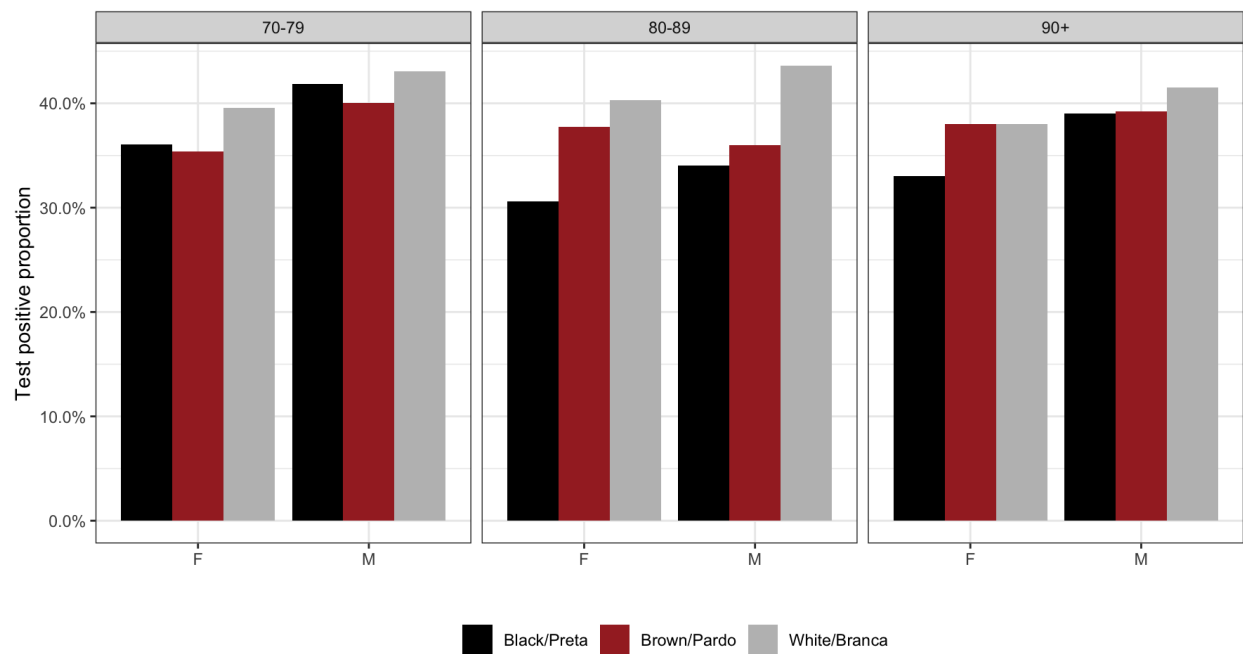
Figure 3: PCR positive proportion by age, sex and self-reported race (from