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# Seroprevalence of SARS-CoV-2 in southwest Goiás, Brazil, 2020: a population-based cross-sectional serological study

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## Abstract

**Aim:** The COVID-19 pandemic has been causing significant socioeconomic and health implications worldwide. We aimed to perform a population-based serological survey of SARS-CoV-2 in Quirinópolis, Goiás and to relate the infection to sociodemographic and epidemiological aspects.

**Methods:** Systematic sampling and active search in the community was conducted using a rapid detection kit for specific IgG and IgM. Three collection phases occurred between September and October 2020.

**Results:** In total, 557 volunteers participated in the study. The general seroprevalence rate was 8.1%, with one-off prevalence of 10.4%, 6.7% and 6.8% in each of the three phases. There was a predominance of results for IgG antibodies (88.9%). The bivariate analysis revealed that age < 19 years (OR = 5.0; 95%CI: 2.10-11.90;  $P = 0.0003$ ), search for medical care (OR = 2.21; 95%CI: 1.10-4.31;  $P = 0.03$ ), families of 3-4 residents (OR = 2.34; 95%CI: 1.08-5.05;  $P = 0.03$ ) and the presence of symptoms (OR = 3.59; 95%CI: 1.92-6.78;  $P < 0.001$ ) were associated with antibody detection. The symptoms related to the detection of antibodies were mainly fever (OR = 10.49; 95%CI:



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4.52-23.93;  $P < 0.001$ ) and abnormalities in taste (OR = 13.57; 95%CI: 6.15-29.84;  $P < 0.001$ ) and smell (OR = 13.30; 95%CI: 5.36-32.99;  $P < 0.001$ ).

**Conclusion:** The high seroprevalence and predominance of IgG antibodies indicated intense circulation of the virus. These data suggest late application of protective measures, as well as population behaviour that favours viral dissemination. In this aspect, young people < 19 years of age were important, possibly due to greater exposure. The majority detection of mild symptoms was a reflection of the active search in the community.

**Keywords:** Serology, epidemiological survey, COVID-19, risk factors

## INTRODUCTION

On 8 December 2019, a novel coronavirus was identified for the first time in Wuhan, Hubei Province, China, causing serious respiratory syndromes and leading to higher hospitalisation rates and deaths, being later called SARS-CoV-2<sup>[1]</sup>. At the present time, widespread distribution of vaccines is restricted to a few countries; in Brazil, complete immunisation is still incipient. The clinical management protocol is highly variable and based on the patient's risk factors. Additionally, the virus has a dynamic of fast and efficient transmission, originated primarily from asymptomatic individuals<sup>[2,3]</sup>.

COVID-19 cases and deaths continue to increase worldwide, and the Americas account for the highest number of COVID-19 cases in the world. In Brazil, one of the countries with the lowest number of tests performed in the world, the Ministry of Health confirmed the first case on 25 February 2020 and the first report of self-sustaining cases on 5 March 2020. Therefore, COVID-19 is significantly underreported, thus resulting in an increase in the number of symptomatic cases and hampering the application of effective public policies, mainly in terms of social restriction<sup>[4-7]</sup>.

Moreover, information on socioeconomic, demographic and epidemiological determinants are not routinely investigated<sup>[8]</sup>. Black populations and ethnic minorities, for example, are most severely affected due to social inequities and availability of medical care resources<sup>[9]</sup>. Besides, cardiovascular diseases, diabetes and advanced age are prerequisites for severe infection<sup>[8,10]</sup>.

Within this context, serological surveys can provide important results in vast regions where COVID-19 investigations are not implemented. Furthermore, the high sociodemographic heterogeneity of the country may affect myriad epidemiological data on the disease<sup>[11]</sup>.

Antibody detection tests are known to have limitations, especially when compared to viral genome detection tests. Even so, rapid serological tests can represent a valuable research tool for SARS-CoV-2, considering its practicality, speed and ease of handling, especially when there is no practical application of molecular tests.

