

## Original Research Article

# What did we learn after the 2020 pandemic? Seroprevalence of SARS-CoV-2 infection in a North region of Portugal during 3rd lockdown

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## ABSTRACT

**Background:** Serological studies of antibody prevalence in response to infection with the acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are useful to monitor the epidemic progression of the disease and to evaluate infection rates. In this study, we estimated the prevalence of anti-SARS-CoV-2 antibodies (IgM and IgG) in a Portuguese sub-district, during National lockdown - January-March 2021.

**Methods:** In the seroepidemiological survey participants were tested for SARS-CoV-2 antibodies (IgM and IgG), in blood samples. The estimated seroprevalence and results were stratified by age, gender, education, occupational exposure, symptoms and previous SARS-CoV-2 infection.

**Results:** Seroprevalence was 41.3%, (15.7% IgG positive, 11.6% IgM positive and 14% positive for both). The majority (74%) of the participants were working presentially and did the serological assay because they had either symptoms / positive contact (90%) or had a previous positive antigen test (36%). From all the seropositive cases only 44% were symptomatic. Our results show that seroprevalence of SARS -CoV-2 is high in the North, in parallel with the National Surveillance System. Seroprevalence was higher in woman than man and in adults older than 21. Almost all the people with symptoms or a positive contact had a positive test and were working at their regular places of work reflecting the danger of occupational exposure.

**Conclusions:** These results suggest that, between January and March 2021, the restriction conditions were effective but unable to stop SARS-CoV-2 epidemic. It is essential to assess SARS-CoV-2 seroprevalence to monitor population immunity and if it lasts, specially from new COVID 19 variants.

**Keywords:** SARS-CoV2 infection, Seroprevalence, Portugal, Public health, Antibody response, COVID-19

## INTRODUCTION

The infection by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) achieved a pandemic situation worldwide resulting in an elevated number of deaths.<sup>1</sup> Portugal adopted general lockdown restrictions due to the third epidemic wave of SARS-CoV-2 that caused in the 1st week of January 2021 a peak of 20.1% deaths.<sup>2</sup> It was not allowed to circulate in public spaces and

roads, with the exception of authorized travel. The schools and all non-essential economic services were closed and according to Portuguese government recommendations, anyone who could work from home should do so.<sup>3</sup>

Last year, after the first epidemic wave, the early containment measures lead to a large reduction in the number of cases/deaths by COVID-19, comparing to other European countries, like Italy and Spain.<sup>4</sup> However, after

this period, there was no further plans to prevent a new outbreak, like epidemiologic surveillance and mass test screening. Serologic studies evaluating SARS-CoV2 prevalence were scarce making difficult to predict new outbreaks.<sup>5,6</sup> By November 18th, the relaxation in lockdown measures added to the fact that community surveillance was under-estimated caused a second peak in the numbers of deaths by COVID-19.<sup>7</sup> The implementation of the essential surveillance WHO strategies for COVID 19 recommended were not adapted to the country reality (WHO, 2020).<sup>8</sup> In this context, surveillance was limited to RT-PCR antigen SARS-CoV2 testing, only available at hospital/primary care clinics for symptomatic patients. The general population had to pay private testing which was very expensive at the time. Though, there was a big proportion of asymptomatic patients that contributed to the transmission. As a consequence, by March 2021 Portugal reached the third epidemic wave.<sup>7</sup> By this time, other diagnostic tests were available, such as serologic tests, more affordable and accessible for the community. Nevertheless, the reality of the confirmed cases and progression of the virus was affected by the high regional heterogeneity in the country, being the north of Portugal, particularly affected.<sup>9</sup>

Serological assays are included in surveillance studies, not only to evaluate antibody presence against SARS-CoV-2 in population, but also to monitor long-lasting immunity.<sup>10</sup> They are particularly important in the context of epidemiologic studies and in the case of SARS COV 2 infection can be used in the surveillance of asymptomatic carriers.<sup>11</sup>

According to WHO the seroepidemiologic investigation should be adapted to each country, to understand if the restrictive conditions are in line with the strategy adopted, at least until global vaccination occurs.<sup>12</sup>

In this study, our aims were to access the seroprevalence of anti-SARS-CoV-2 antibodies (IgM and IgG) in four parishes of Sto Tirso sub-district, in the North of Portugal, during confinement measures (22nd January-16th March 2021). We aimed to evaluate if restriction conditions were effective and in the cases of infection if seroconversion occurred.

