PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Anti-SARS-CoV-2 antibodies among indigenous populations of the Brazilian Amazon: a cross-sectional study
AUTHORS	Lima, Carlos Neandro; Abreu, Isabella; Rodrigues, Eliene Putira; Freitas, Vanessa; Botelho, Bruno José; Souza, Sandra; Cayres-Vallinoto, Izaura; Guerreiro, João; Ishak, Ricardo; Vallinoto, Antonio

VERSION 1 – REVIEW

REVIEWER	Jose Varona HM Hospitales, Internal Medicine
REVIEW RETURNED	08-Aug-2021

GENERAL COMMENTS	This is an interesting study that evaluates seroprevalence in different indigenous peoples of the Amazon. The high seroprevalence in the study time interval (October 2020-January 2021) is interesting. Among the points to improve, I would highlight the following: - The estimation of mortality seems a weak point of the study and should support the best statistical analysis in this regard. - It is necessary to clarify how the selection sampling of the analyzed population was carried out. It is necessary to know, at least approximately, what percentage of the total population was analyzed and the results were the selection criteria for the sample. - The role of IgM seropositivity needs to be clarified. It is contradictory in most studies. In my opinion, I think it brings more confusion than certainty, but the role that the authors attribute to it as a marker of current incidents is interesting. In any case, this must be justified and supported by significant biographical references in this regard, counting on microbiological teas that document the current infection such as PCR or antigen. - I think it is necessary to compare the seroprevalence obtained in the groups analyzed in the study with the seroprevalence in other regions (both America and other continents)

REVIEWER	Stefani Thomas University of Minnesota, Laboratory Medicine and Pathology
REVIEW RETURNED	16-Aug-2021

GENERAL COMMENTS	Lima et al. conducted a cross-sectional study to investigate the prevalence of anti-spike IgG and IgM antibodies among six

	indigenous ethnic groups living in northern Brazil from Oct. 2020 – Jan. 2021. The results indicated that SARS-CoV-2 was widespread among the indigenous populations investigated, but the mortality was low. This is a succinct, descriptive study; however, several important details are not provided, which calls into question the validity of the results. The points described below should be addressed: 1. Results section of Abstract, "Herd immunity was probably attained"; and 2nd to the last paragraph of the Discussion, "This raises another important question in regard to herd protective immunity". Although there is a lack of consensus in the scientific community regarding the specific definition of herd immunity, the authors should provide the definition of herd immunity that they are using for this study. 2. The Introduction is rather brief (3 sentences) and should be expanded. At the very least, the authors should include some text addressing the goals of their study. 3. In the last sentence of the Introduction, the authors refer to previous reports from their laboratory that address "the distinct cultural and health aspect of the Amazonian peoples, the impact of the virus among native peoples, and the importance of performing serosurveys among these groups to determine the spectrum of the illness among them." Rather than providing general statements regarding the objectives of their previous studies, it would be helpful to include statements addressing the results from these studies. 4. In the 3rd paragraph of the Methods, additional details regarding the geographic regions of interest would be helpful: What is the population of each village? What are the basic demographics of the population of each village (not just the demographics of the subset of the population that was included in their study)? What modes of transportation are available in these villages? 5. The 2nd to the last paragraph of the Methods section mentions that "All individuals were clinically evaluated". This statement requires clarification. What type of clinical evaluation did the individuals undergo? 6. Discussion: "Two methodologies were used and the results obtained were not significantly different." The authors do not present the results from any statistical tests that were conducted to compare the performance of the Guangzhou Wondfo Biotech rapid test with the Euroimmun ELISA. In the absence of comparative diagnostic performance data (sensitivity, specificity, etc.), the previously mentioned statement cannot be justified. 7. Table 1: Please explain the deaths in the context of the study. It is unclear how these deaths were observed given that the study design was a cross-sectional seroepidemiological surveillance study. 8. Results: This section is also rather brief (5 sentences). 9. No justification is provided for measuring IgM in addition to IgG antibodies. The authors should cite studies that have documented the significance of these antibodies. For example, it has been shown that IgM and IgG antibodies can increase nearly simultaneously, whereas IgM antibodies decay more rapidly than IgG. 10. Discussion: Spell out "SESAI".

Reviewer: 1

Dr. Jose Varona, HM Hospitales

Comments to the Author:

This is an interesting study that evaluates seroprevalence in different indigenous peoples of the Amazon.

The high seroprevalence in the study time interval (October 2020-January 2021) is interesting.

Among the points to improve, I would highlight the following:

- The estimation of mortality seems a weak point of the study and should support the best statistical analysis in this regard.