Therefore, the collection of data from serological surveys may elucidate questions about the dynamics and impact of the pandemic in specific territories. Consequently, prophylactic measures and strategies for reducing this impact could be better implemented. In this context, the present study aimed to conduct a population-based serological survey of SARS-CoV-2 in the municipality of Quirinópolis, Goiás, and to relate the infection with the sociodemographic and epidemiological aspects of the patients.

## METHODS

This was a cross-sectional, population-based study, performed through systematic sampling. All areas served by the Basic Health Units of the municipality were selected in this study, covering the entire municipality. One to two households were selected per block according to a pre-established route for collection. Posteriorly, one resident per household was randomly chosen for serological diagnosis by means of the rapid test, using an immunochromatographic kit for SARS-CoV-2 (detection of IgM and IgG). If the sample was positive, all residents present at the time of collection were invited to participate in the study.

The study was divided into three phases of data collection, with an interval of three weeks between each phase to assess the dynamics of viral circulation over time. The first sampling took place in the first week of September (first phase), in which the script of the data collection was established. Subsequently, the second phase was performed in the last week of September and the third at last at the end of October. For these two subsequent collections, neighbouring houses to those drawn in the first phase were chosen. Buildings and commercial establishments, as well as homes with a single resident, were excluded.

The serological survey was carried out in the municipality of Quirinópolis, located in the southwest of the state of Goiás, with a population of approximately 46,788 inhabitants. Regarding the organisation of the Basic Health Units (BHU), the municipality has 13 BHU serving the city, each unit representing about 3800 people.

The team that conducted the data collection worked in pairs and was constituted of employees from the Quirinópolis City Hall. One employee was the community health agent, who was responsible for the initial approach, including a brief explanation of the research objectives, presentation of the free and informed consent term, invitation to participate and, in the case of acceptance of the participation, selection of the member to be approached and application of the questionnaire. Another member of the team, a nurse and/or a dentist, performed a digital puncture to conduct the antibody detection test.

A rapid immunochromatography test kit was used to detect IgG and IgM antibodies for COVID-19: “COVID-19 IgG/IgM”, produced by QINGDAO HIGHTOP BIOTECH Co., Ltd. (Shandong, China). The sensitivity and specificity of the test are 94.14% and 93.91%, respectively. The use of the kit and the interpretation of the data were performed according to the manufacturer’s instructions.

The questionnaire contained sociodemographic and clinical information. Clinical symptoms of the last two weeks, risk factors (comorbidities), risk behaviour (smoking and alcohol consumption), recent trips made by the participant or by a member of the household, social isolation measures, number of residents in the household and use of health services were investigated.

For the sample calculation, a margin of error of 5%, a confidence level of 95%, the total population of the studied area (according to the 2014 IBGE census: 46,788 inhabitants) and a prevalence value of 50% were considered. The use of this prevalence aimed to optimise the sample, thus obtaining a more conservative value. This strategy should be used in situations in which the real prevalence is not known, or when official statistics possibly differ from reality in the population, as in this study, in which the prevalence, according to the official epidemiological bulletin, was 0.6%. Using the formula described by Arango (2001)<sup>[12]</sup>, we obtained the minimum sample number of 381 participants. The samples were collected independently of signs and symptoms of COVID-19 of the participants.

For data analysis, a specific database was constructed using the Epi Info 7.2.2 program. Frequency, significance and association measures were also calculated through this program.

The analysis was conducted according to the following steps: (1) verification of data consistency; (2) distribution of frequencies for all variables surveyed, characterising the population studied according to sociodemographic aspects, comorbidities, risk behaviours and clinical symptoms; and (3) bivariate analysis, where comparisons for two proportions were analysed by Fisher's exact test or Chi-square test ( $\alpha = 5\%$ ). To quantify the association between possible associated factors, Odds Ratio (OR) with a 95% confidence interval was used. For variables with more than two categories, a dummy variable was used by means of logistic regression.

This proposal obtained a favourable ethical opinion from the National Council of Ethics in Research (protocol number 4,260,760/2020; Certificate of Presentation of Ethical Appreciation 36260720.5.0000.8113). A signed informed consent form was obtained from each participant, as determined by the National Council of Ethics in Research with Human Beings (Ministry of Health, Brazil).

