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Prevalence of SARS-CoV-2 infection among the Brazilian Amazon indigenous populations

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ABSTRACT

Objectives The emergence of SARS-CoV-2 and its spread at a pandemic level generated a serious warning over the impact of the infection on vulnerable indigenous populations of the Brazilian Amazon. Thus, the present study aimed to perform seroepidemiological survey for antibodies anti-SARS-CoV-2 in those populations.

Methods We performed a cross-sectional study to investigate the prevalence of anti-spike (S1) IgG antibodies among six indigenous ethnic groups living in the State of Pará (Northern Brazil). The villages of Xikrin do Bacajá, Assurini, Araweté, Parakanã, Munduruku and Kararaô were visited from October 2020 to January 2021 and 1,185 individuals, of both sexes, were enrolled in the investigation. Sera were tested for the presence of anti-SARS-CoV-2 IgM and IgG antibodies using two assays (a lateral flow rapid test and an ELISA assay).

Results The prevalence of IgM and IgG antibodies was 6.9% and 68,3%, respectively, ranging from 0 to 79.6% with significant differences between ages in two communities

(Araweté and Munduruku) and a case fatality rate of 0.86%. Herd immunity was probably attained but the presence of IgM positivity showed ongoing cases.

Conclusion SARS-CoV-2 was rapidly spread among the indigenous populations investigated, but it carried a low mortality. It is necessary to expand the serological investigations towards other communities in the Amazon region of Brazil.

Keywords: SARS-CoV-2, COVID-19, Indigenous peoples, Amazon.

Strengths and limitations of this study

- Our previous reports called the attention to the distinct cultural and health aspects of the Amazonian peoples, the impact of the virus among native peoples, and the importance of performing seroepidemiological surveys among vulnerable populations.
- The results showed that SARS-CoV-2 infection reached the different indigenous peoples living in the Brazilian Amazon region in an almost homogeneous way.

- The results highlight that SARS-CoV-2 infection hit the indigenous populations as rapidly as the virus entered the Amazon region, without the predicted mortality.
- The high IgG prevalence suggest the herd immunity was probably attained but the presence of IgM positivity showed ongoing cases.
- Furthermore, seroepidemiological surveys are of paramount importance for monitoring the outcome of the national contingency plan for the prevention and control of the epidemic.

INTRODUCTION

The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the first cases of Coronavirus Diseases 2019 (COVID-19) were reported by November 2019, in Wuhan, China.[1] The rapid spread of the virus determined its classification as pandemic by WHO[2] and because of the high morbimortality it highlighted the burden to vulnerable populations, including native indigenous peoples living in the Brazilian Amazonian who are susceptible to the virus and could be devastated by their immunological frailty. [3-6]

Two previous reports form our laboratory calls the attention to the distinct cultural and health aspects 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. [5 7]

METHODS

In 2020, a large cross-sectional seroepidemiological surveillance among indigenous ethnic groups within Para State (Northern Brazil, Amazon), started to investigate the prevalence of anti-SARS-CoV-2 IgM and IgG antibodies and the impact of the virus on the health of communities. The study was approved by the leaders of the communities and by the National Research Ethics Committee (*Comissão Nacional de Ética em Pesquisa* – CONEP; CAAE: 33470020.0.1001.0018), in accordance with the Declarations of Helsinki.

From October 2020 to January 2021, multiprofessional health care expeditions were prepared, composed by our team, the staff of the Health Department of the State of Para (*Secretaria de Saúde do Estado do Pará – SESPA*) and the Special Indigenous

Health Districts of Altamira and Santarém (*Distrito Sanitário Especial Indígena – DSEI*) of the Special Secretariat of Indigenous Health (*Secretaria Especial de Saúde Indígena – SESAI-MS*). Six ethnic groups were visited while presenting active cases of COVID-19, before starting the vaccination campaign among indigenous communities.

The groups investigated were from the Tupi (Asurini do Koatinemo, Araweté, Parakanã, and Munduruku) and Jê (Xikrin do Bacajá and Kararaô) linguistic trunks. The groups are geographically located in the State of Pará, Northern of Brazil (Figure 1). The Araweté is located at the margin of the Ipixuna stream, a tributary of the right margin of the Middle Xingu. Asurini is situated on the banks of the Igarapé Ipiaçava, a tributary of the right bank of the Xingu. Kararaô is also at the margin of the Xingu stream. Parakanã and Xikrin villages are located on the margin of the Xingu River, between the municipalities of São Felix do Xingu and Senador José Porfírio. Munduruku villages are located southwest, by the Tapajós river, in the municipalities of Santarém, Itaituba, and Jacareacanga).

The investigation included 1,185 individuals of both sexes (552 male and 633 female), randomly selected from the Araweté (n=508), Asurini (n=08), Munduruku (n=317), Parakanã (n=210), Xikrin (98) and Kararaô (n=44). All individuals were clinically evaluated and a blood sample (5 mL) was drawn to test for the presence of anti-SARS-CoV-2 IgM and IgG antibodies.

The prevalence of anti-SARS-CoV-2 IgG and IgM antibodies used a rapid test (lateral flow method; Guangzhou Wondfo Biotech Co., China) and an enzyme-linked immunosorbent assay (ELISA; Anti-SARS-CoV-2 S1 IgG, Euroimmun, Brazil), according to the manufacturer's recommendation.

Patient and public involvement

Neither patients, parents nor the public were involved in the design, conduct or reporting of this research.

RESULTS

Anti-SARS-CoV-2 IgG was detected among 503 (68.3%) individuals by the rapid test and 815 (68.8%) when tested by the ELISA (Table 1). Eight Asurini did not show antibodies, but among the other villages, it ranged (by the Elisa test) from 51.7%

(Munduruku) to 79.5% (Araweté and Kararaô). No statistical significance was observed between the tests. It is worth mentioning that IgM antibodies detected among 51 (6.9%) individuals from three villages. There was no statistical significance of prevalence values according to sex (χ^2= 0.001; p=0.9793), but in the Araweté the frequencies were significantly lower among those >31 years old (p=0.0065) 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).

Table 1. Prevalence of anti-SARS-CoV-2 (S1) IgG antibodies among Indigenous villages

Population	Age range	Sex		Rapid Test results				ELISA IgG anti-Spike S1 results				N Deaths
		Male N (%)	Female N (%)	N tested	Negative N (%)	IgM N (%)	IgG N (%)	N tested	Negative N (%)	Ind N (%)	IgG N (%)	
Xikrin do Bacajá[7]	02 a 84	51 (52.0)	47 (48.0)	95	38 (40)	nt	57 (60)	98	25 (25.5)	0 (0)	73 (74.5)	01
Asurini	17 a 42	0 (0)	8 (100)	0	nt	nt	nt	08	7 (87.5)	1 (12.5)	0 (0)	0
Araweté	8m a 84	258 (50.8)	250 (49.2)	236	29 (12.3)	11 (4.7)	196 (83)	508	92 (18.1)	12 (2.4)	404 (79.5)	0
Parakanã	7m to 95 y	106 (50.5)	104 (49.5)	195	06 (3.1)	39 (20)	150 (76.9)	210	65 (30.9)	06 (2.8)	139 (66.2)	01
Munduruku	7 m to 89 y	116 (36.6)	201 (63.4)	166	96 (57.8)	nt	70 (42.2)	317	139 (43.9)	14 (4.4)	164 (51.7)	05
Kararaô	1 a 94	21 (47.7)	23 (52.3)	44	13 (29.5)	1 (2.3)	30 (68.2)	44	9 (20.4)	0 (0)	35 (79.5)	0
Total	7m a 95 y	552 (46.6)	633 (53.4)	736	182 (24.7)	51 (6.9)	503 (68.3)	1.185	337 (28.4)	33 (2.8)	815 (68.8)	07

M (months); Y (years), nt (not tested); [7] Rodrigues et al. (2021); Ind: Indeterminate

Table 2. Age and sex distribution to anti-SARS-CoV-2 (S1) IgG antibodies among Indigenous villages

Age	**Xikrin[7]**		**Asurini**		**Araweté**		**Parakanã**		**Munduruku**		**Kararaô**		**Total**
	male	female	male	female	male	female	male	female	male	female	male	female	**n/ total (%)**
0-6	5	2	0	0	44	27	10	6	7	6	3	1	111/1,185 (9.4)
>6 a 16	15	11	0	0	61	56	18	18	8	17	6	8	218/1,185 (18.4)
>16 a 31	9	13	0	0	56	66	12	21	14	44	2	6	241/1,185 (20.3)
>31	10	8	0	0	45	49	27	27	26	42	3	6	243/1,185 (20.5)
*not informed	0	0	0	0	0	0	0	0	1	1	0	0	2/1,185 (0.2)
Total	39	34	0	0	206	198	67	72	55	109	14	21	815/1,185 (68.8)

[7] Rodrigues et al. (2021); Age distribution: (Overall: χ^2=8.2; p=0.042); (Xikrin: G=7.8; p=0,0514); (Araweté: χ^2=12.3; p=0.0065); (Parakanã: χ^2=3.9; p=0.2753); (Munduruku: χ^2=23.8; p<0.0001); (Kararaô: G=4.4; p=0.2213). G - G test; χ^2 - Qui-square.

