

## RESEARCH ARTICLE

# A study of the evolution of the third COVID-19 pandemic wave in the Athens metropolitan area, Greece, through two cross-sectional seroepidemiological surveys: March, June 2021

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## Abstract

We studied the third coronavirus disease 2019 (COVID-19) pandemic wave in Athens metropolitan area (3 738 901 inhabitants) through two seroepidemiological surveys. Persons presenting in 12 healthcare facilities across Athens in March and June 2021 were studied (764 and 901, respectively). Immunoglobulin G antibodies against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike protein were measured by a chemiluminescent microparticle immunoassay. In March the seroprevalence rate was 11.6%, meaning that 435 208 residents of Athens had evidence of immunity. The respective values in June were 55.7% and 2 082 568 residents. The highest seroprevalence rates attributed to SARS-CoV-2 infection were recorded in persons <18 years (16.3% in March and 31.6% in June), while immunity was mainly vaccine-induced in persons 18–64 years and >65 years. Infection-attributed immunity also increased in older-age groups. Wide ranges in seroprevalence rates were noted across areas in March and June. The highest seroprevalence rates were recorded in Piraeus (47.2%) and West Attica (37.5%). However, the highest increase (>5 times) occurred in Piraeus and the South Section of Athens, which are among the most densely populated areas in Athens. In both study periods, history of COVID-19 or febrile episode, and having a cohabitant with COVID-19 were associated with increased risk for seropositivity among unvaccinated persons ( $p$  values <0.001 for all). Residing in Piraeus, the South Section or West Attica was associated with increased risk for seropositivity in June ( $p$  values <0.001). Wide heterogeneity in seroprevalence rates was found across areas in Athens, which is mainly attributed to population density. The impact of population mobility and socioeconomic status should be explored.

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#### KEYWORDS

antibodies, epidemiology, Greece, SARS-CoV-2, seroprevalence

## 1 | INTRODUCTION

Almost 2 years after the emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the declaration of the coronavirus disease 2019 (COVID-19) pandemic, humanity continues to face its considerable health, societal, and economic consequences.<sup>1</sup> As of November 9, 2021, more than 249 million laboratory-confirmed cases and more than 5 million deaths have been reported globally.<sup>2</sup> Pandemics are often heterogeneous in terms of spatial and temporal evolution and wide differences may occur between and within populations.<sup>3–6</sup> These differences are mainly driven by varying levels of population immunity in the context of consecutive pandemic waves, but also by population density, human mobility networks, and the mixture and magnitude of implemented countermeasures which may shift the characteristics of the pandemic.<sup>3–6</sup> Mass vaccination is the key countermeasure to limit COVID-19 associated morbidity and mortality and to speed the return to normality.<sup>7</sup> The first COVID-19 vaccines were developed and granted emergency use authorization within less than a year after the declaration of the pandemic.<sup>8</sup> Currently, there are several COVID-19 vaccines in use globally.<sup>8</sup>

In Greece, the first COVID-19 case was detected on February 26, 2020.<sup>9</sup> Following a small wave in March–May 2020, Greece experienced two large waves in September–December 2020 and February–June 2021, respectively.<sup>10</sup> Overall, as of November 9, 2021, 801 208 cases and 16 414 deaths have been notified in Greece.<sup>10</sup> Yet, there are many more infections than those officially documented, as many infected persons remain asymptomatic or develop mild symptoms and therefore go unnoticed.<sup>9,11,12</sup> Seroprevalence studies can capture past infections in a population at a specific time, including asymptomatic or mildly symptomatic infections.<sup>13–15</sup> These studies can also detect vaccine-induced immunity and, therefore, can be used to estimate the overall immunity levels of a population. However, to the best of our knowledge, the overwhelming majority of seroprevalence surveys worldwide concerned either the first pandemic wave or special population groups such as healthcare personnel, while on several occasions they relied on residual clinical specimens.<sup>11,16,17</sup>

Although it is highly likely that SARS-CoV-2 will become endemic in the coming years, there are uncertainties regarding transition dynamics from COVID-19 pandemic to endemicity, the emergence of new variants, and the expected concomitant pressure of seasonal influenza on healthcare services.<sup>18,19</sup> Regarding Greece, this is critical given that as of November 11, 2021, 62% of the general population is fully vaccinated, rendering this country 23rd in the ranking of European countries.<sup>20</sup> In that respect, we conducted a prospective, two time-point seroepidemiological study in the Athens metropolitan area where one-third of the population of Greece resides. Our objective was to study the evolution of the third COVID-19 pandemic wave and factors influencing the risk for SARS-CoV-2 seropositivity.