## RESULTS

In total, 557 individuals participated in the study, distributed in all sectors of the municipality [Figure 1]: 202 samples for the first phase (21 positive cases), 178 for the second phase (12 positive samples) and 177 samples for the third phase (12 positive samples). After extrapolating the minimum sample number, the expected sample error changed from 5% to 4%.

Regarding the sociodemographic characteristics, the majority were female (66.4%), adults aged 20-59 years (64.7%), self-declared to be brown- or black-skinned (61.6%) and with education up to 11 years old (84.3%). Further, 33.9% of the participants live in houses with  $\leq 2$  people, 51.4% with 3-4 people and 14.7% with  $\geq 5$  people.

Seroprevalence in the three phases is shown in Figure 2. Both IgG positive and IgM cases were analysed. There was no significant difference in relation to seropositivity between the phases. The general seroprevalence (considering the three phases of collection) of the population against SARS-CoV-2 was 8.1% (95%CI: 6.1-10.6), with 45 positive samples widely distributed in the municipality detected. Results with IgG antibodies were predominant, being detected in 88.9% of the cases. However, considering the positive cases, 26 (57.8%) were detected in only 10 households (minimum-maximum: 2-4 cases).

The bivariate analysis between sociodemographic factors and detection of antibodies revealed that age, use of health services in the two weeks prior to collection and number of residents at home were significant in the comparison between positive and negative cases [Table 1]. Youths up to 19 years old were five times more likely to have had contact with the virus than adults aged 20-59 years old. The demand for health services in the two weeks prior to the survey and the presence of 3-4 residents at home had more than twice the chance of contact with the virus [Table 1].

Twenty-six (57.8%) infected individuals showed clinical symptoms, being more than three times as likely to have been in contact with the virus [Table 2]. Most of the symptoms recorded were mild. Among "other symptoms", the most common were headache (11/30; 36.7%) and runny nose (4/30; 13.3%). Only palpitations, vomiting and dyspnoea were not associated. Individuals with fever, alteration of taste and alteration of sense of smell were 10-13 times more likely to have had contact with the virus [Table 2].

**Table 1. Bivariate analysis between sociodemographic factors and positive IgG/IgM antibodies for SARS-CoV-2, Quirinópolis, GO, 2020 (n = 557)**

Variables	Positive IgG/IgM	Bivariate analysis	
		OR (95%CI)	Value of P <sup>a</sup>
<b>Sex</b>			
Female	28/370 (7.6%)	1	0.51
Male	17/187 (9.1%)	1.22 (0.63-2.28)	
<b>Age range</b>			
Youths (up to 19 years)	9/34 (26.5%)	5.00 (2.10-11.90)	0.0003
Adults (20-59 years)	24/357 (6.7%)	1	
Elderly (60 or more)	12/161 (7.4%)	1.11 (0.54-2.29)	0.76
<b>Self-declared ethnicity</b>			
White	20/211 (9.5%)	1	0.33
Brown/Black	24/339 (7.1%)	0.72 (0.39-1.36)	
<b>Education</b>			
0 to 4 years of study	1/32 (3.13%)	1	
5 to 8 years of study	14/214 (2.27%)	2.16 (0.27-17.05)	0.46
9 to 11 years of study	23/213 (10.80%)	3.74 (0.48-28.74)	0.20
12 years of study or more	6/86 (6.98%)	2.32 (0.26-20.06)	0.44
<b>Higher education level</b>			
5 to 8 years of study	7/99 (7.1%)	1	
9 to 11 years of study	18/208 (8.65%)	1.24 (0.50-3.08)	0.63
12 years of study or more	14/144 (9.72%)	1.41 (0.54-3.64)	0.47
<b>Smoking</b>			
No	40/466 (8.6%)	1	0.25
Yes	3/77 (3.9%)	0.43 (0.10-1.29)	
<b>Do any residents smoke?</b>			
No	37/455 (8.1%)	1	0.94
Yes	8/101 (7.9%)	0.97 (0.41-2.09)	
<b>Alcohol consumption?</b>			
No	29/407 (7.1%)	1	0.22
Yes	16/149 (10.7%)	1.56 (0.80-2.96)	
<b>Travel in the last 15 days?</b>			
No	37/464 (8.0%)	1	0.93
Yes	8/90 (8.9%)	1.12 (0.47-2.43)	
<b>Have you used health services in the past two weeks?</b>			
No	31/449 (6.90%)	1	0.03
Yes	14/99 (14.14%)	2.21 (1.10-4.31)	
<b>Social isolation measures (Self-assessment)</b>			
Not adopted	0/13 (0.0%)	-	0.97
Mild/Moderate	32/409 (7.8%)	0.71 (0.36-1.41)	0.33
Total	13/123 (10.6%)	1	
<b>Number of residents in the household</b>			
Up to 2	9/189 (4.8%)	1	
3-4	30/286 (10.5%)	2.34 (1.08-5.05)	0.03
More than 5	6/82 (7.3%)	1.57 (0.54-4.59)	0.40

