



Seroprevalence of anti-SARS-CoV-2 antibodies among children and their parents in Greece

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Abstract

School closures were enforced as measures to restrain the COVID-19 pandemic, based on the assumption that young children may play a key role in SARS-CoV-2 spread. This study aims to determine the prevalence of SARS-CoV-2 IgG antibodies in children and corresponding parents, in order to improve surveillance and estimate the prevalence of asymptomatic or subclinical COVID-19 cases. A prospective multicenter study was conducted between March and June 2021 in Greece. Children admitted to the hospital or examined in outpatient clinics for reasons other than COVID-19 and their parents were tested for anti-Spike SARS-CoV-2 IgG in serum. A questionnaire about clinical and demographic data was completed. The study included 823 participants: 427 children and 396 corresponding parents. The overall seroprevalence was 16.4% in parents and 13.8% in children. Among families with ≥ 1 seropositive child or parent, the combination of a seropositive parent and a corresponding seronegative child was 29.6%, a seronegative parent and a corresponding seropositive child was 24.7%, and a seropositive child with a corresponding seropositive parent was 45.7%. Age, level of education, and school or work attendance were not significantly associated with increased seropositivity. On the contrary, ethnic minority of Roma, close contact with known COVID-19 case, previous symptoms consistent with COVID-19, and mass gatherings were risk factors for seropositivity.

Conclusion: The spread of SARS-CoV-2 during a period of lockdown in Greece was low in children and comparable to adults most likely due to intrafamilial transmission. Accordingly, it is unlikely that children have boosted virus transmission.

What is Known:

- In the earliest months of the pandemic, it was demonstrated that children had significantly lower seroprevalence rates than the older age groups, due to the fact that children had decreased exposure to the virus, because of early public health interventions, such as school and day care closure.
- Later, further studies reported that children have similar incidence rate of SARS-CoV-2 infection compared to adults in households and community settings.

What is New:

- In this seroprevalence study, the spread of SARS-CoV-2 infection during a period of lockdown in Greece with the predominance of the Alpha-variant was particularly low in children and comparable to adults, most likely due to intrafamilial transmission.
- These study findings will be useful for decisions regarding non-pharmaceutical interventions during the pandemic, and especially, to guide in designing and implementing appropriate containment measures for schools and social gatherings.

Keywords Seroprevalence · COVID-19 · Children · Parents · Seroepidemiology · Household transmission

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Abbreviations

CI	Confidence interval
CMIA	Chemiluminescent microparticle immunoassay
COVID-19	Coronavirus disease 2019

EUA	Emergency use authorization
IgG	Immunoglobulin G
IQR	Interquartile range
OR	Odds ratios
PR	Proportional ratio
RBD	Receptor-binding domain
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of the novel disease 2019 (COVID-19), has spread rapidly around the world since first identified in Wuhan, China, in December 2019 [1]. COVID-19 pandemic is characterized by sustained human-to-human transmission, threatening the global public health and resulting in worldwide severe morbidity and mortality [2]. The global range and high fatality rate of this pandemic are causing economic damage, related to public panic and aggressive policy measures to contain the disease worldwide, and it has made SARS-CoV-2 the focus of the scientific world. The first COVID-19 case was reported in Greece on 26 February 2020 and on 10 March 2020, with a total of 89 active COVID-19 cases and 0 death; the Greek government decided to suspend the operation of all educational institutions throughout the country [3]. Gradually, restrictive measures were extended and resulted in a general lockdown on the 23rd of March, when 649 active cases and two deaths had been recorded [4]. In November 2020, the Greek government decided a new school closure due to the second pandemic wave with gradual opening of the educational institutions in April 2021.

Seroprevalence studies are important tools for determining the true extent of an outbreak, mapping its distribution, identifying hotspots and at-risk groups needing special attention, and designing public health strategies to reduce community transmission [5, 6]. Also, serosurveys can reveal the proportion of asymptomatic or subclinical infections in the general population, resulting in proper public health measures and control strategies [5, 6]. For this purpose, the immunoassays must have high sensitivity and specificity, in order to avoid the misleading public health interventions by overestimating the level of immunity and prematurely easing restrictions [7]. It is worth noting that seroprevalence of antibodies to SARS-CoV-2 varies with country, study population, time of sampling, and laboratory testing method [7].