## 2 | METHODS

### 2.1 | Sample size estimation

The resident population in the Athens metropolitan area amounted to 3 738 901 inhabitants (2011 census).<sup>21</sup> With a 15% expected seroprevalence rate against SARS-CoV-2, it was estimated that a sample of 625 persons was required for each cross-sectional seroepidemiological survey for a 2.7% margin of error, 90% power, and 95% confidence interval (CI) (power analysis).

### 2.2 | Enrolment of participants, data collection, and sera sampling

We selected 12 healthcare facilities (4 hospitals and 8 primary healthcare centers) across Athens metropolitan area based on geographical and population catch areas. The infection control committees of the hospitals and the directors of the primary healthcare centers were invited and agreed to participate in the study. The participating healthcare facilities were requested to enroll a pre-defined number of persons (60–80 per healthcare facility) from March 1 through 22, 2021 (3-week window) (Figure 1). At that time, the Alpha (B.1.1.7) SARS-CoV-2 variant

predominated in Greece. Participants were selected randomly among asymptomatic persons referred for blood sampling, irrespective of demographic or other characteristics, history of SARS-CoV-2 infection or COVID-19 vaccination, or reason for blood testing. Symptomatic persons, persons whose permanent residence was out of the Athens metropolitan area, and healthcare personnel were excluded from the study. Attention was requested to enroll persons across all age groups. Demographic, clinical, and epidemiological data were collected anonymously using one standardized questionnaire per participant. A febrile episode was defined as the onset of fever ( $>38^{\circ}\text{C}$ ) twice within 24 h. COVID-19 was defined as a case with compatible symptoms confirmed by a positive SARS-CoV-2 real time-PCR. A cohabitant was defined as a person living permanently in the same residence. The survey and sera sampling was conducted concomitantly and prospectively. The study was repeated from June 1 through 22, 2021 implementing the same methodology as in March (Figure 1). During June 2021, the Alpha (B.1.1.7) and the Delta (B.1.617.2) SARS-CoV-2 variants co-circulated, however, the Delta variant predominated rapidly over the Alpha variant. Consent was requested by participants or, in the case of children  $<18$  years old, by their parents or guardians. The study was approved by the Ethics Committee of the National and Kapodistrian University of Athens.

### 2.3 | Laboratory diagnosis

Serum samples were sent to the Department of Microbiology of National and Kapodistrian University of Athens for testing. The presence of immunoglobulin G (IgG)-specific antibodies against the SARS-CoV-2 spike protein was determined by a chemiluminescent microparticle immunoassay (SARS-CoV-2 IgG II Quant, Abbott). Immunity against SARS-CoV-2 (either from past infection or from vaccination) was defined as a cut-off value of  $\geq 50$  AU/ml. Negative and positive controls were included in all assays.