<sup>a</sup>Chi-square test. OR: Odds ratio; CI: confidence interval.

**Table 2. Bivariate analysis between the presence of symptoms and positive IgG/IgM antibodies for SARS-CoV-2, Quirinópolis, GO, 2020 (n = 557)**

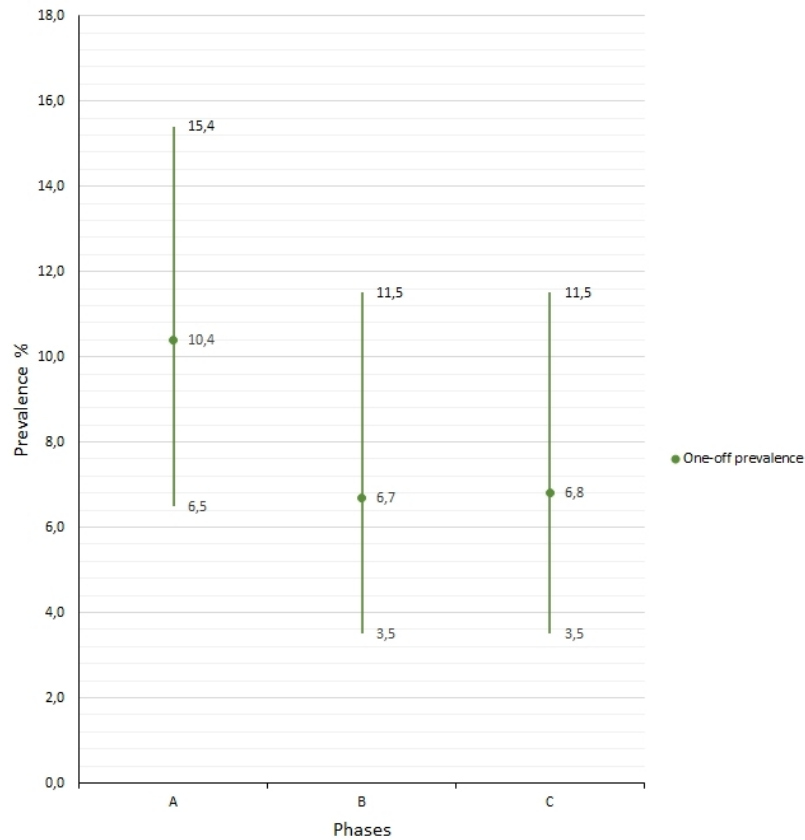
Variables	Positive IgG/IgM (%)	Bivariate analysis	
		OR (95%CI)	Value of P <sup>a</sup>
<b>Presence of symptoms</b>			
No	19/390 (4.9%)	1	< 0.001
Yes	26/167 (15.6%)	3.59 (1.92-6.78)	
<b>Presence of specific symptoms</b>			
<b>Cough</b>			
No	29/467 (6.2%)	1	< 0.001
Yes	16/90 (17.8%)	3.25 (1.65-6.26)	
<b>Fever</b>			
No	33/528 (6.3%)	1	< 0.001
Yes	12/29 (41.4%)	10.49 (4.52-23.93)	
<b>Palpitations</b>			
No	44/537 (8.2%)	1	0.92 <sup>f</sup>
Yes	1/20 (5.0%)	0.59 (0.02-3.34)	
<b>Sore throat</b>			
No	35/490 (7.1%)	1	0.05
Yes	10/67 (14.9%)	2.27 (1.02-4.75)	
<b>Dyspnoea</b>			
No	41/526 (7.8%)	1	0.30 <sup>f</sup>
Yes	4/31 (12.9%)	1.75 (0.50-4.93)	
<b>Alteration of taste</b>			
No	30/524 (5.7%)	1	< 0.001
Yes	15/33 (45.5%)	13.57 (6.15-29.84)	
<b>Alteration of smell</b>			
No	34/534 (6.4%)	1	< 0.001
Yes	11/23 (47.8%)	13.3 (5.36-32.99)	
<b>Vomiting</b>			
No	42/545 (7.7%)	1	0.06 <sup>f</sup>
Yes	3/12 (25.0%)	3.97 (0.84-14.67)	
<b>Diarrhoea</b>			
No	34/520 (6.5%)	1	< 0.001 <sup>f</sup>
Yes	11/37 (29.7%)	6.0 (2.65-13.11)	
<b>Other symptoms</b>			
No	37/527 (7.0%)	1	< 0.001 <sup>f</sup>
Yes	8/30 (26.7%)	4.79 (1.89-11.34)	