The main clinical manifestations reported among infected individuals were coughing, dyspnea, coryza, fever, fatigue, diarrhea, ear pain, headache, chest and back pain. There were seven deaths among the 815 infected persons showing a case fatality rate of 0.86%.

DISCUSSION

Introduction of new infectious agents among vulnerable indigenous peoples is thought to be a heavy burden because of the low genetic variability among genes that control the immune response,[8] an important element working as a selective pressure over indigenous peoples since the initial colonization process of the Amazon region of Brazil. SARS-CoV-2, brought the question again as to how the virus would affect native peoples? Despite the theoretical arguments for the possible speculative devastation of indigenous groups,[6] there was no confirmed evidence of susceptibility to Covid-19 in the presence of coinfections and pre-existing conditions, including obesity and malnutrition as previously suggested.[4 5]

Most of the indigenous peoples of the Amazon region of Brazil, including those of the present study, present endemic diseases as malaria, tuberculosis, virus hepatitis and, to HTLV-2 hyperendemic infections among Xikrin, Kararaô, Munduruku and Parakanã.[9] Presently, there is no scientific evidence for host modulation of SARS-CoV-2 in the presence of these coinfections. Environmental and social conditions are important factors that could impact on COVID-19 among indigenous communities,[3 4] including the lack of drinking water and malnutrition, which might had been potentialized after the prophylactic isolation measures.

In a previous report the quality of tests to measure the presence of antibodies was raised.[7] It is important that antibodies to SARS-CoV-2 are detected using tests with greater sensitivity and specificity to obtain accurate prevalence rates. Rapid tests show low sensitivity and yield false negative results and false-positive results due to cross-reactions. Two methodologies were used and the results obtained were not significantly different.

In contrast to the genetic and socio-environmental vulnerability the results showed SARS-CoV-2 infection evenly distributed, with a high prevalence of the virus and few reported deaths, confirming the official results by SESAI in its COVID-19 epidemiological bulletin.[10]

The prevalence of anti-SARS-CoV-2 IgG antibodies shows a high dissemination of the virus spread favored by inherent social and cultural difficulties of keeping social distance, sharing households with other families and wearing masks correctly. In spite of the high prevalence of infection, during the investigation they were still facing the epidemic, showed by the detection of IgM antibodies in three villages. It is relevant to mention that three villages are more than 100Km apart from each other and there is no simple way of communication among them. This raises another important question in regard to herd protective immunity. Apparently, the communities achieved herd immunity levels,[11] similarly as found in Manaus, the capital of the Amazonas State.[12] However, differently from what is seen among urban areas,[13] virgin soil epidemics generally exhaust susceptible individuals before it comes to an end,[14] and the present epidemic is still not totally explained and understood.

The results show new information over the SARS-CoV-2 epidemic among indigenous peoples and provides the Brazilian government with tools to establish adequate measures to control the epidemic among Brazilian indigenous communities in the Amazon region.

CONCLUSION

SARS-CoV-2 infection reached the indigenous populations from the State of Para as rapid as the virus entered the Amazon region, confirming our previous alert to the need of serological studies for surveillance, minimizing the burden of the epidemic and to promote indigenous health policies.

In spite of the suggestion of high mortality and chaos facing the Amerindian populations (*6*), the majority of the cases was asymptomatic or mild, with low fatality rate, supporting analysis that shows that the mortality associated with epidemics following contact of Amazonian indigenous communities with urban communities has decreased in recent years.[15]

Finally, continuing seroepidemiological surveys are of paramount importance for monitoring the outcome of the national contingency plan for the prevention and control of the epidemic, by a mass vaccination program for indigenous peoples started by February 2021.

Acknowledgments We thank the Indigenous populations for authorizing the study and the institutions that provided technical support for the development and implementation of this study. Our especial gratitude to Vilson Monteiro and Hailton Monteiro for laboratorial assistance during the expeditions to Indigenous villages.

Contributor ACRV, JFG and RI: conception of idea, data analysis, writing and editing of manuscript; ACRV, RI, JFG, INA, BJSB and CNCL: Acquisition, analysis, or interpretation of data; CNCL, RI, IMVCV: Drafting of the manuscript; ACRV, RI, JFG and IMVCV: Critical revision of the manuscript for important intellectual content; SSL: Statistical analysis; ACRV: Obtained funding; VOF and EPSR: technical and material support.

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Competing interests None declared

Patient and public involvement Neither patients, parents nor the public were involved in the design, conduct or reporting of this research.

Patient consent for publication Not requirable

Ethics approval The study was approved by the leaders of the communities and by the National Research Ethics Committee (*Comissão Nacional de Ética em Pesquisa* – CONEP; CAAE: 33470020.0.1001.0018), in accordance with the Declarations of Helsinki.

Data availability statement Data are available upon reasonable request (vallinoto@ufpa.br)

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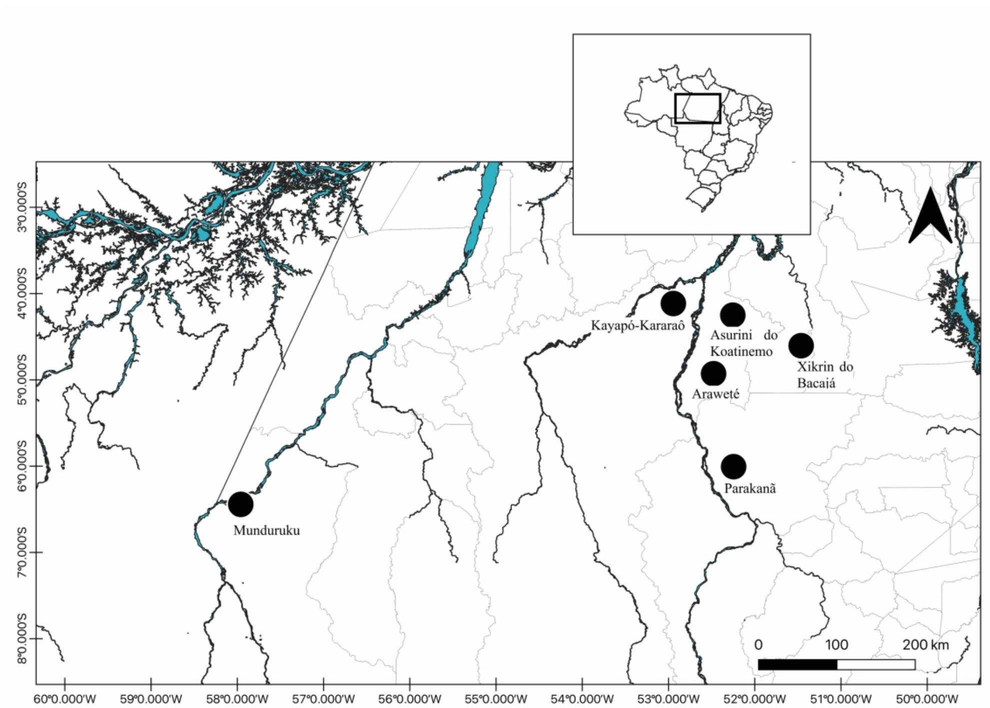
Figure 1. Legend.

Figure 1. Map showing the geographical location of the indigenous communities investigated enrolled in the present study.



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(Araweté, Xikrin and Munduruku) and a virulence rate of 0.86%. Herd immunity was probably attained but the presence of IgM positivity suggests ongoing cases.

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Strengths and limitations of this study

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- The results showed that SARS-CoV-2 infection reached the different indigenous peoples living in the Brazilian Amazon region in an almost homogeneous way.