### 2.4 | COVID-19 vaccination campaign in Greece

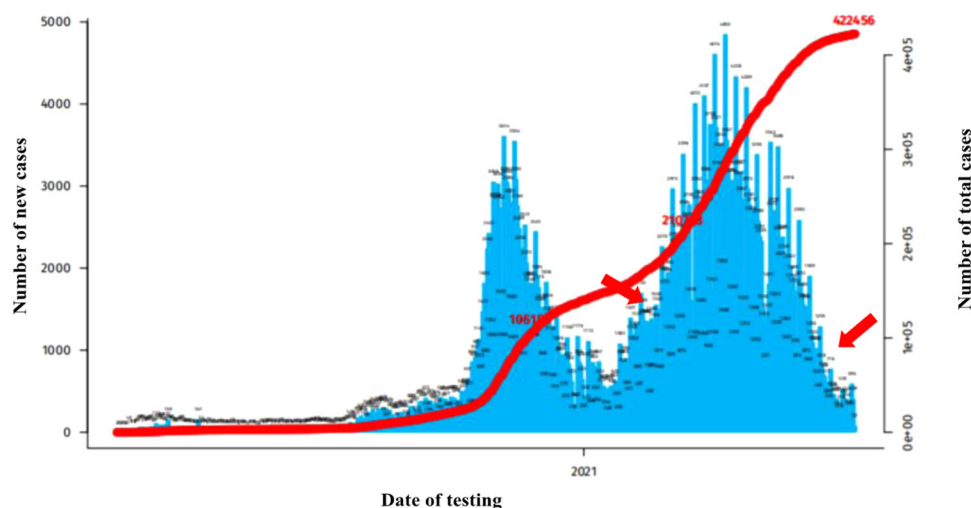
The COVID-19 vaccination campaign started on December 28, 2020. Healthcare personnel were prioritized, followed by the elderly ( $>80$  years and  $>70$ – $79$  years), persons with co-morbidities, and younger age groups. The following vaccines were used: Comirnaty (Pfizer-BioNTech messenger RNA [mRNA] BNT162b2 vaccine) from late December 2020, Spikevax (Moderna mRNA-1273 vaccine) from January 2021, Vaxzevria (Oxford/Astra Zeneca ChAdOx1-S vaccine) from February 2021, and Janssen (Johnson & Johnson recombinant viral vector adenovirus vaccine) from May 2021. Most vaccinations were accomplished with the two mRNA vaccines.

### 2.5 | Statistical analysis

Frequencies and percentages were used for categorical variables. Comparisons between groups were performed by using the two-tailed *t*-test for continuous variables and the chi-square test for categorical variables. CIs were estimated. All *p* values of  $\leq 0.05$  were considered statistically significant. The statistical analysis was conducted using the IBM SPSS Statistics for Windows, Version 26.0.: IBM Corp.

## 3 | RESULTS

A total of 1665 persons were studied, 764 in March 2021 and 901 in June 2021. Of them, 1192 were enrolled in primary healthcare centers and 473 in hospitals, with no significant difference in enrollment site by studied period (data not shown). Table 1 shows the characteristics of tested persons by study period. The two groups differed in the distribution of areas of residence (*p* value = 0.004). In addition, more persons had a history of COVID-19, a cohabitant with a history of COVID-19, or had been vaccinated against COVID-19 in



**FIGURE 1** Laboratory-confirmed SARS-CoV-2 cases in Greece, June 30, 2021 (National Public Health Organization.<sup>11</sup>) Arrows indicate the two sampling periods. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

**TABLE 1** Characteristics of tested persons by study period

Characteristic	March 2021 n = 764 (%)	June 2021 n = 901 (%)	p value
Mean age, years (range)	58.3 (0.1–93)	55.7 (0.1–94)	0.291
Males	340 (44.5)	378 (42.0)	0.235
Comorbidities <sup>a</sup>	357 (46.7)	428 (47.5)	0.768
Mean no. of underlying diseases (range)	1.8 (1–9)	1.7 (1–7)	0.147
Residence area			
East Attica	144 (18.8)	168 (18.6)	0.004
North Section of Athens	31 (4.0)	26 (2.9)	
West Attica	7 (1.0)	10 (1.1)	
West Section of Athens	126 (16.5)	195 (21.6)	
Central Section of Athens	271 (35.5)	252 (28.0)	
South Section of Athens	119 (15.6)	143 (15.9)	
Piraeus	66 (8.6)	107 (11.9)	
Having cohabitants	606 (79.3)	727 (80.7)	0.499
Mean no. of cohabitants (range)	2.2 (1–16)	2.2 (1–10)	0.437
History of febrile episode <sup>b</sup>	55 (7.2)	86 (9.5)	0.087
History of COVID-19	23 (3.0)	74 (8.2)	<0.001
COVID-19 vaccination	60 (7.9)	468 (51.9)	<0.001
One dose	43 (71.6)	165 (35.3)	
Two doses	17 (28.4)	303 (64.7)	
Cohabitant with COVID-19	22 (2.9)	69 (7.6)	<0.001

Abbreviation: COVID-19, coronavirus disease 2019.