<sup>a</sup>Chi-square test or <sup>f</sup>Fisher's exact test. OR: Odds ratio; CI: confidence interval.

## DISCUSSION

The serological survey was an approach of remarkable epidemiological value, considering its importance for the control of the pandemic; its extensive, fast and easy applicability; and the modest costs in comparison to the molecular approach<sup>[13,14]</sup>. Additionally, serological tests by sampling, with active search, can show how the records of COVID-19 are underestimated and, sometimes, inaccurate in relation to the official data<sup>[4]</sup> or even to the statistics derived from the so-called “mass testing”, especially if performed without methodological criteria<sup>[15]</sup>. Furthermore, these tests provide conditions for a better glimpse of the epidemiological situation of the virus in regions where the application of molecular investigative methods is





**Figure 2.** One-off prevalence and 95% confidence interval, according to each stage of the serological survey.

further demonstrates the effectiveness of this type of approach<sup>[13,14]</sup>.

In general, more than 8% of the studied population had contact with SARS-CoV-2, with no concentration of cases in a specific territory. The homogeneous sociodemographic characteristics of the studied region may have contributed to an equitable distribution of cases. In any case, we considered that seroprevalence in the municipality is high when compared to data from the most extensive serological survey conducted in Brazil to date, performed in three stages: 1.9% (95%CI: 1.7-2.1), 3.1% (95%CI: 2.8%-3.4%) and 3.8% (95%CI: 3.5%-4.2%), respectively. It was < 2% in the municipalities surveyed in the Midwest region<sup>[11]</sup>. The prevalence of 8.1% in the population is also substantially higher than the cases confirmed by the local government. In fact, in addition to low testing rates, investigations of this nature may demonstrate how underestimated the detection of SARS-CoV-2 is<sup>[4-6]</sup>. Another element that further elucidates the situation of underestimated cases is the imminent viral spread between asymptomatic and pre-symptomatic cases<sup>[18]</sup>. This aspect is rarely investigated in small municipalities.

Besides being high, seroprevalence in the three phases of this survey remained stable during the surveyed months, with no significant difference. This stability possibly may reflect the behavioural dynamics of the population, as well as the non-application of public policies to promote measures of social restriction. In this case, there may have been a delay in advancing such measures and for the population to assume a behaviour to contain viral spread. Indeed, social restriction measures have been shown to be effective in containing the dissemination of COVID-19<sup>[7,19,20]</sup>. Moreover, political and managerial decisions to contain the pandemic have an influence on the epidemiological situation<sup>[21]</sup>.