The risk of transmission from symptomatic and pre-symptomatic patients is considered to be higher. However, asymptomatic infection is common, especially in the pediatric population, and the potential of viral transmission

from asymptomatic patients, who may have similar viral loads with symptomatic cases, has been reported [8–10]. Furthermore, studies have shown that as much as 44% of SARS-CoV-2 transmission can take place when individuals are pre-symptomatic or asymptomatic [11]. Generally, mildly affected or asymptomatic individuals are not screened. As a result, the number of confirmed SARS-CoV-2 infections is largely underestimated [12]. In this context, seroprevalence surveys are of utmost importance to assess the proportion of the population that has already developed antibodies against the virus and might potentially be protected against subsequent infection [13]. As recommended by the WHO, monitoring changes of seroprevalence over time is also crucial at the beginning of an epidemic to anticipate its dynamics and plan an adequate public health response [14]. Also, studying the seroprevalence in both children and parents may be a useful tool to evaluate intrafamilial transmission and the possibility of children to be the drivers of the COVID-19 transmission. In the earliest months of the pandemic, it was demonstrated that children (5–9 years) had significantly lower seroprevalence rates than the older age groups, suggesting that infection was less prevalent in children than in adolescents and adults [15]. These results may have been due to the fact that children had decreased exposure to the virus, because of early public health interventions, such as school and day care closure. Later, further studies reported that children have similar incidence rate of SARS-CoV-2 infection compared to adults in households and community settings [16–18].

The purpose of this study was to estimate the prevalence of SARS-CoV-2 IgG antibodies in children and their parents in order to examine the extent of potential underdiagnosis of asymptomatic or subclinical infections. This information may also help elucidate the role of children in the transmission of infection within the family.

Material and methods

Study design and participants

A period prevalence, multicenter, prospective study was conducted from March 1, 2021 to June 30, 2021 with the participation of seven university departments of pediatrics that were distributed throughout the country, representing all 7 Regional Health Directorates of Greece. Children and adolescents 0–16 years of age and their parents presenting in the emergency department or in the outpatient clinics or admitted to the hospital for any reason were candidates for enrollment in the study.

It is worth noting that on 10 March 2020, the operation of all educational institutions throughout the country was suspended. In May 2020, the schools were open for 1 month, and in September 2020, the schools re-opened, but in November 2020, a new school closure was performed due to the second pandemic wave with gradual opening of the educational in April 2021. Therefore, the study period included a period after the school opening and the general lockdown implemented in Greece. The Alpha-variant was the predominant variant during the study period, while in July 2021, the Delta variant arose representing the most prevalent variant.

The study participation was random and voluntary. Informed consent was obtained from all the participants at the time of enrollment. Children or parents, who had symptoms consistent with acute COVID-19, according to the Centers for Disease Control and Prevention (CDC) definition [19] and/or a positive SARS-CoV-2 PCR test at the time of enrollment were excluded, in order to avoid the overestimation of the SARS-CoV-2 infection prevalence. In addition, parents who had received COVID-19 vaccination before enrollment and participants with congenital or acquired immunodeficiency were also excluded from the study. On the other hand, we included the participants with previous symptoms consistent with COVID-19 or previous SARS-CoV-2 infection, in order to estimate the true prevalence of SARS-CoV-2 IgG antibodies, representing the prevalence in the community and to examine the extent of potential underdiagnosis of asymptomatic or subclinical infections. Siblings or more than one parent per household consisting of more than one pair of children-parents residing in the same household were eligible. Parents were asked to complete a questionnaire on age, sex, ethnic origin, medical history, level of education, close contact with a confirmed COVID-19 case, occupation, school, previous travel, or symptoms compatible with COVID-19. Clinical, demographic, and epidemiological data of the parents and their children were recorded.

Close contact with a confirmed COVID-19 case is defined as the person who is within 6 feet of a case infected with SARS-CoV-2 for a cumulative total of 15 min or more over a 24-h period, starting from 2 days before illness onset (or, for asymptomatic patients, 2 days prior to test specimen collection) until the time the patient is isolated [20]. Previous travel is defined as any travel history outside the person's community by different means of public transportation, such as airplane, ship, train, or bus during the period from the pandemic's start to the time of enrollment. School attendance is defined as the duration that children attended school, during the interval from the beginning of the outbreak until the study recruitment. In addition, the implementation of preventive measures in schools included mask wearing, while regular mass testing was implemented in April 2021, after the re-opening of schools. Data on mask wearing at school was reported by the participants, in order to evaluate if this

is a risk factor for the increased seroprevalence. Finally, the participants were questioned if they attend frequently social gatherings and about the number of persons in these gatherings. If there were different group sizes in the gatherings, the highest number was recorded.

Serological testing

Venous blood samples were collected for the purpose of the study, and immunoglobulin G (IgG) against SARS-CoV-2 was detected in serum samples by using emergency use authorization (EUA)-approved and CE-marked SARS-CoV-2 IgG II Quant assay kit (Abbott Laboratories, Chicago, USA), according to the manufacturer's instructions. The SARS-CoV-2 IgG II Quant assay is an automated, two-step chemiluminescent microparticle immunoassay (CMIA) used for the qualitative and quantitative determination of IgG antibodies to the receptor-binding domain (RBD) of the S1 subunit of the spike protein of SARS-CoV-2 in human serum on the ARCHITECT i Systems. The analytical measurement interval is stated as 21 to 40,000 AU/ml, and positivity cutoff is ≥ 50 AU/ml.