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- The high IgG prevalence suggest the herd immunity was probably attained but the presence of IgM positivity showed ongoing cases.
- Furthermore, seroepidemiological surveys are of paramount importance for monitoring the outcome of the national contingency plan for the prevention and control of the epidemic.

INTRODUCTION

The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the first cases of Coronavirus Diseases 2019 (COVID-19) were reported by November 2019, in Wuhan, China.[1] The rapid spread of the virus determined its classification as pandemic by WHO[2] and because of the high morbimortality it highlighted the burden to vulnerable populations, including native indigenous peoples living in the Brazilian Amazonian who are susceptible to the virus and could be devastated by their immunological frailty.[3-6]

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]

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.

METHODS

In 2020, a large cross-sectional seroepidemiological surveillance among indigenous ethnic groups within Para State (Northern Brazil, Amazon), started to investigate the prevalence of anti-SARS-CoV-2 IgM and IgG antibodies and the impact of the virus on the health of communities. The study was approved by the leaders of the communities and by the National Research Ethics Committee (*Comissão Nacional de Ética em Pesquisa* – CONEP; CAAE: 33470020.0.1001.0018), in accordance with the Declarations of Helsinki.

From October 2020 to January 2021, multiprofessional health care expeditions were prepared, composed by our team, the staff of the Health Department of the State of Para (*Secretaria de Saúde do Estado do Pará – SESPA*) and the Special Indigenous Health Districts of Altamira and Santarém (*Distrito Sanitário Especial Indígena – DSEI*) of the Special Secretariat of Indigenous Health (*Secretaria Especial de Saúde Indígena – SESAI-MS*). Six ethnic groups were visited while presenting active cases of COVID-19, before starting the vaccination campaign among indigenous communities. 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.

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 (Figure 1). 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).

A blood sample (5 mL) was drawn to test for the presence of anti-SARS-CoV-2 IgM and IgG antibodies. The prevalence of anti-SARS-CoV-2 IgG and IgM antibodies used a rapid test (lateral flow method; Guangzhou Wondfo Biotech Co., China) and an enzyme-linked immunosorbent assay (ELISA; Anti-SARS-CoV-2 S1 IgG, Euroimmun, Brazil), according to the manufacturer's recommendation. 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.

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.

Patient and public involvement

Neither patients, parents nor the public were involved in the design, conduct or reporting of this research.

RESULTS

A total of 1,187 subjects was investigated, being 552 males (46.5%) and 635 females (53.5%). The age ranged from 1 to 95 years old (mean of 26.2, standard deviation of 19.9).

Anti-SARS-CoV-2 IgG was detected among 505 (68.1%) individuals by the rapid test and 815 (68.7%) when tested by the ELISA (Table 1). Eight Asurini did not show antibodies, but among the other villages, it ranged (by the Elisa test) from 51.7% (Munduruku) to 79.5% (Araweté and Kararaô). 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. Additionally, it is worth mentioning that IgM antibodies was detected among 51 (6.9%) individuals from three villages.

There was no statistical significance of IgG prevalence values according to sex (Overall: χ^2 =0.001, p=0.9793; Xikrin: χ^2= 0.056, p=0.8129; Araweté: χ^2=0.003, p=0.9554; Parakanã: χ^2=1.022, p=0.3121; Munduruku: χ^2=1.496, p=0.2213; Kararaô: χ^2=0.0278, p=0.0642); 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).

Table 1. Prevalence of anti-SARS-CoV-2 (S1) IgG antibodies among Indigenous villages

Population	Age range	Sex		Rapid Test results				ELISA IgG anti-Spike S1 results				N Deaths
		Male N (%)	Female N (%)	N tested	Negative N (%)	IgM N (%)	IgG N (%)	N tested	Negative N (%)	Ind N (%)	IgG N (%)	
Xikrin do Bacajá[7]	02 a 84	51 (51.0)	49 (49.0)	100	42 (42)	nt	58 (58)	100	27 (27)	0 (0)	73 (73)	01
Asurini	17 a 42	0 (0)	8 (100)	0	nt	nt	nt	08	7 (87.5)	1 (12.5)	0 (0)	0
Araweté	8m a 84	258 (50.8)	250 (49.2)	236	29 (12.3)	11 (4.7)	196 (83)	508	92 (18.1)	12 (2.4)	404 (79.5)	0
Parakanã	7m to 95 y	106 (50.5)	104 (49.5)	195	06 (3.1)	39 (20)	150 (76.9)	210	65 (30.9)	06 (2.8)	139 (66.2)	01
Munduruku	7 m to 89 y	116 (36.6)	201 (63.4)	166	96 (57.8)	nt	70 (42.2)	317	139 (43.9)	14 (4.4)	164 (51.7)	05
Kararaô	1 a 94	21 (47.7)	23 (52.3)	44	13 (29.5)	1 (2.3)	30 (68.2)	44	9 (20.4)	0 (0)	35 (79.5)	0
Total	7m a 95 y	552 (46.5)	635 (53.5)	741	185 (25.0)	51 (6.9)	505 (68.1)	1.187	339 (28.5)	33 (2.8)	815 (68.7)	07

M (months); Y (years), nt (not tested); [7] Rodrigues et al. (2021); Ind: Indeterminate

Table 2. Age and sex distribution to anti-SARS-CoV-2 (S1) IgG antibodies among Indigenous villages

Age	Xikrin[7]		Asurini		Araweté		Parakanã		Munduruku		Kararaô		Total
	male	female	male	female	male	female	male	female	male	female	male	female	**n/ total (%)**
0-6	5	2	0	0	44	27	10	6	6	5	3	1	109/1,187 (9.2)
>6 a 16	15	11	0	0	61	56	18	18	8	17	6	8	218/1,187 (18.4)
>16 a 31	9	13	0	0	56	66	12	21	14	44	2	6	243/1,187 (20.5)
>31	10	8	0	0	45	49	27	27	26	42	3	6	243/1,187 (20.5)
*not informed	0	0	0	0	0	0	0	0	1	1	0	0	2/1,187 (0.2)
Total	39	34	0	0	206	198	67	72	55	109	14	21	815/1,187 (68.7)

[7] Rodrigues et al. (2021); Age distribution: (Overall: χ^2=8.2; p=0.042); (Xikrin: G=8.15; p=0,0198); (Araweté: χ^2=12.3; p=0.0065); (Parakanã: χ^2=3.9; p=0.2753); (Munduruku: χ^2=23.8; p<0.0001); (Kararaô: G=4.4; p=0.2213). G - G test; χ^2 - Qui-square.

The main clinical manifestations reported among infected individuals were coughing, dyspnea, coryza, fever, fatigue, diarrhea, ear pain, headache, chest and back pain. There were seven deaths among the 815 infected persons showing a virulence rate of 0.86%.

DISCUSSION

The high prevalence of IgG anti-SARS-CoV-2 antibodies reported herein show that the new corovavirus infection reached indigenous populations in a wide way. Introduction of new infectious agents among vulnerable indigenous peoples is thought to be a heavy burden because of the low genetic variability among genes that control the immune response,[9] an important element working as a selective pressure over indigenous peoples since the initial colonization process of the Amazon region of Brazil. SARS-CoV-2 brought the question again as to how the virus would affect native peoples? Despite the theoretical arguments for the possible speculative devastation of indigenous groups,[6] there was no confirmed evidence of susceptibility to Covid-19 in the presence of coinfections and pre-existing conditions, including obesity and malnutrition as previously suggested.[4 5]

Most of the indigenous peoples of the Amazon region of Brazil, including those of the present study, present endemic diseases as malaria, tuberculosis, virus hepatitis and, to HTLV-2 hyperendemic infections among Xikrin, Kararaô, Munduruku and Parakanã.[10] Presently, there is no scientific evidence for host modulation of SARS-CoV-2 in the presence of these coinfections, but researches in order to investigate these coinfections are of paramount importance for a better understanding of the outcome of SARS-CoV-2 infection. Furthermore, environmental and social conditions are important factors that could impact on COVID-19 among indigenous communities,[3 4] including the lack of drinking water and malnutrition, which might had been potentialized after the prophylactic isolation measures.

In our previous report the quality of tests to measure the presence of antibodies was raised.[7] It is important that antibodies to SARS-CoV-2 are detected using tests with greater sensitivity and specificity to obtain accurate prevalence rates. Rapid tests usually show low sensitivity and yield false negative results and false-positive results due to cross-reactions. In the present study, two methodologies were used and the agreement of the results of the rapid test in relation to ELISA was good and the IgG prevalence values similar.