Actually, we estimated the virulence rate considering the case of death to each village over the number of individuals that were infected with SARS-CoV-2, according to the seropositivity to IgG. We changed it in the text.

- It is necessary to clarify how the selection sampling of the analyzed population was carried out. It is necessary to know, at least approximately, what percentage of the total population was analyzed and the results were the selection criteria for the sample.

The indigenous populations are usually of small population size; we work with a representative sample of at least 50% of the population. Our group performed a multi-professional health approach in the Indigenous villages and, we attended the majority of the individuals living in the village during the visits. Examining 1,185 individuals is something hard to achieve considering the difficulties found trying to reach the Indigenous area located in the Amazon region.

- The role of IgM seropositivity needs to be clarified. It is contradictory in most studies. In my opinion, I think it brings more confusion than certainty, but the role that the authors attribute to it as a marker of current incidents is interesting. In any case, this must be justified and supported by significant biographical references in this regard, counting on microbiological teas that document the current infection such as PCR or antigen.

The main objective was to identify the seroprevalence of anti-SARS-CoV-2 IgG antibodies. However, as we also performed a rapid test, it was possible to detect IgM reactivity in some individuals. We agree that the IgM detection is contradictory, but we used this information as a reference to indicate either that the virus was still circulating among the examined individuals or were in the acute phase of infection/disease.

- I think it is necessary to compare the seroprevalence obtained in the groups analyzed in the study with the seroprevalence in other regions (both America and other continents).

The results were compared with those obtained in Manaus (capital of the Amazonas State) and with a nationwide serological household surveys in Brazil (references 13 and 14). Other references were included and a new paragraph.

"*The high seroprevalence of IgG anti-SARS-CoV-2 antibodies reported herein among vulnerable Amazon Indigenous peoples, is comparable to our recent finding among Venezuelan indigenous Warao refugees residing in Belem city, the capital of Para State, where the infection was detected among 83% of the subjects living in conditions of vulnerability.[16] Similar results were reported among indigenous people living in the surrounding area of Manaus, where the number of individuals sharing households was a risk for virus infection.[17]* "

Finally, we would like to thank you for all comments and suggestion which certainly improved the final version of the manuscript.

Reviewer: 2

Dr. Stefani Thomas, University of Minnesota

Comments to the Author:

Lima et al. conducted a cross-sectional study to investigate the prevalence of anti-spike IgG and IgM antibodies among six indigenous ethnic groups living in northern Brazil from Oct. 2020 – Jan. 2021. The results indicated that SARS-CoV-2 was widespread among the indigenous populations investigated, but the mortality was low. This is a succinct, descriptive study; however, several important details are not provided, which calls into question the validity of the results. The points described below should be addressed:

1.   Results section of Abstract, "Herd immunity was probably attained"; and 2nd to the last paragraph of the Discussion, "This raises another important question in regard to herd protective immunity". Although there is a lack of consensus in the scientific community regarding the specific definition of herd immunity, the authors should provide the definition of herd immunity that they are using for this study.

The definition used as reference of herd immunity was that described in the article by Omer et al. (*JAMA* 2020. https://doi.org/10.1001/jama.2020.20892), which assumes that when the population shows no immunity, all individuals are equally susceptible to infection herd immunity threshold for SARS-CoV-2 would be expected to be in the range between 50% and 60% in the absence of any interventions.

In order to clarify this issue and following your suggestion we rephrased the sentence below:

"Apparently, the communities achieved herd immunity levels, when reaching at least 60% seropositivity for IgG,[11]..."

2.   The Introduction is rather brief (3 sentences) and should be expanded. At the very least, the authors should include some text addressing the goals of their study.

You are correct. We followed your suggestion and added a new paragraph ~~in~~ at the end of the Introduction.

"Considering the vulnerability of indigenous peoples that inhabit the Brazilian Amazon region, their supposed inability to respond immunologically to new pathogens that emerge in the community and, assuming the possible negative impact that the Covid-19 pandemic may have on these communities, the present study aimed to carry out a seroepidemiological investigation in indigenous populations located in the State of Pará, through the screening of anti-SARS-CoV-2 antibodies."

3.   In the last sentence of the Introduction, the authors refer to previous reports from their laboratory that address "the distinct cultural and health aspect of the Amazonian peoples, the impact of the virus among native peoples, and the importance of performing serosurveys among these groups to determine the spectrum of the illness among them." Rather than providing general statements regarding the objectives of their previous studies, it would be helpful to include statements addressing the results from these studies.