## METHODS

The study was conducted in a Community pharmacy-Farmácia Popular, S. Martinho do Campo, with 121 participants, belonging to four parishes of Stº Tirso Sub-district, an area of 27.15 km<sup>2</sup> and 18 294 habitants (Figure 1), from January to March 2021.

The selection criteria of the participants were all the people who came to the Community pharmacy to perform serological tests for COVID- 19, asked to participate in the study and to complete the questionnaire. This one covered their main demographic characteristics, work situation, information about contact with COVID19 positive cases,

signs and symptoms of infection and previous positive SARS-CoV2 infection. The symptoms considered in the survey were fever, headache, caught, dyspnea, and asthenia. The pre-existing comorbidities were also analyzed: hypertension, diabetes, cardiovascular diseases, respiratory, auto-immune and oncologic diseases.

Fingertip blood samples were collected and the presence of IgM and IgG against SARS-CoV2 was determined using a commercial lateral flow assay (OnSite™). The test, according to manufacturer's information, has a sensitivity 96.86% and a specificity of 99.39%.

## Study type

The type of the study was descriptive and analytical.

## Data analysis

Descriptive statistics was performed for each of the variables: age, gender, education, occupational exposure and COVID19 symptoms after a contact or positive previous test. We determined seroprevalence as the proportion of the population who had a positive result for IgM and/or IgG. The frequency of the qualitative variables according to seroprevalence was expressed as a percentage.

## Informed consent and ethical approval

Each participant gave written informed consent before participation in the seroepidemiological study, except the ones younger than 18 years old that had parents' legal consent. The study received Ethical approval from the Ethics Commission of Health Sciences Faculty, Fernando Pessoa University in Oporto.

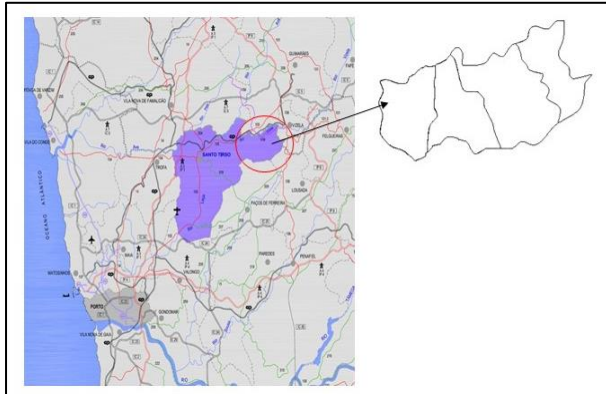
## RESULTS

In the study performed between 22th January 2021 and 16th March 2021, there were 121 participants.

Overall 68 (56.2%) participants were woman and 53 (43.8%) were man. Seven (5.8%) were aged<20 years, 54 (44.6) were aged [21-40] years, 39 (32.2%) were aged [41-60], 18 (14,9%) were aged [61-80] and 3 (2.5%) were older than 80 years. Regarding education levels, 86 (69.1%) had Basic formation and the others, 35 (28.9%), had higher education. Of all participants, 60 (49.6%) reported symptoms compactible with SARS-CoV-2 infection, 46 (38%) reported contact with a positive case and the remain 15 (12.4%) were curious about their serologic profile. Eighty-five (70 %) of the participants worked presentially and 36 (30%) worked from home (Table 1).

Seroprevalence of SARS-CoV-2 infection (positive for IgM and/or IgG) was 50 (41.3%): 15.7% IgG positive, 11.6% IgM positive and 14% positive for both IgM and IgG (Table 2). Overall, seroprevalence was higher in women 60% compared to men (40%). Considering groups

of age, seroprevalence was higher among individuals between 21 and 60 years old (80%), comparing with participants older than 61 years (14%) and younger than 21 years old (6%).