In contrast to the genetic and socio-environmental vulnerability the results showed SARS-CoV-2 infection evenly distributed, with a high prevalence of the virus and few reported deaths, confirming the official results by Special Secretariat of Indigenous Health (SESAI) in its COVID-19 epidemiological bulletin.[11]

The prevalence of anti-SARS-CoV-2 IgG antibodies shows a high dissemination of the virus spread favored by inherent social and cultural difficulties of keeping social distance, sharing households with other families and wearing masks correctly. In spite of the high prevalence of infection, during the investigation they were still facing the epidemic, showed by the detection of IgM antibodies in three villages. It is relevant to mention that three villages are more than 100Km apart from each other and there is no simple way of communication among them. This raises another important question in regard to herd protective immunity. Apparently, the communities achieved herd immunity levels, when reaching at least 60% seropositivity for IgG,[12] similarly as found in Manaus, the capital of the Amazonas State.[13] However, differently from what is seen among urban areas,[14] virgin soil epidemics generally exhaust susceptible individuals before it comes to an end,[15] and the present epidemic is still not totally explained and understood. 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]

The results show new information over the SARS-CoV-2 epidemic among indigenous peoples and provides the Brazilian government with information to establish adequate measures to control the epidemic among Brazilian indigenous communities in the Amazon region.

CONCLUSION

SARS-CoV-2 infection reached the indigenous populations from the State of Para as rapid as the virus entered the Amazon region, confirming our previous alert to the need of serological studies for surveillance, minimizing the burden of the epidemic and to promote indigenous health policies.[5]

In spite of the suggestion of high mortality and chaos facing the Amerindian populations,[6] the majority of the cases was asymptomatic or mild, with low fatality rate, supporting analysis that shows that the mortality associated with epidemics following contact of Amazonian indigenous communities with urban communities has decreased in recent years.[18]

Finally, continuing seroepidemiological surveys are of paramount importance for monitoring the outcome of the national contingency plan for the prevention and control of the epidemic, by a mass vaccination program for indigenous peoples started by February 2021.

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Competing interests None declared

Patient and public involvement Neither patients, parents nor the public were involved in the design, conduct or reporting of this research.

Patient consent for publication Not requirable

Ethics approval The study was approved by the leaders of the communities and by the National Research Ethics Committee (*Comissão Nacional de Ética em Pesquisa* – CONEP; CAAE: 33470020.0.1001.0018), in accordance with the Declarations of Helsinki.

Data availability statement Data are available upon reasonable request (vallinoto@ufpa.br)

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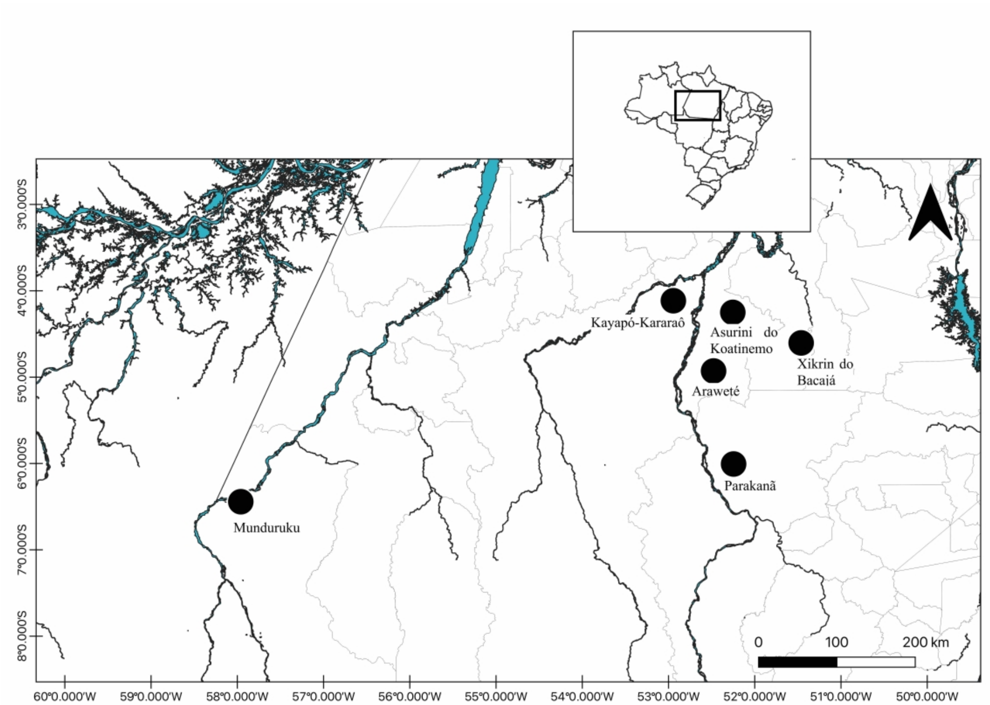
Figure Legend.

Figure 1. Map showing the geographical location of the indigenous communities investigated enrolled in the present study.



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Prevalence of anti-SARS-CoV-2 antibodies among the Brazilian Amazon indigenous populations

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ABSTRACT

Objectives The emergence of SARS-CoV-2 and its spread at a pandemic level generated a serious warning over the impact of the infection on vulnerable indigenous populations of the Brazilian Amazon. Thus, the present study aimed to perform seroepidemiological survey for antibodies anti-SARS-CoV-2 in those populations.

Design We performed a cross-sectional study to investigate the prevalence of anti-spike (S1) IgG antibodies.

Setting Six indigenous ethnic groups living in the State of Pará (Northern Brazil) were investigated. The villages of Xikrin do Bacajá, Assurini, Araweté, Parakanã, Munduruku and Kararaô were visited from October 2020 to January 2021

Participants A total of 1,185 individuals, of both sexes, were enrolled in the investigation.

Method Plasma were tested for the presence of anti-SARS-CoV-2 IgM and IgG antibodies using two assays (a lateral flow rapid test and an ELISA assay).

Results The prevalence of IgM and IgG antibodies was 6.9% and 68,1%, respectively, ranging from 0 to 79.6% with significant differences (p<0.001) between ages in two communities (Araweté, Xikrin and Munduruku) and a virulence rate of 0.86%. Herd immunity was probably attained, similarly as found in other communities of Amazon.

Conclusions SARS-CoV-2 was rapidly spread among the indigenous populations investigated, but it carried a low mortality. It is necessary to expand the serological investigations towards other communities in the Amazon region of Brazil.

Keywords: SARS-CoV-2, COVID-19, Indigenous peoples, Amazon.

Strengths and limitations of this study

- Our previous reports called the attention to the distinct cultural and health aspects of the Amazonian peoples, the impact of the virus among native peoples, and the importance of performing seroepidemiological surveys among vulnerable populations.

- The results showed that SARS-CoV-2 infection reached the different indigenous peoples living in the Brazilian Amazon region in an almost homogeneous way.
- The results highlight that SARS-CoV-2 infection hit the indigenous populations as rapidly as the virus entered the Amazon region, without the predicted mortality.
- The high IgG prevalence suggest the herd immunity was probably attained but the presence of IgM positivity showed ongoing cases.
- Furthermore, seroepidemiological surveys are of paramount importance for monitoring the outcome of the national contingency plan for the prevention and control of the epidemic.

INTRODUCTION

The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the first cases of Coronavirus Diseases 2019 (COVID-19) were reported by November 2019, in Wuhan, China.[1] The rapid spread of the virus determined its classification as pandemic by WHO[2] and because of the high morbimortality it highlighted the burden to vulnerable populations, including native indigenous peoples living in the Brazilian Amazonian who are susceptible to the virus and could be devastated by their immunological frailty.[3-6] 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]

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.

METHODS

Type of study and ethic aspects

In 2020, a large cross-sectional seroepidemiological surveillance among indigenous ethnic groups within Para State (Northern Brazil, Amazon), started to investigate the prevalence of anti-SARS-CoV-2 IgM and IgG antibodies and the impact of the virus on the health of communities. The study was approved by the leaders of the communities and by the National Research Ethics Committee (*Comissão Nacional de Ética em Pesquisa* – CONEP; CAAE: 33470020.0.1001.0018), in accordance with the Declarations of Helsinki.