<sup>a</sup>Co-morbidities included arterial hypertension, diabetes mellitus, chronic cardiovascular disease, chronic pulmonary disease, chronic renal disease, chronic hepatic disease, obesity, malignancy, and immunosuppression.

<sup>b</sup>From September 2020.

June compared with persons tested in March (*p* values <0.001 for all comparisons).

### 3.1 | Estimation of seroprevalence rate against SARS-CoV-2 in March 2021

In March 2021, 89 persons tested seropositive against SARS-CoV-2, therefore the overall seroprevalence rate was 11.6% (CI: 9.5%–14.1%). This implies that at that time approximately 435 208 residents of the Athens metropolitan area had serologic evidence of immunity against SARS-CoV-2. Table 2 shows seroprevalence rates against SARS-CoV-2 according to participants' characteristics regardless of history of COVID-19 vaccination. History of febrile episode, past diagnosis of COVID-19, and diagnosis of COVID-19 in a

**TABLE 2** Seroprevalence rates against SARS-CoV-2 according to characteristics of tested persons regardless of history of COVID-19 vaccination, March and June 2021

Characteristic	Seroprevalence rate <sup>a</sup>		p value
	March (n = 764)	June (n = 901)	
Age (years)			
<18	16.3%	31.6%	0.475
18–64	9.6%	48.6%	<0.001
>65	14.4%	69.2%	<0.001
Gender			
Male	14.2%	56.9%	<0.001
Female	9.5%	55.5%	<0.001
Residence			
East Attica	13.9%	56.5%	<0.001
North Section of Athens	20.0%	46.2%	0.293
West Attica	14.3%	50.0%	0.549
West Section of Athens	8.7%	49.2%	0.011
Central Section of Athens	10.4%	52.4%	<0.001
South Section of Athens	8.4%	60.8%	0.001
Piraeus	19.7%	70.1%	<0.001
History of febrile episode <sup>b</sup>			
Yes	27.3%	68.6%	<0.001
No	10.5%	54.4%	0.001
History of COVID-19			
Yes	91.3%	91.8%	0.942
No	9.2%	52.5%	<0.001
Cohabitant with COVID-19			
Yes	68.2%	75.4%	0.579
No	10.0%	54.1%	<0.001

Abbreviations: COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

<sup>a</sup>Against SARS-CoV-2.

<sup>b</sup>From September 2020.

cohabitant were significantly associated with increased risk for seropositivity (*p* values <0.001 for all comparisons).

Of the 89 persons who tested seropositive against SARS-CoV-2 in March 2021, 54 (52.1%) were not vaccinated, 21 (32.2%) had received one dose of COVID-19 vaccine, while 14 (15.7%) had received two doses. Table 3 shows seroprevalence rates of unvaccinated tested persons according to their characteristics. History of COVID-19, history of febrile episode, and having a cohabitant with COVID-19 were significantly associated with an increased risk for seropositivity against SARS-CoV-2 among unvaccinated persons in March (*p* values <0.001 for all comparisons). Age, gender, and area of

**TABLE 3** Seroprevalence rates against SARS-CoV-2 according to characteristics of tested persons with no history of COVID-19 vaccination, March and June 2021