**Figure 1: Geographical location of the sample collection area.**

**Table 1: Sample characterization regarding gender, age, education, motivation to do the test and occupational exposure.**

Characteristics	N	Percentage
<b>Gender</b>		
Female	68	56.2
Male	53	43.8
<b>Age group</b>		
≤20	7	5.8
21-40	54	44.6
41-60	39	32.2
61-80	18	14.9
≥81	3	2.5
<b>Education level</b>		
Basic education	86	69.1
Higher education	35	28.9
<b>Motivation to do the test</b>		
Symptoms compatible with SARS-CoV-19 infection	60	49.6
Contact with a COVID 19 - positive case	46	38
Curiosity about serologic profile	15	12.4
<b>Occupational exposure</b>		
Working presentially	85	70
Working from home	36	30

The IgG and/or IgM positive cases belong to the group of people working presentially (74%). From the total of positive cases, 44% had symptoms of SARS-CoV-2 infection and 46% had contact with an infected person. Among the seropositive cases, 36% had a previous SARS-CoV-2 positive test. This one was done in January/February 2021 (33,3% of the cases) or during

2020 (66,7%). Considering only the cases IgM and IgG positive all had a previous SARS-CoV-2 positive test, although half of them tested positive before the study survey.

**Table 2: Anti-SARS-CoV-2 IgG and IgM seroprevalence.**

Characteristics	IgG n (%)	IgM n(%)	IgG and IgM n (%)
<b>Overall sample</b>	19 (15.7)	14 (11.6)	17 (14)
<b>Gender</b>			
Female	16 (32)	6 (12)	8 (16)
Male	3 (6)	8 (16)	9 (18)
<b>Age group</b>			
≤20	0 (0)	1 (2)	2 (2)
21-60	16 (32)	12 (24)	12 (24)
≥61	3 (6)	1 (2)	3 (6)
<b>Education levels</b>			
Basic education	10 (20)	10 (20)	11 (22)
Higher education	9 (18)	4 (8)	6 (12)
<b>Occupational exposure</b>			
Working presentially	16 (32)	11 (22)	10 (20)
Working from home	3 (6)	3(6)	7 (14)
<b>Motivation to do the test</b>			
Symptoms compatible with SARS-CoV-2 infection	7 (14)	3 (6)	12 (24)
Contact with a COVID 19-positive case	7 (14)	11 (22)	5 (10)
Curiosity	5 (10)	0 (0)	0 (0)

From all, only 36% presented specific comorbidities, namely hypertension, diabetes and cardiovascular diseases but also 35% of the negative cases did.

## DISCUSSION

Our results depict the effects of the third epidemic wave and comparing to 2020 the effect of the total lockdown in a country with several affected areas, especially in the North.

The 43% of seroprevalence of SARS-CoV-2 observed in this study, concerning only a sub-district of Portugal, actually reflect the high levels of infection and, consequently, the mortality numbers officially referred for the north of the country.<sup>9,13</sup> In the North, particularly in non-urban areas, there are major industries that require in person work, though the risk of exposure to SARS-CoV-2 is always big.<sup>14,15</sup> Also, this can relate to a higher level of risk factors (comorbidities) or just due to the population size.

Seroprevalence was higher in woman than man, a difference of 20%, although there isn't report of gender differences in the proportion rates of people with COVID-19; in fact, males have a high rate of mortality after infection.<sup>16</sup> This may reflect the occupational exposure and the disproportion that exist between woman and men working in essential sectors like factory workers, grocery store workers and retail.<sup>17</sup> When looking to seroprevalence by age group, in spite of reports that make no association between them<sup>5</sup>, we have a high seroprevalence in individuals between 21 and 60 years as reported by Pagani.<sup>18</sup> It can suggest that in younger people the susceptibility to infection is lower, explained by the fact that ACE receptors expression increases with age and by the lockdown measures imposed by the government.<sup>19,20</sup> In people older than 61 seroprevalence decreased. As a high-risk group they maintained a strict isolation during this period, waiting for the vaccine to SARS-CoV 2 to be available for them which started in February 2021. Another hypothesis is the lower capacity to generate immunity in older ages, even with the vaccine.<sup>21</sup>