Indeed, you are correct about the general statement. At the present, we are dealing and processing the enormous amount of clinical, epidemiological, demographic, social and behavioral information collected during the visits to the villages. For instances, there is some indications over the influence of HTLV-2 co-infected persons and the impact on COVID-19. We hope to have a more precise in few months as the expeditions are still occurring. An important information is the different modulation of SARS-CoV-2 infection by the indigenous populations (which was previously discussed in a letter (Why did they not die? - Austin J Public Health Epidemiol - Volume 8 Issue 3 - 2021), which goes in the opposite direction of the expected doom of the virus among isolated communities). In anyway, we included in the discussion one recent publication on COVID-19 in Indigenous individuals living in an urban context in Manaus, capital of the Amazonas State.

Additionally, we changed the paragraph as follow:

"Three previous reports from our laboratory calls the attention to the distinct cultural (sharing households), health aspects (coinfections and malnutrition) and the modulation of infection among these Amazonian peoples, the impact of the virus among native peoples, and the importance of performing serosurveys among these groups to determine the spectrum of the illness among them.[5] [7] [8]"

4.   In the 3rd paragraph of the Methods, additional details regarding the geographic regions of interest would be helpful: What is the population of each village? What are the basic demographics of the population of each village (not just the demographics of the subset of the population that was included in their study)? What modes of transportation are available in these villages?

We included the following paragraph in order to attend your suggestion:

"Access to the indigenous peoples of the basin of the middle Xingu River (Asurini, Araweté and Parakanã), Iriri River (Kararaô) and Tapajós River (Munduruku) is almost exclusively fluvial, while access to the Xikrin people, located in the Bacajá River channel, is currently preferably done by road. The indigenous people studied were:

(i) Araweté, a Tupi-Guarani-speaking people, population of 589 inhabitants, currently distributed in 22 villages with populations from 7 to 71, located on the banks of the Xingú River and the Ipixuna stream, right bank tributary of the middle Xingu River, in the municipality of Altamira (PA). A total of 508 people from all villages were sampled (-4,8853, -52,4281);

(ii) Asurini do Xingu, from the Tupi-Guarani family, totaling 260 individuals distributed in five villages on the banks of the middle Xingu River (-4,2449, -52,2380); eight people from Kwatinemu village (n = 139) were studied;

(iii) Parakanã, a Tupi-Guarani indigenous people who live in the Apyterewa land, in the municipalities of Altamira and São Félix do Xingu, in the Xingu basin, Pará, with a population of 782 people living in 16 small villages with population ranging from 17 to 94, of which 210 people were studied (-5,6904, -52,0037);

(iv) Xikrin do Bacajá, people of the Kayapó (or Mebengokré) language, Jê linguistic family, who live in 19 small villages (populations from 11 to 141; current population of 1,051 inhabitants, 3.7160, -53.0546) in the middle Bacajá basin, municipalities of Senador José Porfírio and Anapú, PA; One hundred individuals from seven villages were sampled: Kenkrô (39/61), Bakajá (23/109), Mrotdjam (1/128), Pykatum (4/59), Rapkô (7/60), Pytatko (1941) and Moinorô (13/77);

(v) Kararaô, another Jê-speaking Kayapó subgroup, living in four small villages (Kararaô, n = 15; Kruakrô, n = 15; Pidjôdjã, n = 42 and Rikrekô, n = 11), located in the lower Iriri river and in the middle Xingu river, in Altamira (PA). A sample of 44 individuals was collected from a total population of 83 (-3,9112, -52,8044);

(vi) Munduruku, an indigenous people belonging to the Munduruku linguistic family, from the Tupi trunk, lives in the southwest of Pará, in the Tapajós river channel and tributaries, in the municipalities of Santarém, Itaituba and Jacareacanga. Population of 10,629 distributed in 133 villages. A total of 317 individuals were sampled, 213 from the Nova Karapanatuba village (213/414) and the others from smaller villages around Nova Karapanatuba, in Jacareacanga. (-3.9112, -52.8044). Access to the indigenous peoples of the basin of the middle Xingu River (Asurini, Araweté and Parakanã), Iriri River (Kararaô) and Tapajós River (Munduruku) is almost exclusively fluvial, while access to the Xikrin people, located in the Bacajá River channel, is currently preferably done by road.*"*

5.   The 2nd to the last paragraph of the Methods section mentions that "All individuals were clinically evaluated". This statement requires clarification. What type of clinical evaluation did the individuals undergo?

The follow sentence was included:

"The indigenous people underwent a standard clinical examination that could be performed under field conditions, consisting of anamnesis and physical examination (inspection, palpation, percussion and auscultation), with assessment of anthropometric data, blood pressure, body temperature and digital oximetry, in addition to laboratory support for blood count and biochemical, microbiological and parasitological exams."