Characteristic	Seroprevalence rate <sup>a</sup>		p value
	March (n = 704)	June (n = 433)	
<b>Age (years)</b>			
<18	16.3%	31.6%	0.475
18–64	7.4%	23.1%	0.057
>65	6.6%	19.6%	0.287
<b>Gender</b>			
Male	8.8%	23.7%	0.120
Female	6.6%	22.5%	0.084
<b>Residence area</b>			
East Attica	9.8%	10.4%	0.967
North Section of Athens	4.3%	9.1%	0.923
West Attica	14.3%	37.5%	0.708
West Section of Athens	7.5%	19.0%	0.436
Central Section of Athens	7.5%	22.8%	0.166
South Section of Athens	5.4%	30.0%	0.225
Piraeus	9.1%	47.2%	0.133
<b>History of febrile episode<sup>b</sup></b>			
Yes	27.3%	59.7%	0.036
No	6.0%	17.0%	0.108
<b>History of COVID-19</b>			
Yes	90.9%	91.7%	0.913
No	5.0%	12.1%	0.279
<b>Cohabitant with COVID-19</b>			
Yes	70.0%	71.2%	0.933
No	5.9%	16.5%	0.113

Abbreviations: COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

<sup>a</sup>Against SARS-CoV-2.

<sup>b</sup>From September 2020.

residence were not associated with increased risk for seropositivity in March, regardless of history of COVID-19 vaccination.

### 3.2 | Estimation of seroprevalence rate against SARS-CoV-2 in June 2021

In June 2021, 502 persons tested seropositive against SARS-CoV-2. The overall seroprevalence rate at that time was 55.7% (CI: 52.4%–58.9%). It is estimated that approximately 2 082 568 residents of the Athens metropolitan area had serologic evidence of immunity against SARS-CoV-2 at that time. The difference between

the overall seroprevalence rates in March and June was statistically significant ( $p$  value <0.001). The following factors were significantly associated with increased risk for seropositivity: age ( $p$  value <0.001), history of COVID-19 ( $p$  value <0.001), history of febrile episode ( $p$  value = 0.011), having a cohabitant with a history of COVID-19 ( $p$  value = 0.001), and area of residence ( $p$  value = 0.013).

Of the 502 persons who tested seropositive against SARS-CoV-2 in June, 111 (22.1%) and 291 (58%) had received one or two doses of COVID-19 vaccines, respectively, while 100 (19.9%) were not vaccinated. In June 2021, the following factors were significantly associated with increased risk for seropositivity among unvaccinated persons: history of COVID-19, history of febrile episode, having a cohabitant with COVID-19, and area of residence ( $p$  values <0.001 for all comparisons). In contrast, age and gender were not associated with increased risk for seropositivity.

### 3.3 | Evolution of the third COVID-19 pandemic wave in the Athens metropolitan area

Persons <18 years old had the highest seroprevalence rates attributed to natural SARS-CoV-2 infection in both studied periods (16.3% in March and 31.6% in June) (Table 3). In contrast, serologically proven immunity against SARS-CoV-2 was mainly vaccine-induced in persons 18–64 years and ≥65 years (Tables 2 and 3). Nevertheless, seroprevalence attributed to natural SARS-CoV-2 infection also increased during the third pandemic wave in older-age groups (Table 3).

Wide ranges in seroprevalence rates against SARS-CoV-2 attributed to natural infection were noted across studied areas (Table 3). In March 2021, West Attica had the highest seroprevalence rates against SARS-CoV-2 (14.3%) attributed to natural infection (Table 3). In June 2021, the highest seroprevalence rate among unvaccinated persons was noted in Piraeus (47.2%), followed by West Attica (37.5%). However, the highest increase of seroprevalence due to natural SARS-CoV-2 infection was recorded in the South Section of Athens and Piraeus (5.5 and 5.18 times, respectively).