data extracted on April 07, 2021)
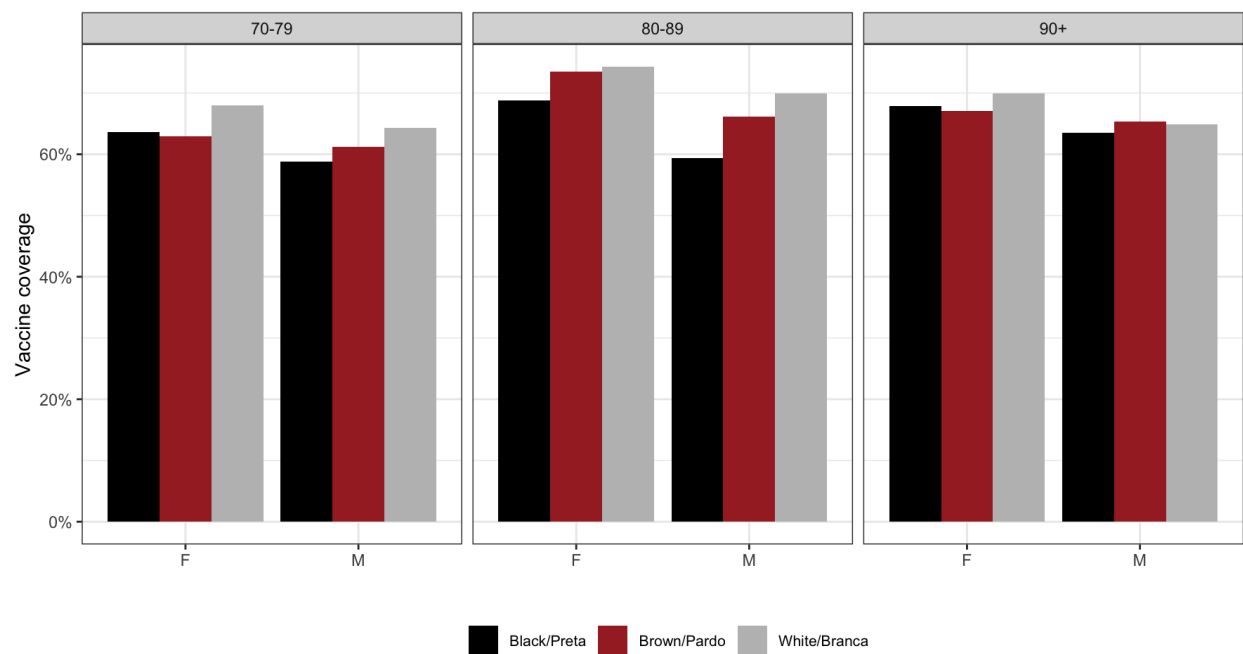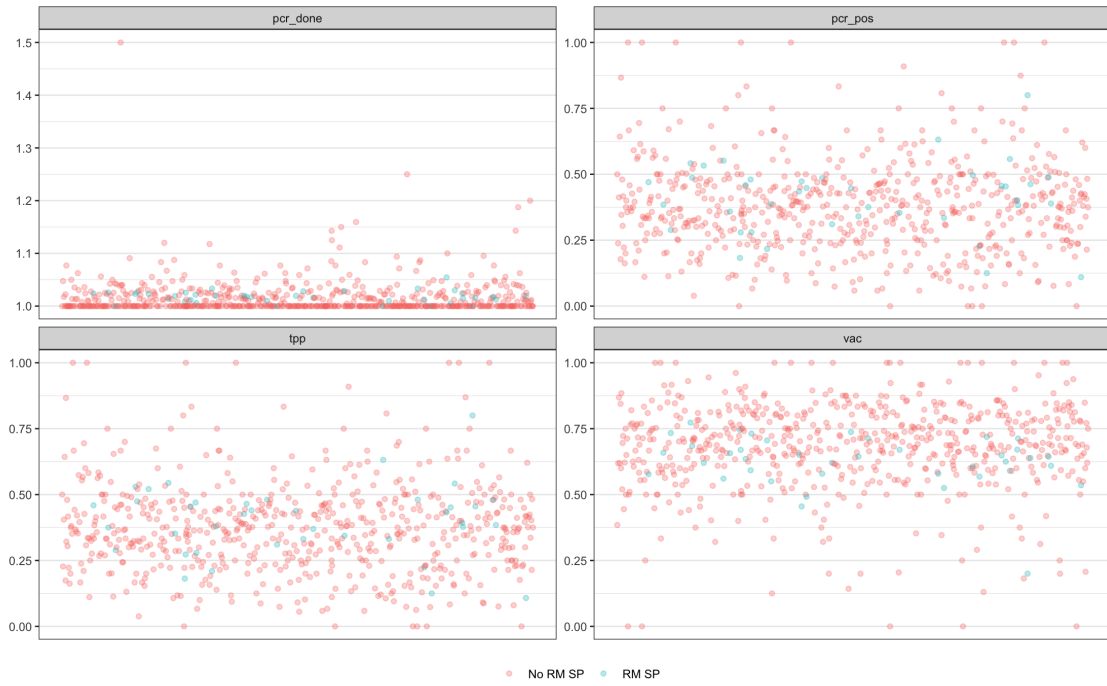