Study population

From October 2020 to January 2021, multiprofessional health care expeditions were prepared, composed by our team, the staff of the Health Department of the State of Para (*Secretaria de Saúde do Estado do Pará – SESPA*) and the Special Indigenous Health Districts of Altamira and Santarém (*Distrito Sanitário Especial Indígena – DSEI*) of the Special Secretariat of Indigenous Health (*Secretaria Especial de Saúde Indígena – SESAI-MS*). Six ethnic groups were visited while presenting active cases of COVID-19, before starting the vaccination campaign among indigenous communities. 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.

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 (Figure 1). 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 (-4,8853, -52,4281), right bank tributary of the middle Xingu River, in the municipality of Altamira (PA). A total of 508 people from all villages were sampled;

(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 (-5,6904, -52,0037), 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;

(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, in the middle Bacajá basin (-3.7160, -53.0546), 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 (-3,9112, -52,8044), in Altamira (PA). A sample of 44 individuals was collected from a total population of 83;

(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 (-3.9112, -52.8044). 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 (PA).

Anti-SARS-CoV-2 antibody assays

A blood sample (5 mL) was drawn and the plasma tested for the presence of anti-SARS-CoV-2 IgM and IgG antibodies. The prevalence of anti-SARS-CoV-2 IgM and IgG antibodies used a rapid test (lateral flow method; Guangzhou Wondfo Biotech Co., China) and an enzyme-linked immunosorbent assay (ELISA; Anti-SARS-CoV-2 S1 IgG, Euroimmun, Brazil), respectively, according to the manufacturer’s recommendation.

Data analysis

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.

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.

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Neither patients, parents nor the public were involved in the design, conduct or reporting of this research.

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Munduruku a lower frequency among those <6 years old and greater ($p<0.0001$) among those older than 31 years old (Table 2).

Table 1. Prevalence of anti-SARS-CoV-2 (S1) IgG antibodies among Indigenous villages

Population	Age range	Sex		Rapid Test results				ELISA IgG anti-Spike S1 results				N Deaths
		Male N (%)	Female N (%)	N tested	Negative N (%)	IgM N (%)	IgG N (%)	N tested	Negative N (%)	Ind N (%)	IgG N (%)	
Xikrin do Bacajá[7]	02 a 84	51 (51.0)	49 (49.0)	100	42 (42)	nt	58 (58)	100	27 (27)	0 (0)	73 (73)	01
Asurini	17 a 42	0 (0)	8 (100)	0	nt	nt	nt	08	7 (87.5)	1 (12.5)	0 (0)	0
Araweté	8m a 84	258 (50.8)	250 (49.2)	236	29 (12.3)	11 (4.7)	196 (83)	508	92 (18.1)	12 (2.4)	404 (79.5)	0
Parakanã	7m to 95 y	106 (50.5)	104 (49.5)	195	06 (3.1)	39 (20)	150 (76.9)	210	65 (30.9)	06 (2.8)	139 (66.2)	01
Munduruku	7 m to 89 y	116 (36.6)	201 (63.4)	166	96 (57.8)	nt	70 (42.2)	317	139 (43.9)	14 (4.4)	164 (51.7)	05
Kararaô	1 a 94	21 (47.7)	23 (52.3)	44	13 (29.5)	1 (2.3)	30 (68.2)	44	9 (20.4)	0 (0)	35 (79.5)	0
Total	7m a 95 y	552 (46.5)	635 (53.5)	741	185 (25.0)	51 (6.9)	505 (68.1)	1.187	339 (28.5)	33 (2.8)	815 (68.7)	07

M (months); Y (years), nt (not tested); [7] Rodrigues et al. (2021); Ind: Indeterminate

Table 2. Age and sex distribution to anti-SARS-CoV-2 (S1) IgG antibodies among Indigenous villages

Age	Xikrin[7]		Asurini		Araweté		Parakanã		Munduruku		Kararaô		Total
	male	female	male	female	male	female	male	female	male	female	male	female	n/ total (%)
0-6	5	2	0	0	44	27	10	6	6	5	3	1	109/1,187 (9.2)
>6 a 16	15	11	0	0	61	56	18	18	8	17	6	8	218/1,187 (18.4)
>16 a 31	9	13	0	0	56	66	12	21	14	44	2	6	243/1,187 (20.5)
>31	10	8	0	0	45	49	27	27	26	42	3	6	243/1,187 (20.5)
*not informed	0	0	0	0	0	0	0	0	1	1	0	0	2/1,187 (0.2)
Total	39	34	0	0	206	198	67	72	55	109	14	21	815/1,187 (68.7)

[7] Rodrigues et al. (2021); Age distribution: (Overall: χ^2=8.2; p=0.042); (Xikrin: G=8.15; p=0,0198); (Araweté: χ^2=12.3; p=0.0065); (Parakanã: χ^2=3.9; p=0.2753); (Munduruku: χ^2=23.8; p<0.0001); (Kararaô: G=4.4; p=0.2213). G - G test; χ^2 - Chi-square.

The main clinical manifestations reported among infected individuals were coughing, dyspnea, coryza, fever, fatigue, diarrhea, ear pain, headache, chest and back pain. There were seven deaths among the 815 infected persons showing a virulence rate of 0.86%.

DISCUSSION

The high prevalence of IgG anti-SARS-CoV-2 antibodies reported herein show that the new Corovavirus infection reached indigenous populations in a wide way. Introduction of new infectious agents among vulnerable indigenous peoples is thought to be a heavy burden because of the low genetic variability among genes that control the immune response,[9] an important element working as a selective pressure over indigenous peoples since the initial colonization process of the Amazon region of Brazil. SARS-CoV-2 brought the question again as to how the virus would affect native peoples? Despite the theoretical arguments for the possible speculative devastation of indigenous groups,[6] there was no confirmed evidence of susceptibility to Covid-19 in the presence of coinfections and pre-existing conditions, including obesity and malnutrition as previously suggested.[4 5]

Most of the indigenous peoples of the Amazon region of Brazil, including those of the present study, present endemic diseases as malaria, tuberculosis, virus hepatitis and, to HTLV-2 hyperendemic infections among Xikrin, Kararaô, Munduruku and Parakanã.[10] Presently, there is no scientific evidence for host modulation of SARS-CoV-2 in the presence of these coinfections, but researches in order to investigate these coinfections are of paramount importance for a better understanding of the outcome of SARS-CoV-2 infection. Furthermore, environmental and social conditions are important factors that could impact on COVID-19 among indigenous communities,[3 4] including the lack of drinking water and malnutrition, which might had been potentialized after the prophylactic isolation measures.

In our previous report the quality of tests to measure the presence of antibodies was raised.[7] It is important that antibodies to SARS-CoV-2 are detected using tests with greater sensitivity and specificity to obtain accurate prevalence rates. Rapid tests usually show low sensitivity and yield false negative results and false-positive results due to cross-reactions. In the present study, two methodologies were used and the agreement of the results of the rapid test in relation to ELISA was good and the IgG prevalence values similar.

In contrast to the genetic and socio-environmental vulnerability the results showed SARS-CoV-2 infection evenly distributed, with a high prevalence of the virus and few reported deaths, confirming the official results by Special Secretariat of Indigenous Health (SESAI) in its COVID-19 epidemiological bulletin.[11]

The prevalence of anti-SARS-CoV-2 IgG antibodies shows a high dissemination of the virus spread favored by inherent social and cultural difficulties of keeping social distance, sharing households with other families and wearing masks correctly

The detection of IgM antibody 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]

It is relevant to mention that three villages are more than 100Km apart from each other and there is no simple way of communication among them. This raises another important question in regard to herd protective immunity. Apparently, the communities achieved herd immunity levels, when reaching at least 60% seropositivity for IgG,[13] similarly as found in Manaus, the capital of the Amazonas State.[14] However, differently from what is seen among urban areas,[15] virgin soil epidemics generally exhaust susceptible individuals before it comes to an end,[16] and the present epidemic is still not totally explained and understood. 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.[17] 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.[18]

The results show new information over the SARS-CoV-2 epidemic among indigenous peoples and provides the Brazilian government with information to establish adequate measures to control the epidemic among Brazilian indigenous communities in the Amazon region.

CONCLUSION

SARS-CoV-2 infection reached the indigenous populations from the State of Para as rapid as the virus entered the Amazon region, confirming our previous alert to the need of serological studies for surveillance, minimizing the burden of the epidemic and to promote indigenous health policies.[5]

In spite of the suggestion of high mortality and chaos facing the Amerindian populations,[6] the majority of the cases was asymptomatic or mild, with low fatality rate, supporting analysis that shows that the mortality associated with epidemics following contact of Amazonian indigenous communities with urban communities has decreased in recent years.[19]

Finally, continuing seroepidemiological surveys are of paramount importance for monitoring the outcome of the national contingency plan for the prevention and control of the epidemic, by a mass vaccination program for indigenous peoples started by February 2021.