## 4 | DISCUSSION

We conducted a two-point seroprevalence survey in March and June 2021 to study the evolution of the third COVID-19 pandemic wave in the Athens metropolitan area, which is mostly urban, with significant variations in population density and socioeconomic status of residents. To the best of our knowledge, this is among the few prospectively conducted seroepidemiological surveys not focusing on risk groups, and the only one conducted in the third pandemic wave, after the implementation of mass COVID-19 vaccination programs. Seroprevalence surveys at the regional level can yield valuable insights to guide future intervention policies but also prepare the healthcare system. This is imperative given the emergence of new variants, the upcoming influenza season, and the suboptimal

COVID-19 vaccine uptake rates in Greece, which can fuel additional waves of severe cases, hospitalizations, and deaths.<sup>22</sup>

We estimated that the seroprevalence rate against SARS-CoV-2 increased sharply from 11.6% in March 2021 to 55.7% in June 2021. In particular, we estimated that approximately 435 208 residents in Athens had serologic evidence of immunity in March. This number was raised fivefold (to 2 082 568 residents) within a period of 3 months. This evolution is mainly attributed to the fast pace of the national vaccination program, which should be maintained until the vast majority of the population is vaccinated. High (31.5%) seroprevalence rates after the first pandemic wave were detected in the urban area of Chelsea, Massachusetts.<sup>23</sup> In contrast, negligible seroprevalence rates (<0.5%) against SARS-CoV-2 were estimated after the first pandemic wave in Greece, which is attributed to the early implementation of nationwide lockdown.<sup>24</sup> In our study, although half of the seropositive cases in March were linked to natural infection, serologically proved immunity was mainly vaccine-driven in June. At that time, mass vaccination campaigns targeting all persons  $\geq 25$  years old were fully implemented across the country. In our study, the association between older age and increased rates of seropositivity not attributed to natural infection is explained by the fact that persons >70 years were prioritized for COVID-19 vaccination in early 2021. Furthermore, the demographic profile of tested persons seeking healthcare may explain the increased vaccination rates recorded in our study groups.

Another finding of the current study is the increase of seroprevalence rates among persons <18 years from 16.3% to 31.6% within 3 months, demonstrating the high transmissibility of the novel coronavirus, despite the fact that all schools and educational activities were almost continuously closed from early November 2020 through early May 2021. During this 6-month period strict lockdown policies were implemented nationwide, while the COVID-19 stringency index ranged from 78.70 to 88.89.<sup>25</sup> Nevertheless, recent studies suggest that restrictions applied for a long period or re-introduced later may weaken the effect on the circulation of SARS-CoV-2 and the number of casualties.<sup>26</sup>

In our study, having a cohabitant with a history of COVID-19 was associated with an increased risk for seropositive status, similarly with other studies.<sup>27</sup> In our study of transmission dynamics of SARS-CoV-2 within Greek families, the median attack rate was 60% (range: 33.4%–100%).<sup>28</sup> High attack rates (up to 75%) within families were also found by others.<sup>29,30</sup> Finally, a history of febrile episode up to 9 months before testing was also predictive of seropositivity, as also shown in other studies.<sup>31,32</sup>

Similarly with past pandemics and the ongoing COVID-19 pandemic,<sup>3–6,33,34</sup> wide differences in seroprevalence rates against SARS-CoV-2 across areas were found in our study. However, we found no statistical significance between the two study periods, which is explained by the rather small number of tested persons per region. The highest burden of infections occurred in Piraeus and West Attica. One could argue that the limited number of tested persons in West Attica warrants cautious interpretation of our findings. Nevertheless, national surveillance data also showed wide

heterogeneity of infection rates across the Athens metropolitan area. During the peak of the third pandemic wave in April 2021, the daily incidence rate of laboratory-confirmed COVID-19 cases was as high as 98 cases per 100 000 residents in West Attica and 78 cases per 100 000 residents in Piraeus.<sup>10</sup> However, in our survey the highest increase (>5 times) occurred in the South Section of Athens and Piraeus (nearby areas). Piraeus and the South Section of Athens are among the most densely populated areas in the Athens metropolitan area (8905 and 7690 residents per km<sup>2</sup>, respectively) which largely explains our findings. On the other hand, West Attica is the most sparsely populated area of Athens (143 residents per km<sup>2</sup>). Our findings indicate that factors other than population density account for the high seroprevalence rate in West Attica. West Attica is the largest industrial area in Athens and almost all logistics enterprises, including food and retail warehouses, are located there. Every day a large number of industrial workers commute to their workplace, either within West Attica or from other regions. There are also many immigrant workers and Roma settlements in this area whose vaccination status may be low. West Attica has also extensive connectivity with all national roads and suburban public transportation systems of Athens. It is possible that despite the strict lockdown, the increased human mobility in association with suboptimal implementation of hygiene measures in workplaces highly facilitated the transmission of SARS-CoV-2 in West Attica. Socioeconomic status might also be associated with risk for SARS-CoV-2 infection, as shown in other studies.<sup>35</sup>