6.   Discussion: "Two methodologies were used and the results obtained were not significantly different." The authors do not present the results from any statistical tests that were conducted to compare the performance of the Guangzhou Wondfo Biotech rapid test with the Euroimmun ELISA. In the absence of comparative diagnostic performance data (sensitivity, specificity, etc.), the previously mentioned statement cannot be justified.

I apologize for the equivocal. Indeed, both tests showed good agreement (p<0.001). We rephrased the sentence and included the information on the test in Methods and in Results, as follow:

Method:

"The Kappa test was used to assess the agreement between the rapid test results and enzyme-linked immunosorbent assay. The G and chi-square tests were applied to assess the difference in the prevalence of IgG, among the villages, in relation to sex and age..."

Results:

"The overall IgG prevalence obtained by the rapid test and ELISA were similar and the agreement of the results between both tests, as compared, was 80% - classified as good (kappa=0.4987; p<0.001; sensitivity of 82.1% and specificity of 71.6%). It was observed 33 (2.8%) individuals with indeterminate result to anti-SARS-CoV-2 IgG antibodies in ELISA test."

7.   Table 1: Please explain the deaths in the context of the study. It is unclear how these deaths were observed given that the study design was a cross-sectional seroepidemiological surveillance study.

Indeed, it was a cross-sectional study, and the number of deaths were obtained assessing the death registry of the number of the deaths in each village during the period of the study. The number has not been modified until the present.

8. Results: This section is also rather brief (5 sentences).

Ok. Thank for your alert. We included some new data.

9. No justification is provided for measuring IgM in addition to IgG antibodies. The authors should cite studies that have documented the significance of these antibodies. For example, it has been shown that IgM and IgG antibodies can increase nearly simultaneously, whereas IgM antibodies decay more rapidly than IgG.

Our main objective was to identify the seroprevalence of anti-SARS-CoV-2 IgG antibodies. By the time of the visits, we also performed a rapid test to detect IgM reactivity in some individuals. We agree that the IgM detection is contradictory, but we used this information not to infer the real prevalence of active infection, but just as a reference to indicate that the virus was still circulating among the individuals despite the high values of IgG prevalence.

10. Discussion: Spell out "SESAI".

Okay, it was done, but It was already spelled in the Materials and Methods.

"Special Secretariat of Indigenous Health (*Secretaria Especial de Saúde Indígena – SESAI-MS*)"

Finally, we would like to thank you for all comments and suggestion which certainly improved the final version of the manuscript.

VERSION 2 – REVIEW

REVIEWER	Jose Varona HM Hospitales, Internal Medicine
REVIEW RETURNED	24-Oct-2021

GENERAL COMMENTS	It is OK now

REVIEWER	Stefani Thomas University of Minnesota, Laboratory Medicine and Pathology
REVIEW RETURNED	22-Oct-2021

GENERAL COMMENTS	Lima et al. have responded to the majority of the concerns raised by the reviewers following the first round of evaluation; however, a couple of outstanding issues remain that should be addressed before recommending the manuscript for publication in BMJ Open. 1. The significance of IgM vs. IgG in the context of the course of SARS-CoV-2 infection is unclear. Studies have shown a brief

| | window (~4-7 days) between the onset IgM and IgG seropositivity. Additionally, the duration of IgG seropositivity exceeds that of IgM seropositivity. The authors are strongly encouraged to re-evaluate their interpretation of IgM seropositivity. For example, in the abstract the authors state, "The high IgG prevalence suggest[s] the herd immunity was probably attained but the presence of IgM positivity showed ongoing cases."

2. The clarity of the Methods section could be enhanced by adding sub-section headings such as "Study population", "SARS-CoV-2 antibody assays", and "Data analysis".

3. The authors provided thorough responses to the comment from the previous review regarding adding details about the geographic regions of interest and the population sampling method. In the description of the populations in the methods section, the numbers in parentheses following the locations should be defined. For example, "A total of 508 people from all villages were sampled (-4,8853, -52,4281)." Are these numbers longitude and latitude coordinates?

4. In the methods section, please specify the sample type that was used for the ELISA (serum, plasma, whole blood?)

5. Results section: "The overall IgG prevalence obtained by the rapid test and ELISA were similar…sensitivity of 82.1% and specificity of 71.6%." Do these numbers for sensitivity and specificity refer to the rapid test or to the ELISA?