Statistical analysis

Seroprevalence with 95% confidence interval (CI) was estimated for each prefecture [21]. Continuous variables are expressed as medians and interquartile range (IQR) and categorical variables as frequencies and relative frequencies.

The relationship between the main outcome measure (SARS-CoV-2 infection) and participants' characteristics was assessed using either chi-square analysis or Fisher's exact test for categorical variables and Mann–Whitney U test for continuous variables. Mann–Whitney U test was performed for continuous data since there was deviation from normal distribution (Shapiro–Wilk normality test). In univariate analysis, the percentage of infected and the proportional ratio (PR) with 95% CIs are presented. Multivariate analysis was performed using a logistic regression for binary outcomes (positive vs negative), and odds ratios (OR) with 95% CIs were calculated. Variables with $P < 0.05$ on univariate analyses were included in the final model. All tests were 2-sided, and a p value < 0.05 was considered to indicate statistical significance. Statistical analysis was performed using Excel and IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY, USA).

Results

A total of 823 participants were enrolled, 427 children (males: 224 (52.5%) and females: 203 (47.5%)) and 396 corresponding parents (males: 97 (24.5%) and females: 299 (75.5%)). A

total of 363 pairs of one parent and one child, 21 families with 3 members (either 1 child and 2 parents or 2 children and 1 parent) and only 1 family with 2 parents and 2 children, as well as 30 children without parents were included in the study. The children without parents who participated in the study were immigrants or parents who had refused to give blood. The median age of children was 8.1 years (IQR 5.4), and the median age of the parents was 39.4 years (IQR 9.7). A rate of 5.9% of the children and 6.3% of the parents belonged to the Roma minority, and 14.1% of the children and 15.4% of the parents were immigrants. Previous COVID-19 was reported in 24 (5.6%) children and 29 parents (7.3%), previous symptoms consistent with COVID-19 in 107 (25.1%) children and 79 (19.9%) parents, while close contact with confirmed COVID-19 case in 62 (14.5%) children and 62 (15.7%) parents. Tables 1 and 2 summarize the demographic and clinical characteristics of all the individuals included in the study.

A total of 59 children and 65 parents were seropositive with a seroprevalence of 13.8% and 16.4%, respectively. Moreover, 58 children and 62 parents were seropositive from the 363 pairs of parent/child, and 1 child and 3 parents were seropositive from the group that included 21 families with 3 members. In total, there were 81 families with at least one seropositive individual, either child or parent. In detail, there were only seropositive

children in 20 families, and only seropositive parents in 24 families, while there was seropositivity in both children and parents in 37 families. So, the combination of a seropositive parent and a corresponding seronegative child was 29.6%, the combination of a seronegative parent and a corresponding seropositive child was 24.7%, and the combination of a seropositive child and a corresponding seropositive parent was 45.7%. In order to clarify whether seroprevalence increased more in children than in adults over the course of the study, we tried to compare the seroprevalence in parents and children between the 2 time periods, March/April and May/June. During the period May/June, the seroprevalence in both children and parents increased. In parents, there was a statistically significant increase in seroprevalence from March/April (10.3%) to May/June (22.8%) (PR (95% CI): 2.58 (1.47–4.53), $p=0.001$) (Suppl. Table 1). On the other hand, in children seroprevalence increased from March/April (11.1%) to May/June (16.4%) (PR (95% CI): 1.59 (0.91–2.79), $p=0.103$) (Suppl. Table 1), but the difference was not statistically significant.

Table 3 describes the seroprevalence differences in children related to their demographic and clinical characteristics. Previous COVID-19 symptoms were present in 21/59 (35.6%) seropositive children, while previous known SARS-CoV-2 infection was reported in 18/59 (30.5%) of them. There was

Table 1 Demographics and clinical characteristics of the children and parents participating in the study