Figure 4: Vaccine coverage by age, sex and self-reported race (from data extracted on April 07, 2021)

Panel A. Indicators by Municipality

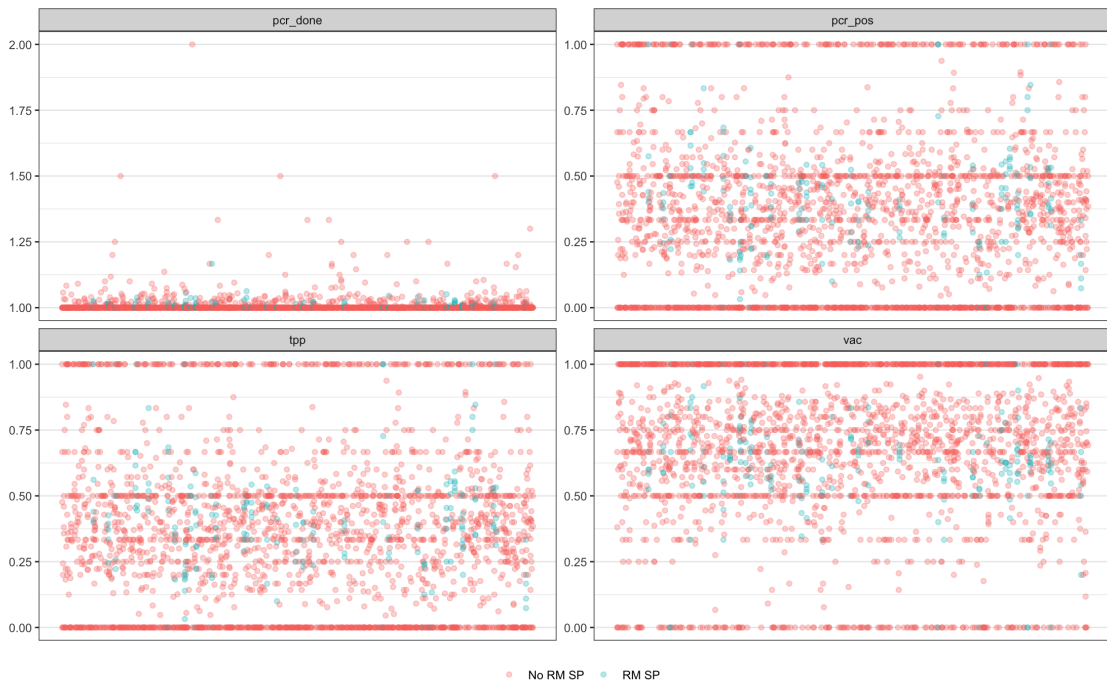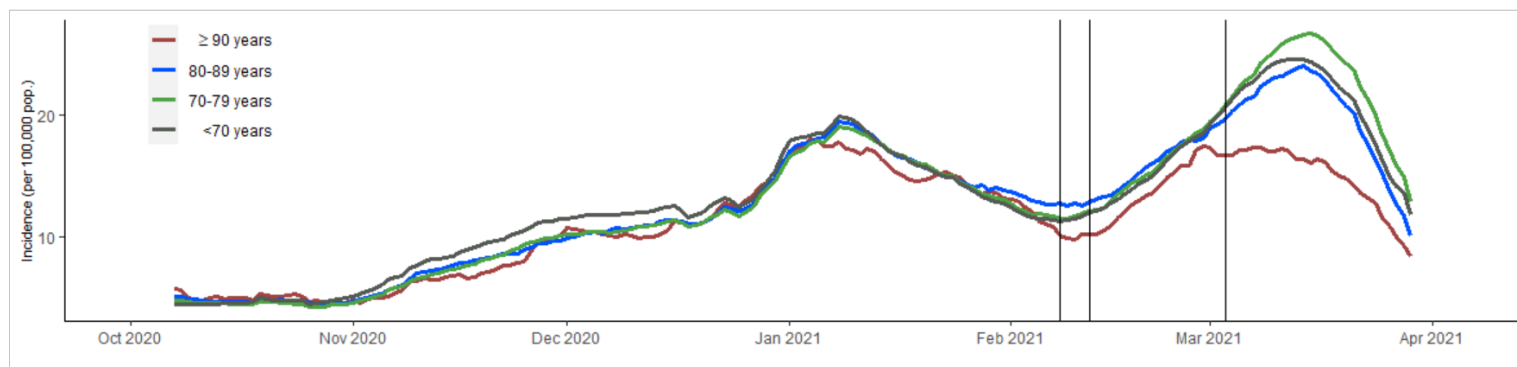Panel B. Indicators by Municipality and Race

Figure 5: PCR testing rate (pcr_done), PCR positive testing rate (pcr_pos), positivity proportion (tpp) and vaccine coverage (vac) by each municipality (A) and municipality and race (B). RM SP denotes metropolitan area of São Paulo city (from data extracted on April 07, 2021)