6. The 2nd to last paragraph of the Results section is a bit confusing. The groups used for comparison are unclear: age, indigenous population, sex? This paragraph should be re-written to improve the clarity. For example, "…but in the Araweté and Xikrin the frequencies were significantly lower among those >31 years old (p=0.0065 and p=0.0198) and in the Munduruku a lower frequency among those <6 years old and greater (p<0.0001) among those older than 31 years old (Table 2)."

7. Table 2 legend: Change "Qui-square" to "Chi-square."

8. 2nd to last sentence of the Results section: "The main clinical manifestations reported among infected individuals were coughing, dyspnea, …" How were the authors able to determine whether the study participants had active infection? Antibody tests do not detect active infection. The sentence in question could be deleted without detracting from the significance of the manuscript. |

VERSION 2 – AUTHOR RESPONSE

Lima et al. have responded to the majority of the concerns raised by the reviewers following the first round of evaluation; however, a couple of outstanding issues remain that should be addressed before recommending the manuscript for publication in BMJ Open.

1. The significance of IgM vs. IgG in the context of the course of SARS-CoV-2 infection is unclear. Studies have shown a brief window (~4-7 days) between the onset IgM and IgG seropositivity. Additionally, the duration of IgG seropositivity exceeds that of IgM seropositivity. The authors are strongly encouraged to re-evaluate their interpretation of IgM seropositivity. For example, in the abstract the authors state, "The high IgG

prevalence suggest[s] the herd immunity was probably attained but the presence of IgM

positivity showed ongoing cases."

OK. WE CHANGED THE ABSTRACT AND THE PARAGRATH BELLOW.

"The detection of IgM antibodies in three villages might suggest recent infection in the villages. But the confirmation could only be done by antigen or nucleic acid tests, which were not available at the time of the study. Additionally, recent report has shown IgM persistence for up to 8 months post-Covid, pointing to the need for no longer using IgM as a diagnostic criterion

for acute or recent COVID-19.[12]"

2. The clarity of the Methods section could be enhanced by adding sub-section headings

such as "Study population", "SARS-CoV-2 antibody assays", and "Data analysis".

IT WAS DONE.

3. The authors provided thorough responses to the comment from the previous review

regarding adding details about the geographic regions of interest and the population

sampling method. In the description of the populations in the methods section, the

numbers in parentheses following the locations should be defined. For example, "A total

of 508 people from all villages were sampled (-4,8853, -52,4281)." Are these numbers

longitude and latitude coordinates?

THEY MEAN THE LONGITUDE AND LATITUDE COORDINATES. IT WAS REPOSITIONED IN THE PARAGRATH.

4. In the methods section, please specify the sample type that was used for the ELISA

(serum, plasma, whole blood?)

IT WAS DONE.

5. Results section: "The overall IgG prevalence obtained by the rapid test and ELISA were similar…sensitivity of 82.1% and specificity of 71.6%." Do these numbers for sensitivity and specificity refer to the rapid test or to the ELISA?

WE TEST THE SENSITIVITY AND SPECIFICITY OF THE RAPID TEST REGARDING THE ELISA, WHICH WE USE AS A REFERENCE METHOD FOR ANTIBODY DETECTION, BECAUSE THEY HAVE KNOWNALLY GREATER SENSITIVITY AND SPECIFICITY.

6. The 2nd to last paragraph of the Results section is a bit confusing. The groups used for comparison are unclear: age, indigenous population, sex? This paragraph should be rewritten to improve the clarity. For example, "…but in the Araweté and Xikrin the frequencies were significantly lower among those >31 years old (p=0.0065 and p=0.0198) and in the Munduruku a lower frequency among those <6 years old and greater (p<0.0001) among those older than 31 years old (Table 2)."

WE CHANGED A LITTLE BIT THE SENTENCE. WE BELIEVE IT IS FINE NOW.

7. Table 2 legend: Change "Qui-square" to "Chi-square."

IT WAS DONE.

8. 2nd to last sentence of the Results section: "The main clinical manifestations reported among infected individuals were coughing, dyspnea, …" How were the authors able to determine whether the study participants had active infection? Antibody tests do not detect active infection. The sentence in question could be deleted without detracting from the significance of the manuscript.

AS WE REPORTED BEFORE, THE INDIVIDUALS WERE EXAMINED BY A MEDICAL TEAM IN LOCO. THE SYMPTOMS REPORTED WERE OBSERVED AND RECORDED BY PHYSICIANS DURING THE VISIT TO THE VILLAGES. THE AUTHORS BELIEVE THAT THIS REPORT IN THE ARTICLE IS IMPORTANT.

WE THANK YOU FOR YOUR COMMENTS AND SUGGESTIONS.