Characteristics		Children ($n = 427$)	Parents ($n = 396$)
Sex	Male	224 (52.5%)	97 (24.5%)
	Female	203 (47.5%)	299 (75.5%)
Age (years) (median (IQR))		8.1 (5.4)	(9.7)
Age groups			
0–1 years		58 (13.5%)	
2–5 years		91 (21.3%)	
6–12 years		166 (38.9%)	
13–16 years		108 (25.3%)	
Ethnic minority		99 (23.2%)	88 (22.2%)
Roma		25 (5.9%)	25 (6.3%)
Immigrants		60 (14.1%)	61 (15.4%)
No		342 (80.1%)	310 (78.3%)
Residency in a camp		13 (3.1%)	9 (2.3%)
Level of education	No education	26 (7.9%)	0 (0.0%)
	Kindergarten	37 (11.2%)	0 (0.0%)
	Pre-school	38 (11.5%)	0 (0.0%)
	Elementary school	121 (36.7%)	37 (10.7%)
	High school	106 (25.8%)	166 (8.1%)
	University	2 (0.6%)	127 (36.6%)
	Master degree	-	15 (4.3%)
PhD		-	2 (0.6%)
Underlying disease*		103 (24.1%)	84 (21.2%)
Previous known SARS-CoV-2 infection		24 (5.6%)	29 (7.3%)

*Underlying disease: any medical condition or disease that interferes with daily life or activities and requires continuous medical care or treatment, except for the congenital and acquired immunodeficiency

Table 2 Demographics and clinical characteristics of the children and parents participating in the study

Characteristics	Children (<i>n</i> = 427)	Parents (<i>n</i> = 396)
History of symptoms consistent with COVID-19	107 (25.1%)	79 (19.9%)
Close contact with COVID-19 cases	62 (14.5%)	62 (15.7%)
Travel	52 (12.2%)	59 (14.9%)
School attendance	304 (71.2%)	2 (0.5%)
Number of children in the class (median (IQR))	19.4 (4.6)	-
Duration of school attendance (months) (median (IQR))	4.6 (1.6)	-
Wearing mask at school		
Always	137 (52.3%)	
Occasionally	87 (33.2%)	
Rarely	38 (14.5%)	
COVID-19 case in the class	36 (8.4%)	
Number of cases at school (median (IQR))	1.4 (2.5)	
Closure of the class due to COVID-19 case	32 (7.5%)	
Work attendance		213 (53.8%)
Teleworking		49 (22.4%)
Duration of teleworking (months) (median (IQR))		10 (26.1)
Wearing mask at work		
Always		162 (77.9%)
Occasionally		31 (14.9%)
Rarely		15 (7.3%)
Use of public transport	40 (9.4%)	83 (21.0%)
Participation in social gatherings		
Yes	110 (29.8%)	92 (27.1%)
Rarely	115 (31.2%)	122 (35.9%)
Never	144 (39.0%)	126 (37.1%)
Number of persons at gatherings (median (IQR))	4.3 (3.2)	4.5 (3.4)
Number of families at gatherings (median (IQR))	2.2 (1.5)	2.0 (1.4)
Wearing mask at gatherings	11 (5.7%)	14 (7.4%)
Frequent visits by relatives	274 (64.1%)	254 (64.1%)
Number of family members (median (IQR))	4.4 (1.4)	4.3 (1.4)

no correlation between age and seroprevalence ($p=0.841$), as well as between the level of education and seropositivity ($p>0.05$). School attendance was not a risk factor for seropositivity ($p=0.22$), but known close contact with a confirmed COVID-19 case in the class, resulting in closure of the class, was associated with seropositivity (PR (95% CI): 2.35 (1.25–4.42), $p=0.01$). Frequent gatherings with other people, close contact with a confirmed COVID-19 case, and previous symptoms consistent with COVID-19 were related to seropositivity (PR (95% CI): 2.16 (1.18–3.95), $p=0.008$; PR (95% CI): 4.03 (2.59–6.27), $p<0.001$; and PR (95% CI): 1.66 (1.03–2.70), $p=0.041$, respectively) ($p=0.008$, $p<0.001$, and $p=0.041$, respectively). Children belonging to the ethnic minority of Roma were statistically associated with increased seroprevalence (PR (95% CI): 2.50 (1.33–4.69), $p=0.008$).

Table 4 shows the seroprevalence differences in parents related to their demographic and clinical characteristics. Previous COVID-19 symptoms were present in 48/65 (73.8%) seropositive parents, while previous known SARS-CoV-2

infection was reported in 25/65 (38.5%) of them. Age, level of education, occupation, and teleworking were not correlated with seropositivity ($p=0.084$, $p=0.519$, $p=0.450$, and $p=0.414$). History of previous symptoms compatible with COVID-19 and known close contact with SARS-CoV-2-infected individual were associated with seropositivity (PR (95% CI): 11.37 (6.93–18.64), $p<0.001$ and PR (95% CI): 3.61 (2.38–5.46), $p<0.001$, respectively). Finally, individuals who belonged to the Roma minority were at higher risk to be seropositive (PR (95% CI): 2.18 (1.17–4.08), $p=0.022$).