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Competing interests None declared

Patient and public involvement Neither patients, parents nor the public were involved in the design, conduct or reporting of this research.

Patient consent for publication Not requirable

Ethics approval The study was approved by the leaders of the communities and by the National Research Ethics Committee (*Comissão Nacional de Ética em Pesquisa* –

CONEP; CAAE: 33470020.0.1001.0018), in accordance with the Declarations of Helsinki.

Data availability statement Data are available upon reasonable request (vallinoto@ufpa.br)

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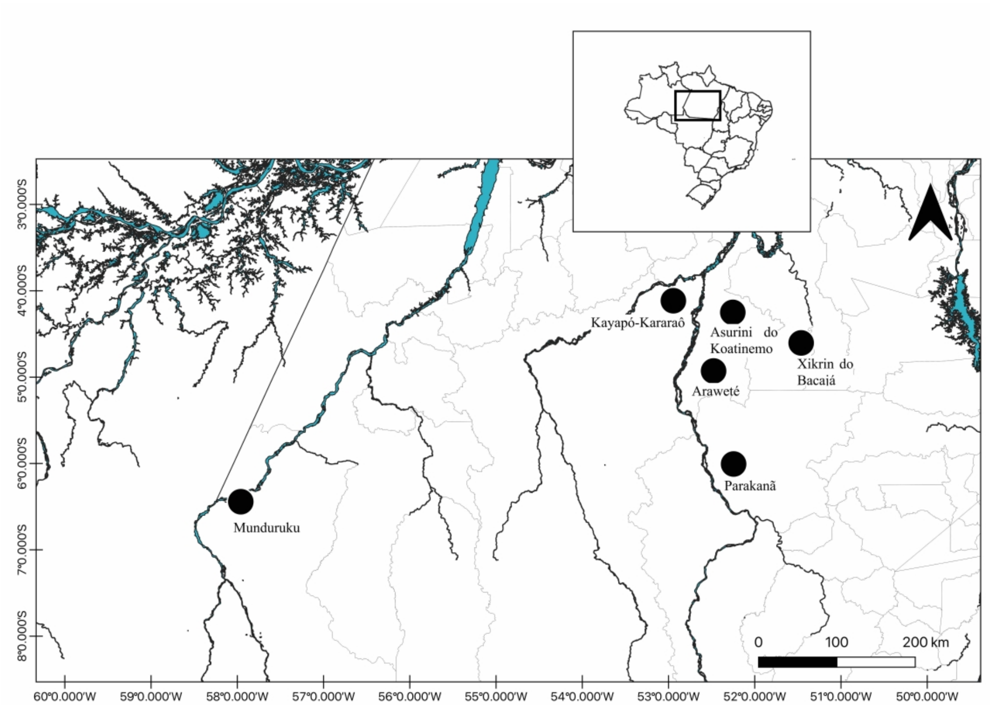
Figure Legend.

Figure 1. Map showing the geographical location of the indigenous communities investigated enrolled in the present study.



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Anti-SARS-CoV-2 antibodies among indigenous populations of the Brazilian Amazon: a cross-sectional study

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ABSTRACT

Objectives The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and its pandemic spread generated serious concern about the impact of the infection on vulnerable indigenous populations of the Brazilian Amazon. Thus, the present study aimed to perform a seroepidemiological survey of anti-SARS-CoV-2 antibodies in those populations.

Setting Six indigenous ethnic groups living in the State of Pará (Northern Brazil) were investigated. The villages of Xikrin do Bacajá, Assurini, Araweté, Parakanã, Munduruku and Kararaô were visited from October 2020 to January 2021.

Design and participants We performed a cross-sectional study to investigate the prevalence of anti-spike (S1) IgG antibodies. Plasma was tested for the presence of anti-SARS-CoV-2 IgM and IgG antibodies using two assays (a lateral flow rapid test and an ELISA). A total of 1,185 individuals of both sexes were enrolled in the study.

Results The prevalence of IgM and IgG antibodies were 6.9% and 68.1%, respectively, ranging from 0% to 79.6%, with significant differences ($p<0.001$) between age groups in three communities (Araweté, Xikrin and Munduruku) and a virulence rate of 0.86%. The overall IgG prevalence obtained by rapid tests and ELISAs were similar, and the agreement of the results between the two tests was 80%, which was classified as good (kappa=0.4987; p<0.001; sensitivity of 82.1% and specificity of 71.6%). Herd immunity was probably attained, similar to that found in other communities of the Amazon.

Conclusions SARS-CoV-2 spread rapidly among the indigenous populations investigated, but it had a low mortality rate. It is necessary to expand serological investigations to other communities in the Amazon region of Brazil.

Keywords: SARS-CoV-2, COVID-19, Indigenous peoples, Amazon.

Strengths and limitations of this study

- The sample size of the present study was high and representative of the populations.

- Serological tests of high sensitivity and specificity were used in the present study.
- Failure to assess infection by RT-PCR was a limitation of the study.

INTRODUCTION

Infection with novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the first cases of coronavirus disease 2019 (COVID-19) were reported by November 2019 in Wuhan, China.[1] The rapid spread of the virus determined its classification as a pandemic by the World Health Organization (WHO)[2,] and the high associated morbimortality highlighted the burden imposed on vulnerable populations, including native indigenous peoples living in the Brazilian Amazon who were susceptible to the virus and could be substantially affected due to their immunological vulnerablity.[3-6] Three previous reports from our laboratory highlight the distinct cultural (sharing households) and health aspects (coinfections and malnutrition) and mode of infection among these Amazonian populations, the impact of the virus among native people, and the importance of performing serosurveys among such populations to determine the spectrum of illness.[5 7 8]

Due to the vulnerability of indigenous people who inhabit the Brazilian Amazon region and their supposed inability to respond immunologically to new pathogens that emerge in the community, the following question was asked: What was the impact of SARS-CoV-2 infection on indigenous people living in the Brazilian Amazon region? Assuming the possible negative impact that the COVID-19 pandemic may have had on these communities, in this study, we carried out a seroepidemiological investigation in indigenous populations located in the State of Pará by performing anti-SARS-CoV-2 antibody screening.

METHODS

Type of study and ethical considerations

In 2020, a large cross-sectional seroepidemiological surveillance study among indigenous ethnic groups within Para State (Northern Brazil, Amazon) was initiated to investigate the prevalence of anti-SARS-CoV-2 IgM and IgG antibodies and the impact of the virus on the health of communities. The study was approved by the leaders of the communities and by the National Research Ethics Committee (*Comissão Nacional de Ética em Pesquisa* – CONEP; CAAE: 33470020.0.1001.0018), in accordance with the Declarations of Helsinki.

Study population

From October 2020 to January 2021, multiprofessional health care expeditions, composed of members of our team and staff of the Health Department of the State of Para (*Secretaria de Saúde do Estado do Pará – SESPA*) and the Special Indigenous Health Districts of Altamira and Santarém (*Distrito Sanitário Especial Indígena – DSEI*) of the Special Secretariat of Indigenous Health (*Secretaria Especial de Saúde Indígena – SESAI-MS*), were established. Six ethnic groups with active cases of COVID-19 in members were visited before initiation of the vaccination campaign among indigenous communities. Indigenous individuals underwent a standard clinical examination that was performed under field conditions; the examination consisted of anamnesis and a physical examination (inspection, palpation, percussion and auscultation), with assessments of anthropometric, blood pressure, body temperature and digital oximetry data. Laboratory support was provided for blood counts and biochemical, microbiological and parasitological exams.

The villages of the indigenous people of the basin of the middle Xingu River (Asurini, Araweté and Parakanã), Iriri River (Kararaô) and Tapajós River (Munduruku) are usually accessed by the river, while the village of the Xikrin people, located in the Bacajá River channel, are accessed by road (Figure 1). The indigenous people studied were as follows:

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

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

(iii) Parakanã: indigenous Tupi-Guarani people who live in the Apyterewa land, in the municipalities of Altamira and São Félix do Xingu, in the Xingu basin (-5,6904, -52,0037), Pará. The total population is 782 people distributed in 16 small villages, with populations ranging from 17 to 94 individuals. Two hundred ten people were examined.