Furthermore, the behaviour of alleged habituation to the pandemic situation may also have contributed to the stability of the cases throughout the three phases of the investigation with high seroprevalence. In this case, the perception of risk regarding the pandemic may assume a relativised character, being variable with time and demographic factors<sup>[22]</sup>.

In the context specifically related to the age factor, we found that young people up to 19 years old were five times more likely to have had contact with the virus than adults aged 20-59 years old. The age factor can be important for modulating behaviours during the pandemic, with younger people being less resilient and more prone to risky attitudes in relation to older people<sup>[22]</sup>. The trivialisation of risk could come from the suggestion that young people without comorbidities may have an incorrect perception that they would not be at risk for developing a serious disease<sup>[10,23]</sup>. Younger individuals can infect family members in the most vulnerable situations, at home, where protective measures against the virus are not practiced.

Additionally, considering that the presence of 3-4 residents (compared with families with up to two residents) at home had more than twice the chance of contact with the virus, intrafamilial transmission was relevant in our study, in accordance with a greater propensity for positive cases in larger families, as previously reported<sup>[24]</sup>. However, this hypothesis must be carefully analysed, considering that there was no difference between houses with up to 2 residents and those with more than 5 residents (although the analysis may have been hampered because of the relatively low sample size).

The importance of unregistered cases should also be taken into account, which contributes to a rapid viral spread. In this regard, it is estimated that 79% of infections originate from undocumented cases of the respiratory disease<sup>[3]</sup>. The structural limitation of the municipality evaluated in this study, regarding the ability to carry out molecular epidemiological surveillance mechanisms, should be considered to understand the high record of cases of SARS-CoV-2 infection, which probably occurred at the beginning of the pandemic, as well as the stability in the three phases of the serological survey. In fact, almost 90% of positive cases were IgG, thereby indicating an intense viral circulation in the past. As already discussed, the lack of measures to contain the pandemic or their late application, such as social distancing, which has proven effective<sup>[7,19,20]</sup>, may be related to this character of intense previous viral circulation in the region.

Higher detection of antibodies to SARS-CoV-2 was also reported among those who sought health services in the two weeks prior to collection, hence reflecting that almost 60% of those infected had clinical symptoms<sup>[4]</sup>. The majority of infected people develop clinical symptoms, which makes them more likely to seek medical attention. In this case, the bivariate analysis revealed that those who had at least one symptom were more likely to have had contact with the virus. In any case, most of the symptoms recorded were mild, which may have been a reflection of the design of this sampling approach: active search in the community, regardless of clinical symptoms.

Individuals with antibodies who lived in households with 3-4 residents, compared to households with up to 2 residents, were twice as likely to have had contact with the virus. These data are consistent with the information that there is a high risk of intrafamilial infection by SARS-CoV-2, also considering the ease of viral transmission<sup>[2,25]</sup>. Furthermore, the virus transmission is particularly accentuated when social restriction measures are not adopted<sup>[7,19,20]</sup>.

Those with fever, alteration of taste and alteration of smell were 10-13 times more likely to have had contact with the virus. The clinical symptoms associated with COVID-19, although non-specific and common to various other respiratory agents, are predominantly related to fever, cough, sore throat, vomiting and

diarrhoea, in addition to loss of smell and taste<sup>[23,26]</sup>. All of these clinical characteristics were also recorded in our study, and, with the exception of vomiting, dyspnoea and palpitations, they were significant in the bivariate analysis comparing infected and non-infected individuals.

In general, antibodies were detected in similar proportions for both those with and without comorbidities (7.6% and 8.4%, respectively). Specifically assessing each comorbidity, only high cholesterol accounted, in a very discreet way, for greater chances of contact with the virus. Thus, this association requires confirmation in studies with larger samples and which can assess the high cholesterol of the individuals by laboratory diagnosis.