Multivariate analysis revealed that known close contact with COVID-19 was associated with increased seropositivity in children (OR (95% CI): 4.82 (1.22–19.00), $p=0.025$). In addition, parents who belonged to the Roma minority as well as those with a history of previous symptoms compatible with SARS-CoV-2 infection were more likely to be positive (OR (95% CI): 6.15 (1.28–29.46), $p<0.023$ and OR (95% CI): 11.70 (3.77–36.32), $p<0.001$, respectively).

Table 3 Seroprevalence differences in children related to their demographic and clinical characteristics

Characteristics	Children (<i>N</i> = 414)	Seropositive N (%) or median (IQR)	Univariate analysis		Multivariate analysis	
			PR (95% CI)	<i>P</i> -value	OR (95% CI)	<i>P</i> -value
Sex	Male	219	29 (13.2)	0.86 (0.54–1.38)	0.534 (C)	
	Female	195	30 (15.4)	Ref		
Age (years)	Seropositive ^a	59	2 (1)		0.841 (M-W)	
	Seronegative ^b	355	1 (1)			
Ethnic minority	Yes	97	15 (15.5)	1.11 (0.65–1.91)	0.696 (C)	
	No	317	44 (13.9)	Ref		
	Roma	24	8 (33.3)	2.50 (1.33–4.69)	0.008 (C)	0.999
	Immigrants	60	7 (11.7)	0.88 (0.41–1.85)	0.725 (C)	0.70 (0.14–3.62)
	No	330	44 (13.3)	Ref		0.673
Residence in a camp ^c	Yes	12	2 (16.7)	1.17 (0.32–4.25)	0.811 (F)	
	No	401	57 (14.2)	Ref		
Level of education	No education	25	6 (24.0)	1.68 (0.48–5.92)	0.408 (C)	
	Kindergarten	35	7 (20.0)	1.40 (0.41–4.84)	0.437 (C)	
	Preschool	38	3 (7.9)	0.55 (0.12–2.50)	0.361 (F)	
	Elementary school	119	11 (9.2)	0.65 (0.20–2.13)	0.442 (C)	
	High school	84	14 (16.7)	1.17 (0.37–3.69)	0.791 (C)	
Symptoms consistent with COVID-19	Yes	103	21 (20.4)	1.66 (1.03–2.70)	0.041 (C)	1.43 (0.41–5.01)
	No	310	38 (12.3)	Ref		0.573
Close contact with COVID-19 ^c	Yes	60	24 (40.0)	4.03 (2.59–6.27)	<0.001 (C)	4.82 (1.22–19.00)
	No	353	35 (9.9)	Ref		0.025
Travel	Yes	49	6 (12.2)	0.84 (0.38–1.86)	0.669 (C)	
	No	365	53 (14.5)	Ref		
School Attendance ^c	Yes	298	39 (13.1)	0.73 (0.45–1.20)	0.220 (C)	
	No	112	20 (17.9)	Ref		
Number of children in the class ^c	Positive	36	20 (5)		0.919 (M-W)	
	Negative	220	20 (7)			
Wearing mask at school ^c	Occasionally	219	31 (14.2)	0.87 (0.39–1.95)	0.742 (C)	
	Rarely	37	6 (16.2)	Ref		
COVID-19 case in the class ^c	Yes	35	10 (28.6)	2.35 (1.25–4.42)	0.010 (C)	
	No	222	27 (12.2)	Ref		
Number of COVID-19 cases at school ^c	Seropositive	16	2 (2)		0.006 (M-W)	1.24 (0.85–1.79)
	Seronegative	124	0 (2)			0.260
Closure of the class due to COVID-19 ^c	Yes	32	9 (28.1)	2.48 (1.22–5.06)	0.014 (C)	1.59 (0.35–7.33)
	No	150	17 (11.3)	Ref		0.550
Use of means of public transport ^c	Yes	39	2 (5.1)	0.48 (0.12–2.00)	0.294 (F)	
	No	150	16 (10.7)	Ref		
Frequent social gatherings ^c	Yes	222	43 (19.4)	2.16 (1.18–3.95)	0.008 (C)	0.89 (0.24–3.20)
	No	134	12 (9.0)	Ref		0.853
Number of persons at gatherings ^c	Seropositive	30	4 (4)		0.304 (M-W)	
	Seronegative	159	4 (3)			
Number of families at gatherings ^c	Seropositive	30	2 (2)		0.808 (M-W)	
	Seronegative	156	2 (2)			
Wearing mask at gatherings ^c	Yes	8	3 (37.5)	2.42 (0.93–6.31)	0.126 (F)	
	No	181	28 (15.5)	Ref		
Number of family members ^c	Seropositive	55	4 (1)		0.133 (M-W)	
	Seronegative	303	4 (1)			

Table 3 (continued)