(iv) Xikrin do Bacajá: people who speak the Kayapó (or Mebengokré) language, Jê linguistic family, and live in 19 small villages (populations from 11 to 141 individuals; current population of 1,051 inhabitants) in the middle Bacajá basin (-3.7160, -53.0546) and municipalities of Senador José Porfírio and Anapú, PA. One hundred individuals from seven villages were examined: 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 distributed 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 (-3,9112, -52,8044), Altamira, PA. Forty-four individuals were examined among a total population of 83 individuals.

(vi) Munduruku: an indigenous population belonging to the Munduruku linguistic family of the Tupi language trunk who lives in southwestern Pará in the Tapajós River channel and tributaries in the municipalities of Santarém, Itaituba and Jacareacanga (-3.9112, -52.8044). The total population of 10,629 is distributed in 133 villages. A total of 317 individuals were examined: 213 from Nova Karapanatuba village (213/414) and 201 from smaller villages around Nova Karapanatuba in Jacareacanga, PA.

Anti-SARS-CoV-2 antibody assays

A blood sample (5 mL) was collected, and the plasma was tested for the presence of anti-SARS-CoV-2 IgM and IgG antibodies. Anti-SARS-CoV-2 IgM and IgG antibodies were detected using a rapid test (lateral flow method; Guangzhou Wondfo Biotech Co., China) and an enzyme-linked immunosorbent assay (ELISA; Anti-SARS-CoV-2 S1 IgG, Euroimmun, Brazil), respectively, according to the manufacturers' recommendations.

Data analysis

The Kappa test was used to assess the agreement between the rapid test and ELISA results. G and chi-square tests were performed to assess difference in the prevalence of IgG among the villages in relation to sex and age.

We estimated the virulence rate as the number of deaths in each village divided by the number of individuals who were infected with SARS-CoV-2, according to IgG seropositivity.

Patient and public involvement

The study subjects, parents of study subjects or the public had no involvement in the design, conduct or reporting of this research.

RESULTS

A total of 1,187 subjects were investigated, including 552 males (46.5%) and 635 females (53.5%). The ages ranged from 1 to 95 years old (mean of 26.2, standard deviation of 19.9 years).

Anti-SARS-CoV-2 IgG was detected in 505 (68.1%) individuals by rapid tests and 815 (68.7%) individuals by ELISAs (Table 1). Eight Asurini individuals were negative for antibodies, but among the other indigenous populations, antibody positivity (by ELISA) ranged from 51.7% (Munduruku) to 79.5% (Araweté and Kararaô). The overall IgG prevalence obtained by rapid tests and ELISAs were similar, and the agreement of the results between the two tests was 80%, which was classified as good (kappa=0.4987; $p<0.001$; sensitivity of 82.1% and specificity of 71.6%). Thirty-three (2.8%) individuals had indeterminate anti-SARS-CoV-2 IgG antibody results by ELISA. Additionally, IgM antibodies were detected in 51 (6.9%) individuals from three villages.

There was no significant difference in the IgG prevalence between the sexes (overall: $\chi^2=0.001$, p=0.9793; Xikrin: $\chi^2= 0.056$, p=0.8129; Araweté: $\chi^2=0.003$, p=0.9554; Parakanã: $\chi^2=1.022$, p=0.3121; Munduruku: $\chi^2=1.496$, p=0.2213; Kararaô: $\chi^2=0.0278$, p=0.0642). Regarding age, in Araweté and Xikrin, the prevalences were significantly lower among those >31 years old (p=0.0065 and p=0.0198), and in Munduruku, the prevalence was lower among those aged <6 years ($p<0.0001$) and more than 31 years (Table 2).

Table 1. Prevalence of anti-SARS-CoV-2 (S1) IgG antibodies among indigenous populations by village

Population	Age range	Sex		Rapid test results				ELISA IgG anti-spike S1 results				N Deaths
		Male N (%)	Female N (%)	N tested	Negative N (%)	IgM N (%)	IgG N (%)	N tested	Negative N (%)	Ind N (%)	IgG N (%)	
Xikrin do Bacajá[7]	2 y to 84 y	51 (51.0)	49 (49.0)	100	42 (42)	nt	58 (58)	100	27 (27)	0 (0)	73 (73)	01
Asurini	17 y to 42 y	0 (0)	8 (100)	0	nt	nt	nt	08	7 (87.5)	1 (12.5)	0 (0)	0
Araweté	8 m to 84 y	258 (50.8)	250 (49.2)	236	29 (12.3)	11 (4.7)	196 (83)	508	92 (18.1)	12 (2.4)	404 (79.5)	0
Parakanã	7 m to 95 y	106 (50.5)	104 (49.5)	195	06 (3.1)	39 (20)	150 (76.9)	210	65 (30.9)	06 (2.8)	139 (66.2)	01
Munduruku	7 m to 89 y	116 (36.6)	201 (63.4)	166	96 (57.8)	nt	70 (42.2)	317	139 (43.9)	14 (4.4)	164 (51.7)	05
Kararaô	1 y to 94 y	21 (47.7)	23 (52.3)	44	13 (29.5)	1 (2.3)	30 (68.2)	44	9 (20.4)	0 (0)	35 (79.5)	0
Total	7 m to 95 y	552 (46.5)	635 (53.5)	741	185 (25.0)	51 (6.9)	505 (68.1)	1.187	339 (28.5)	33 (2.8)	815 (68.7)	07

m (months); y (years), nt (not tested); [7] Rodrigues et al. (2021); Ind: Indeterminate

Table 2. Anti-SARS-CoV-2 (S1) IgG antibodies among indigenous villages by age and sex

Age (y)	Xikrin[7]		Asurini		Araweté		Parakanã		Munduruku		Kararaô		Total
	male	female	male	female	male	female	male	female	male	female	male	female	**n/total (%)**
0-6	5	2	0	0	44	27	10	6	6	5	3	1	109/1,187 (9.2)
>6 to 16	15	11	0	0	61	56	18	18	8	17	6	8	218/1,187 (18.4)
>16 to 31	9	13	0	0	56	66	12	21	14	44	2	6	243/1,187 (20.5)
>31	10	8	0	0	45	49	27	27	26	42	3	6	243/1,187 (20.5)
*not informed	0	0	0	0	0	0	0	0	1	1	0	0	2/1,187 (0.2)
Total	39	34	0	0	206	198	67	72	55	109	14	21	815/1,187 (68.7)

[7] Rodrigues et al. (2021); Age distribution: (Overall: χ^2=8.2; p=0.042); (Xikrin: G=8.15; p=0,0198); (Araweté: χ^2=12.3; p=0.0065); (Parakanã: χ^2=3.9; p=0.2753); (Munduruku: χ^2=23.8; p<0.0001); (Kararaô: G=4.4; p=0.2213). G - G test; χ^2 - chi-square.

The main clinical manifestations reported among infected individuals were coughing, dyspnea, coryza, fever, fatigue, diarrhea, ear pain, headache, and chest and back pain. There were seven deaths among 815 infected persons, resulting in a virulence rate of 0.86%.

DISCUSSION

The high prevalence of IgG anti-SARS-CoV-2 antibodies reported herein shows that novel coronavirus infection broadly impacted indigenous populations. The introduction of new infectious agents among vulnerable indigenous populations is thought to impose a heavy burden because of the low genetic variability among genes that control the immune response,[9] an important selective pressure in indigenous people since the initial colonization of the Amazon region of Brazil.

The pandemic raised the question of how SARS-CoV-2 would affect native peoples. Despite theoretical arguments for the possible devastation of indigenous groups,[6] there was no confirmed evidence of susceptibility to SARS-CoV-2 infection in the presence of coinfections or pre-existing conditions, including obesity and malnutrition, as previously suggested.[4 5]

Most of the indigenous people of the Amazon region of Brazil, including some of those in the present study, namely the Xikrin, Kararaô, Munduruku and Parakanã, are continuously exposed to endemic diseases such as malaria, tuberculosis, virus hepatitis and HTLV-2 hyperendemic infections.[10] Presently, there is no scientific evidence of host modulation of SARS-CoV-2 in the presence of these coinfections, but research to investigate these coinfections is of paramount importance to better understand of the outcomes of SARS-CoV-2 infection. Furthermore, environmental and social conditions, including a lack of drinking water and malnutrition, are important factors that could impact COVID-19 dynamics among indigenous communities,[3 4] and these factors might have been potentiated after prophylactic isolation measures.