Supplementary Figure 1. Reported RT-PCR or Antigen confirmed COVID-19 in the general population of the São Paulo State, Brazil from October 2020 to April 7, 2021. Lines depict moving 14-day averages for case. Vertical lines represent vaccine eligibility by age.
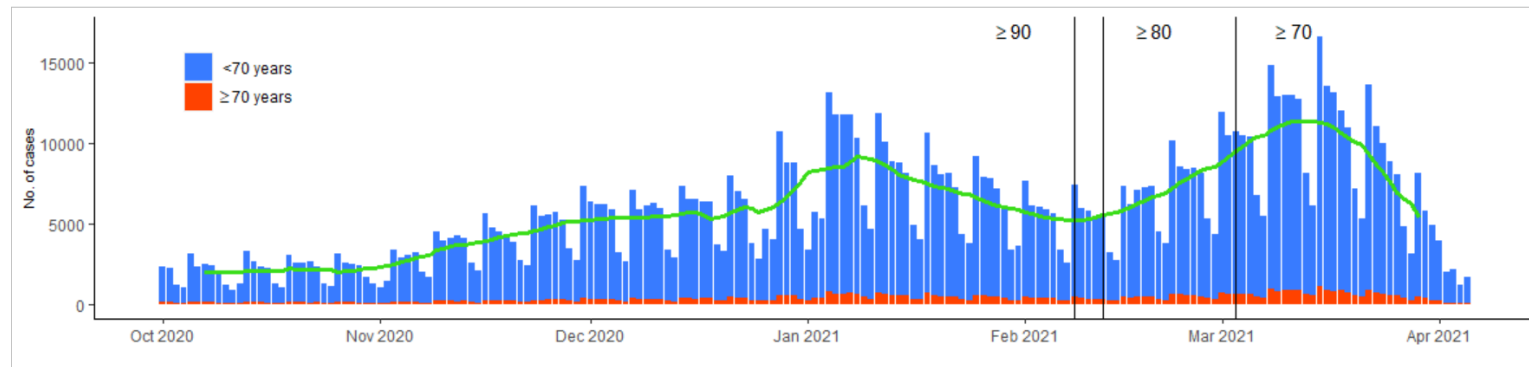
Supplementary Figure 2. Reported RT-PCR or Antigen confirmed COVID-19 rates in the general population of the São Paulo State, Brazil from October 2020 to April 7, 2021. Lines depict rolling averages. Vertical lines represent vaccine eligibility by age.

Table 1: Table of planned analyses

Analysis	Exposure	Outcome
CoronaVac		
Primary outcome, primary exposure	Two-dose regimen of CoronaVac in the period starting 14 days after administration of the 2nd dose	Positive test for SARS-CoV-2, with at least one COVID-19 symptom reported 0-10 days before sample collection date
Primary outcome, secondary exposure (2-dose)	Two-dose regimen of CoronaVac in the period 0-13 days after administration of the 2nd dose	
Primary outcome, secondary exposure (1-dose)	One-dose regimen of CoronaVac, in the period starting 14 days after administration of the 1st dose	
Primary outcome, bias indicator	One-dose regimen of CoronaVac, in the period 0-13 days after administration of the 1st dose	
ChAdOx1		
Primary outcome, primary exposure	One-dose regimen of ChAdOx1 in the period starting 28 days after administration of the 1st dose	Positive test for SARS-CoV-2, with at least one COVID-19 symptom reported 0-10 days before sample collection date
Primary outcome, secondary exposure (2-dose)	Two-dose regimen of ChAdOx1 in the period ≥14 days after administration of the 2nd dose	
Primary outcome, secondary exposure (1-dose)	One-dose regimen of ChAdOx1 in the period 0-13 days after administration of the 1st dose	
Primary outcome, secondary exposure (1-dose)	One-dose regimen of ChAdOx1, in the period starting 14-27 days after administration of the 1st dose	
Primary outcome, secondary exposure (2-dose)	Two-dose regimen of ChAdOx1, in the period starting 0-13 days after administration of the 2nd dose	
Primary outcome, bias indicator	One-dose regimen of ChAdOx1, in the period 0-13 days after administration of the 1st dose	