Characteristics	Children (<i>N</i> = 414)	Seropositive N (%) or median (IQR)	Univariate analysis		Multivariate analysis	
			PR (95% CI)	<i>P</i> -value	OR (95% CI)	<i>P</i> -value
Underlying disease^c	Yes	101	14 (13.9)	0.96 (0.55–1.68)	0.897 (C)	
	No	313	45 (14.4)	Ref		

13 children were excluded from the analysis because their questionnaires were not filled out sufficiently. "Bold" is about the statistical significant *p*-values
C chi-square test, *F* Fisher's exact test, *M-W* Mann–Whitney U test, *CI* confidence interval, *OR* odds ratios, *PR* proportional ratio

^aAge- > seropositive: the median age of the seropositive children

^bAge- > seronegative: the median age of the seronegative children

^cPlease, note that there is missing data

Discussion

Inadequate knowledge about the prevalence of SARS-CoV-2 infection in a certain population challenges public health response. Changing testing strategies has made it very difficult for countries to estimate the proportion of the infected population. Well-designed serosurveys play an important role to determine the prevalence of SARS-CoV-2 infection and its burden in the general population or in selected groups, and they are useful for monitoring transmission trends [13]. In addition, such studies may provide some evidence about the effectiveness of different non-pharmaceutical interventions in a certain population. School closures were enforced as measures to restrain the COVID-19 pandemic, based on the assumption that young children may play a key role in SARS-CoV-2 spread. To date, data on the seroprevalence of children and the household transmission are sparse. This is the first multicenter seroprevalence study of SARS-CoV-2 IgG antibodies in children and corresponding parents in Greece, providing evidence for the potential underdiagnosis of asymptomatic or subclinical COVID-19 cases. The prevalence of infection was found to be comparable in children and adults arguing against the role of children as drivers of transmission.

Our study shows that the seroprevalence in children and parents at the time the study was conducted was 13.8% and 16.4%, respectively, indicating that there was no significant difference between these two groups. Also, there was no difference between the number of families with at least one seropositive child or parent or both of them. These results showed that seroprevalence in children was low and comparable to that of adults possibly implying that children are not the source of the infection neither the drivers of transmission. These findings are similar to those of one serosurvey in children and parents residing in Southwest Germany [22]. However, one seroprevalence study performed in children and family members in Brussels suggested that children may be the primary source of infection and transmission

in households, as children usually have mild or no symptoms and they are underdiagnosed [23]. In the current study, young age was not a significant factor for higher seroprevalence, and children were not identified as the primary case of SARS-CoV-2 infection in households. These findings may contribute to plan for school-based measures, in order to reduce transmission risk within and between classrooms.

Current data about the potential transmission of COVID-19 from children to adults are controversial, as a few studies support a lower transmission rate from children to adults, while other studies show increased infectivity in young children compared to older individuals [24–27]. Our findings about similar infection rates between children and adults are consistent with other household transmission studies [21, 28]. However, some studies, especially those performed in the pre-Omicron period, suggest that children are less susceptible to SARS-CoV-2 infection than adults and particularly in the early phase of the pandemic, when children were largely protected from community exposures due to non-pharmaceutical interventions, including closure of schools and teleworking [29]. In addition, these controversies in the results may be due to the methods of detecting SARS-CoV-2 infection among household contacts, as individuals with asymptomatic or mildly symptomatic infection are rarely tested, resulting in the underestimation of SARS-CoV-2 infections. A recent meta-analysis about child transmission of SARS-CoV-2 showed that children may be less likely to transmit COVID-19 compared to adults, and household transmission remains the most prominent source of child-to-adult and child-to-child transmission [30]. Further studies are needed to be performed, in order to clarify this emergent issue (Fig. 1).

Another important finding of this serosurvey was that people belonging to the ethnic minority of Roma were associated with increased seropositivity. It has been shown that Roma communities are more vulnerable to infectious disease outbreaks, including hepatitis, measles, poliomyelitis, and tuberculosis [31], and Roma children present increased incidence of respiratory infections compared to non-Roma children [32–34].