The occupational exposure of the participants resulted in a higher seroprevalence (74%) versus 26% in remote work, in concurrence with several authors.<sup>17,22</sup> Occupational exposure results from higher exposure to infections- health care workers or from working in the proximity of others. Particularly, in our study, there was an elevated number of workers of essential industries and services (grocery, retail and pharmacy/dentist assistants) We can attribute the higher percentages of infection to close proximity without effective protection from the virus infection.<sup>17</sup> This is also supported by the number of positive cases belonging to participants with basic education (69,1%). Most of them are factory workers, grocery, retail and pharmacy/dentist assistants, exceptions during lockdown restrictions.

Concerning the motive to perform the serologic test 44% of the positive cases presented symptoms compatible with SARS-CoV 2 infection such as cough, dyspnea, asthenia and others 46% of the positive cases had risk contacts, either at work or at home. Our results support the importance of social distancing as one of the most effective means to stop transmission of the virus.

Of notice, if we consider that 44% of the seropositive participants were symptomatic that means that 56% were asymptomatic overlooking the infection and could have transmitted it. The asymptomatic carriers were previously recognized as having an important role in the spread of the pandemic.<sup>11,23</sup> For that, countries like Luxembourg adopted strategies like mass screening of the population by RT-PCR in parallel with the slow relieve of the restriction measures after lockdown.<sup>24</sup> The fact that the asymptomatic persons are carriers of the infection can mean that serologic tests have to be rethought because the dynamics of SARS-CoV 2 infection is still poorly understood.<sup>10</sup> When asked about previous positive antigen test for SARS COV2, in the total of seropositive cases, 36% had already have a previous infection, detected either in January-

February 2021 (33,3%) or before, during 2020 (66,7%). Although confirmation is necessary, this shows that seroconversion occurred as expected.<sup>25</sup>

When we analyzed IgM and IgG positive cases alone, they all had a previous SARS-CoV-2 positive test, half of them in January/February 2021, before the study survey. In our opinion, the ones that had a positive test in 2021 are in fact in the period of seroconversion. The other ones we can speculate that they represent cases of re-infection or maybe cross-reactivity. Given that cross reactivity has been shown in serological testing depending of patients with COVID-19 to nucleocapsid antigens of others SARS-CoV.<sup>26,27</sup> The percentage of positive cases with specific comorbidities (36%) was similar to the other participants with negative serology (35%). Thus, the prevalence of chronic conditions overall was concurrent with the Portuguese population: hypertension, cardiovascular diseases and diabetes.<sup>15</sup>

Serological tests provide the first line of information of the immune response to SARS-CoV2. As mentioned before, being available and cheap for the majority of the population, many of the participants choose to do this test. After March 2021, the rapid antigen tests were available with relative accessible prices for the general population. Still, while mass screening of the population for SARS-CoV2 antigen is not viable serologic tests can constitute the first approach for infection detection.

We are aware of the limitations of this study, namely the relatively small sample size that reflects the period of the study. Note that at that time, Portugal was in a lockdown period and rapid antigens tests of SARS-CoV2 were not yet available in the pharmacies. Also, the study is focused on a particular region of Portugal, a non-urban northern area, thus not representing the general population. Considering serologic test itself, IgM positive cases been confirmed by RT-PCR under the national health system protocols, but we did not had access to that information.

## CONCLUSION

These results suggest that in the period of study (January-March 2021) the restriction conditions were effective and seroconversion occurred. It is essential to study SARS-CoV-2 seroprevalence to monitor population immunity, special asymptomatic cases, and transmission. Nevertheless, also massive screening antigen tests are needed to decide the next strategies to adopt after lockdown. It is also urgent to establish if this immunity is long lasting and protects population from new COVID 19 variants. The vaccine introduction in February 2021 to the general population was a step to stop the disease transmission.

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