Our study has several strengths. We enrolled a large number of asymptomatic persons early in the third pandemic wave and at the end, which allowed us to study in detail the evolution of immunity against SARS-CoV-2. In addition, a clear strength is the fact that we tested persons from all areas of the Athens metropolitan area and of all age groups. A potential limitation is the fact that persons seeking healthcare may not represent the general population. Yet, epidemiological data and serum specimens were collected concomitantly and prospectively, instead of testing residual clinical specimens.

In conclusion, our study provides insight into the evolution of the third COVID-19 pandemic wave in the Athens metropolitan area. Our study showed that the seroprevalence of IgG antibodies against SARS-CoV-2 increased sharply during the 3-month period. This evolution is mainly attributed to the fast pace of the national vaccination program, indicating that it should be maintained until the vast majority of the population is vaccinated. Despite the implemented strict lockdown policies, SARS-CoV-2 circulated widely and infected significant portions across all age groups, and particularly among persons <18 years old. Wide differences in seroprevalence rates were noted among regions. Beyond population density, it is likely that human mobility and connectivity also accounted for the spatial heterogeneity in seroprevalence rates. The role of socioeconomic status should also be explored. Overall, our study can inform policymakers to plan future public health interventions in light of the expected emergence of novel variants of concern, the upcoming influenza season, and the suboptimal vaccination coverage rates against COVID-19 in Greece.

## 5 | STUDY GROUP

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### ACKNOWLEDGEMENTS

We thank the healthcare personnel of the participating healthcare facilities for their assistance in collecting data and blood sampling. We also thank Anastasia Tedoma for technical assistance. The opinions presented in this article are those of the authors, and do not necessarily represent those of their institutions.

### FUNDING INFORMATION

Funding information is not available.

### CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

### AUTHOR CONTRIBUTIONS

**Helena C. Maltezo**: Conception and design of the study, investigation, writing of the manuscript. **Bettina Krumbholz**: Design of the study, review and editing. **Maria Mavrouli**: Laboratory investigation, review and editing. **Maria Tseroni**: Statistical analysis, review and editing. **Maria N. Gamaletsou**: Data collection, review and editing. **Evanthia Botsa**: Data collection, review and editing. **Cleo Anastassopoulou**: Investigation, review and editing. **Aristofanis Gikas**: Data collection, review and editing. **Evanthia Fournarakou**: Data collection, review and editing. **Maria Kavieri**: Data collection, review and editing. **Aikaterini Koureli**: Data collection, review and editing. **Dionysia Mandilara**: Data collection, review and editing. **Aikaterini Marinopoulou**: Data collection, review and editing. **Argyro Theodorikakou**: Data collection, review and editing. **Panagiotis Tsiachris**: Data collection, review and editing. **Anastasia Zarzali**: Data collection, review and editing. **Spyridon Pournaras**: Data collection, review and editing. **Athanasia Lourida**: Data collection, review and editing. **Ioannis Elefsiniotis**: Data collection, review and editing. **Georgia Vrioni**: Investigation, review and editing. **Nikolaos V. Sipsas**: Investigation, review and editing. **Athanasios Tsakris**: Conception and design of the study, investigation, review, writing and editing.

### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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**How to cite this article:** Maltezos HC, Krumbholz B, Mavrouli M, et al. A study of the evolution of the third COVID-19 pandemic wave in the Athens metropolitan area, Greece, through two cross-sectional seroepidemiological surveys: March, June 2021. *J Med Virol*. 2022;94:1465-1472. doi:10.1002/jmv.27465