In our previous report, the quality of tests to measure the presence of antibodies was analyzed.[7] It is important that antibodies to SARS-CoV-2 are detected using tests with good sensitivity and specificity to obtain accurate prevalence rates. Rapid tests usually have low sensitivity and yield false-negative or false-positive results due to cross-reactions. In the present study, two methodologies were used; the agreement of the results between the rapid test and ELISA was good, and the IgG prevalence values were similar.

The results showed that SARS-CoV-2 infections were evenly distributed, with a high viral prevalence but few reported deaths, despite genetic and socioenvironmental vulnerability, confirming the official results of the COVID-19 Epidemiological Bulletin published by the Special Secretariat of Indigenous Health (SESAI).[11]

The prevalence of anti-SARS-CoV-2 IgG antibodies indicated wide dissemination of the virus, caused by inherent social and cultural challenges in social distancing, household isolation and mask wearing.

The detection of IgM antibodies in three villages might suggest recent infection in these villages. However, IgM positivity can be confirmed by only antigen or nucleic acid tests, which were not available at the time of the study. Additionally, a recent report showed IgM persistence for up to 8 months post-SARS-CoV-2 infection, suggesting that IgM positivity should no longer be considered a diagnostic criterion to confirm acute or recent SARS-CoV-2 infection.[12]

Notably, three villages are located more than 100 km away from each other, and there is no simple method of communication among them. This raises another important question regarding herd immunity. Apparently, the communities achieved herd immunity when at least 60% IgG seropositivity was reached,[13] similar to that in Manaus, the capital of Amazonas State.[14] However, unlike in urban areas,[15] in rural areas, virgin soil epidemics generally affect most susceptible individuals before the epidemic ends,[16] and the dynamics of the present epidemic are still not completely understood. 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 infection was detected in 83% of the subjects.[17] 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.[18]

The main limitation of our study was that it was not possible to assess the presence of SARS-CoV-2 infection by RT–qPCR at the time of visits to the villages, but the results provide new information about the SARS-CoV-2 epidemic among indigenous peoples and can provide the Brazilian government with information to establish adequate epidemic control measures among Brazilian indigenous communities in the Amazon region.

CONCLUSION

SARS-CoV-2 infection rapidly spread among indigenous populations in the state of Para in the Amazon region, supporting our previous call for serological surveillance studies to minimize the of burden the epidemic and promote indigenous health policies.[5] Despite the suggestion of high mortality and morbidity among Amerindian populations,[6] the majority of cases were asymptomatic or mild, with a low fatality rate, supporting the suggestion that mortality associated with epidemics that spread from urban communities to Amazonian indigenous communities has decreased in recent years.[19] Finally, continuing seroepidemiological surveys is of paramount importance in monitoring the outcome of the national contingency plan for the prevention and control of the epidemic, which includes a mass vaccination program for indigenous peoples, started in February 2021.

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Contributors ACRV, JFG and RI: study conception, data analysis, manuscript writing and editing; ACRV, RI, JFG, INA, BJSB and CNCL: acquisition, analysis, or interpretation of the data; CNCL, RI, IMVCV: drafting of the manuscript; ACRV, RI, JFG and IMVCV: critical revision of the manuscript for important intellectual content; SSL: statistical analysis; ACRV: obtained funding; VOF and EPSR: technical and material support.

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Competing interests None declared

Patient and public involvement The study subjects, parents of the study subjects or the public were not involved in the design, conduct or reporting of this research.

Patient consent for publication Not requirable

Ethics approval The study was approved by the leaders of the communities and by the National Research Ethics Committee (*Comissão Nacional de Ética em Pesquisa* – CONEP; CAAE: 33470020.0.1001.0018), in accordance with the Declarations of Helsinki.

Data availability statement Data are available upon reasonable request (vallinoto@ufpa.br)

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Figure Legend.

Figure 1. Map showing the geographical location of the indigenous communities enrolled in the present study.

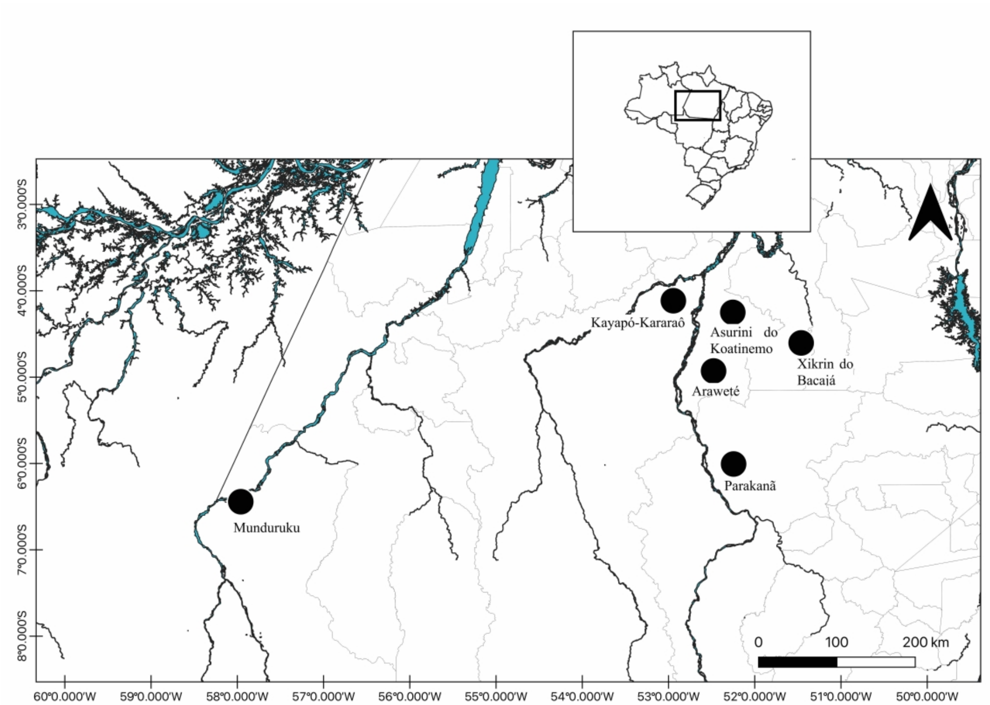

Figure 1. Map showing the geographical location of the indigenous communities enrolled in the present study.

296x209mm (600 x 600 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract Page 1, lines 1 and 2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found Page 2, lines 26 – 36
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported Page 3, lines 55 – 65
Objectives	3	State specific objectives, including any prespecified hypotheses Page 3, lines 66 – 72
Methods		
Study design	4	Present key elements of study design early in the paper Page 4, lines 75 – 81
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection Pages 4 – 6 , lines 84 – 127
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants Pages 4 – 6 , lines 84 – 127
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable Pages 6 and 7 , lines 130 – 134
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group Pages 6 and 7 , lines 84 – 134
Bias	9	Describe any efforts to address potential sources of bias Page 7, lines 136 – 140
Study size	10	Explain how the study size was arrived at Page 7, lines 136 – 140
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why Page 7, lines 136 – 140
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding Page 7, lines 136 – 140
		(*b*) Describe any methods used to examine subgroups and interactions Page 7, lines 136 – 140
		(*c*) Explain how missing data were addressed Page 7, lines 136 – 140
		(*d*) If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses Pages7, lines 136 – 140
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially

		eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed Pages 7 and 8; lines 147 – 162
		(b) Give reasons for non-participation at each stage Pages 7 and 8; lines 147 – 162
		(c) Consider use of a flow diagram Not applicable
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders Pages 7 and 8; lines 147 – 162
		(b) Indicate number of participants with missing data for each variable of interest Pages 7 and 8; lines 147 – 162
Outcome data	15*	Report numbers of outcome events or summary measures Pages 7 and 8; lines 147 – 162
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included Pages 7 and 8; lines 147 – 162
		(*b*) Report category boundaries when continuous variables were categorized Pages 7 and 8; lines 147 – 162
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period Pages 7 and 8; lines 147 – 162
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses Pages 7 and 8; lines 147 – 162
Discussion		
Key results	18	Summarise key results with reference to study objectives Page 11 – 14; lines 178 - 236
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias Page 13; lines 227 - 228
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence Page 13 – 14; lines 234 - 243
Generalisability	21	Discuss the generalisability (external validity) of the study results Page 12 – 14; lines 203 - 226
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based Page 14 – 15; lines 256 - 260

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.