Table 4 Seroprevalence differences in parents related to their demographic and clinical characteristics

Characteristics		Parents (N = 392)	Seropositive N (%) or median (IQR)	Univariate analysis		Multivariate analysis	
				PR (95% CI)	P-value	OR (95% CI)	P-value
Sex	Male	96	12 (12.5)	0.70 (0.39–1.25)	0.216 (C)		
	Female	296	53 (17.9)	Ref			
Age (years)	Seropositive ^a	65	39 (12.0)		0.084 (M-W)	0.99 (0.95–1.04)	0.780
	Seronegative ^b	327	41 (11.0)				
Ethnic minority	Yes	86	18 (20.9)	1.36 (0.84–2.22)	0.220 (C)		
	No	306	47 (15.4)	Ref			
	Roma	24	8 (33.3)	2.18 (1.17–4.08)	0.022 (C)	6.15 (1.28–29.46)	0.023
	Immigrants	60	10 (16.7)	1.09 (0.59–2.04)	0.783 (C)	2.17 (0.56–8.40)	0.262
Residency in a camp ^c	No	308	47 (15.3)	Ref			
	Yes	8	3 (37.5)	2.32 (0.92–5.83)	0.132 (F)		
Level of education ^c	Yes	383	62 (16.2)				
	No	202	33 (16.3)	0.86 (0.54–1.36)	0.519 (C)		
History of symptoms consistent with COVID-19 ^c	BSc, MSc, PhD	142	27 (19.0)				
	Yes	78	48 (61.5)	11.37 (6.93–18.64)	< 0.001 (C)	11.70 (3.77–36.32)	< 0.001
History of close contact with COVID-19 ^c	No	314	17 (5.4)	Ref			
	Yes	61	26 (42.6)	3.61 (2.38–5.46)	< 0.001 (C)	2.54 (0.78–8.27)	0.122
Travel ^c	No	330	39 (11.8)	Ref			
	Yes	58	7 (12.1)	0.69 (0.33–1.44)	0.313 (C)		
Work attendance ^c	No	336	58 (17.3)				
	Yes	211	35 (16.6)	0.84 (0.53–1.33)	0.450 (C)		
Teleworking ^c	No	126	25 (19.8)	Ref			
	Yes	49	10 (20.4)	1.32 (0.68–2.54)	0.414 (C)		
Duration of teleworking (months)	No	168	26 (15.5)				
	Seropositive	8	5.5 (2)		0.805 (M-W)		
Wearing mask at work ^c	Seronegative	37	6 (3)				
	Yes	160	31 (16.2)	0.70 (0.25–2.00)	0.522 (F)		
Use of means of public transportation	Occasionally	13	3 (23.1)	Ref			
	Yes	82	11 (13.4)	0.84 (0.45–1.54)	0.558 (C)		
Parent with a child attending school	No	280	45 (16.1)	Ref			
	Yes	256	44 (17.2)	1.14 (0.64–2.00)	0.658 (C)		
Frequent mass gatherings ^c	No	136	21 (15.4)	Ref			
	Yes	213	42 (19.7)	1.36 (0.82–2.25)	0.229 (C)		
Number of persons at gatherings ^c	No	124	18 (14.5)	Ref			
	Seropositive	27	5 (4)		0.002 (M-W)	1.11 (0.96–1.28)	0.163
Number of families at gatherings ^c	Seronegative	156	4 (3)				
	Seropositive	27	2 (1)		0.942 (M-W)		
Wearing mask at gatherings ^c	Seronegative	153	2 (1)				
	Yes	13	1 (7.7)	0.51 (0.08–3.46)	0.696 (C)		
Number of family members ^c	No	172	26 (15.1)	Ref			
	Seropositive	60	4 (1)		0.616 (M-W)		
Underlying disease	Seronegative	277	4 (1)				
	Yes	83	11 (13.3)	0.76 (0.42–1.38)	0.358 (F)		
	No	309	54 (17.5)	Ref			

Four participants' parents were excluded from the analysis because their questionnaires were not filled out sufficiently. "Bold" is about the statistical significant p-values

C chi-square test, F Fisher's exact test, M-W Mann-Whitney U test, CI confidence interval, OR odds ratios, PR proportional ratio

