Review 1: "Remarkable Variability in SARS-CoV-2 Antibodies across Brazilian Regions: Report on Two Successive Nationwide Serological Household Surveys"

Leandro C. Mendes¹

¹State University of Campinas - Infectious Diseases Department

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**RR:C19 Evidence Scale** rating by reviewer:

- **Reliable.** The main study claims are generally justified by its methods and data. The results and conclusions are likely to be similar to the hypothetical ideal study. There are some minor caveats or limitations, but they would/do not change the major claims of the study. The study provides sufficient strength of evidence on its own that its main claims should be considered actionable, with some room for future revision.

Review:

The manuscript describes nationwide seroprevalence surveys conducted in highly populated urban regions from census units across the entire country of Brazil in two consecutive waves separated by two weeks. Undoubtedly, the work is relevant, original and extremely important. Representative prevalence data for SARS-CoV2 infection is remarkably lacking and is also invaluable not only for epidemiological studies but for ongoing crucial management and policy decisions on different levels.

Site selection, inclusion criteria and contingencies for participation refusal were clearly describe and adequately fit the study objectives. Possible selection biases deriving from the highly urbanized nature of sampling areas were explored as limitations and accounted for in inferential statements in the post-analytical phase. Power calculations were carried out prior to data collection but the authors fail to elaborate on assumptions (simply stating “approximately two standard errors”). Testing was undertaken using a point-of-care lateral flow chromatographic essay. No mention of the antigens used in the test could be found in the manuscript or in the provided link for a webpage by the manufacturer. Polled specificity from independent studies was corrected using the author’s own previous work under the stated assumption that “all the cases in that survey were false positives”, which could not be verified in the referred pre-print manuscript. Additional analysis using uncorrected specificity levels are referenced in supplemental materials not provided for review.

Prevalence data analysis is described as having been carried out using two strategies. The first assumes that both surveys are simple random samples and uses exact binomial tests to calculate confidence intervals and likelihood ratios for comparison of sociodemographic groups. The second strategy, which takes into account the sampling methods used by the authors and inputs test performance data, is described, again, in
supplemental materials not provided for review. Sampling techniques and potential selection bias for sociodemographic factors are crucial in tailoring statistical analysis, especially in large urban centers and, so, it would be interesting to better understand the more complete analytical model.

On a city-level, prevalence results are expressed in the text without confidence intervals. Further, heterogenous results across different regions (including an entire region with zero prevalence) make for challenging interpretation of pooled and longitudinal results. Overall, however, both point estimates and time trends are consistent, highly informative and correlate well with real-world epidemiological data, including outcome reports (such as attributable mortality rates). Social determinants such as wealth and ethnicity were strongly associated with estimated increases in prevalence overtime, also in accordance with previous findings.

Study limitations are briefly discussed and could be further elaborated. Critical methodological information and some important result data are referred to in supplemental materials not provided and could enhance the analytical scope of the main manuscript. Clearly there were great logistic and political challenges faced during data acquisition and they should be acknowledged, however, on page 11, the paragraph starting on line 474 would benefit from revision for clarity and purpose in keeping with the scientific and analytical context of the publication. Figure 06, although illustrative of risk factors for transmission of the virus, exposes individuals’ facial features, raising ethical concerns. English language expert revision is strongly recommended.