Even though the presence of comorbidities, in general, did not differ in our bivariate analysis in relation to symptoms, there was a trend of significance (95%CI: 0.97-2.03; OR = 1.41;  $P = 0.07$ ). Despite the evidence that points to comorbidities as important predictive factors for the worsening of the disease<sup>[27]</sup>, these data reinforce that those with comorbidities may show symptoms, even if mild. In this last aspect, this factor may be related to the sample design of the study. In this case, regardless of symptoms, active search may be related to this, considering that most cases with comorbidities and symptoms of the disease result in more complex medical intervention (hospitalisations). In this context, samples were not collected from those who had already obtained a confirmed laboratory diagnosis, in the case of a more serious disease and who required hospital care. Furthermore, there was no follow-up of positive cases with comorbidities after the study.

In any case, the possible relationship between mild symptoms and antibody detection must be considered because the potential for spreading the virus can be associated both with the perception that the disease is not serious (hence resulting in greater exposure and transmission of the pathogen in the community) and with the socialising behaviour and routine work activities that individuals would have, even with mild symptoms. In fact, measures of social isolation among those who have mild symptoms are effective in containing viral spread<sup>[28]</sup>. Importantly, attention is drawn to the fact that the virus is more efficiently disseminated from symptomatic patients<sup>[15]</sup>, which corroborates the findings of high seroprevalence in the municipality (8.1%), although asymptomatic and pre-symptomatic individuals also play a pivotal epidemiological role<sup>[18]</sup>.

We must consider that the present study had some limitations. Despite having received specific training, the collection of samples was carried out by several community health agents, who know the residents and eventually have close relationships with them. In this case, there may have been a bias in the choice of participants. Moreover, the sensitivity and specificity of the test (94.14% and 93.91%, respectively) may be included as a limitation factor, considering false negative/positive results. In addition, we chose to exclude houses with only one resident at the time of collection, in order to assess intra-family transmission of the virus. This criterion may also have constituted a bias. Finally, testing other family members in positive cases may have introduced a selection bias towards a population with higher prevalences, and neighbour sampling in subsequent surveys may have caused matching regarding socio-economic characteristics. Despite that, the present approach proved to be of great value, considering that it was applied in an area where the optimal routine epidemiological surveillance of SARS-CoV-2 is impractical, as for most Brazilian municipalities. Furthermore, active search in the community, regardless of symptoms, can provide more credible data considering that most of the information is obtained only from hospitalised patients. Finally, these data can be added to others in order to better understand the circulation of the virus in a country as heterogeneous as Brazil, in addition to aggregating information about certain social, clinical and sociodemographic nuances that can influence the epidemiological situation of the virus.

## **Main conclusions**

SARS-CoV-2 was highly prevalent in Quirinópolis, considering that 8% of the population has already come into contact with the virus, four times higher than that demonstrated by the serological survey. In addition, the great majority of positive antibody detection was IgG, which may imply intense past circulation of the virus due to late application of protective measures, as well as unfavourable population behaviour, mostly among young people. The majority of positive cases presented mild symptoms, which may reflect the active search in the community.

## **DECLARATIONS**

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### **Authors' contributions**

Conceptualised and designed the study, carried out the application of the questionnaires for the participants, analysed the results, drafted the manuscript, and approved the final manuscript as submitted: Costa LF, Souza RB, de Oliveira AMM, Limongi JE

### **Availability of data and materials**

Data will be made by authors upon reasonable request.

### **Financial support and sponsorship**

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### **Conflicts of interest**

Lourenço Faria Costa and Roberto Barcelos Souza reports personal fees from Universidade Estadual de Goiás, during the conduct of the study; Antônio Marcos Machado de Oliveira and Jean Ezequiel Limongi reports personal fees from Universidade Federal de Uberlândia.

### **Ethical approval and consent to participate**

This proposal obtained a favourable ethical opinion from the National Council of Ethics in Research (protocol number 4,260,760/2020; Certificate of Presentation of Ethical Appreciation 36260720.5.0000.8113). A signed informed consent form was obtained from each participant, as determined by the National Council of Ethics in Research with Human Beings (Ministry of Health, Brazil).

### **Consent for publication**

Not applicable.

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