^aAge- > seropositive: the median age of the seropositive children

^bAge- > seronegative: the median age of the seronegative children

^cPlease, note that there is missing data

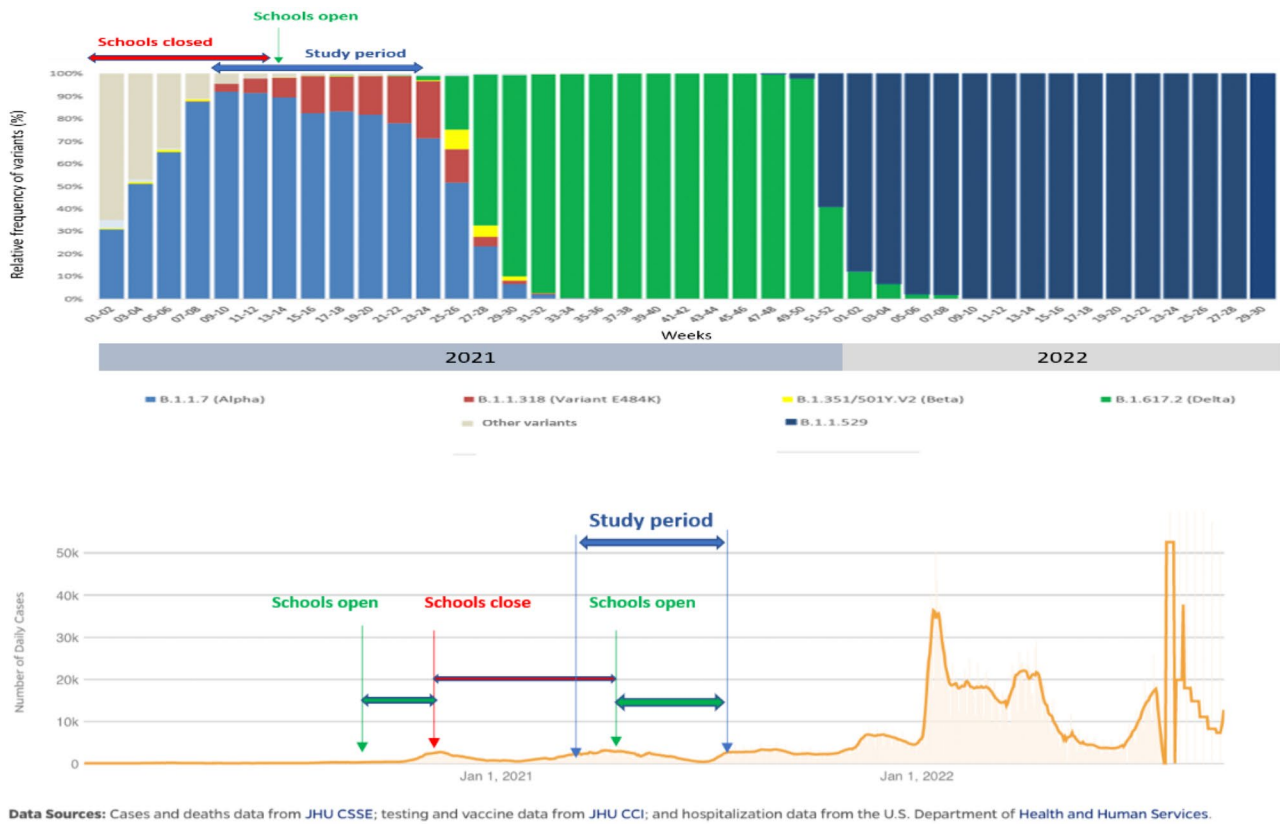


Fig. 1 Graphical overview of the predominant virus variants (adapted from the National Public Health Organization (NPHO), available in <https://eody.gov.gr/epidimiologika-statistika-dedomena/ektheseis-epidimiologikis-epitirisis-loimoxis-apo-ton-sars-cov-2/>). **A** and the course of the pandemic

in Greece (adapted from the Center for Systems Science and Engineering (CSSE) COVID-19 Data at Johns Hopkins University (JHU), available in <https://coronavirus.jhu.edu/region/Greece>) **B**, indicating the closure of schools and the study period

This increased seropositivity may be due to the overcrowded households, poverty, and poor sanitation of this population. This is in line with one study indicating that large household membership and greater household crowding were associated with increased odds for SARS-CoV-2 infection [35].

School attendance for children or absence of teleworking was not associated with increased seropositivity. These data provide more information about how the schools contribute to the spread of the SARS-CoV-2 infection, as major policy decisions on temporary school or class closures have been implemented globally in order to prevent transmission. However, close contact with confirmed COVID-19 case either at school or in the household or at social gatherings are risk factors for higher infectivity. Therefore, the implementation of distancing and contact restriction measures may be the most effective methods to control the spread of the COVID-19. Surprisingly, our study showed that wearing mask is not associated with decreased seropositivity, and this may be related to the fact that these results were based on the information given by the participants, resulting in study bias.

One of the strengths of this study is the prospective design, and that is multicenter, representing all 7 Regional Health

Directorates of the country. However, a potential limitation is the voluntary participation of the individuals participating in the study, leading to potential selection bias in our results. For example, the seroprevalence may be overestimated if the parents were more willing to participate, because they believed that their children and/or themselves had been exposed to SARS-CoV-2 infection. Vaccinated people were excluded from the study, as measurement of anti-N antibodies for SARS-CoV-2 was not performed, and this might have led to another selection bias. Additional limitation is a possible cluster/family effect on the results. Considering that the majority of the family clusters are very small (363 families with a pair of one parent and one child), the correction in the analysis could not be easy and with minimal results. Another limitation is that during the time period before the initiation of the serosurvey, the country was on lockdown, and the schools were closed, resulting in relatively low seroprevalence. In order to clarify this, we compared the seroprevalence between the period March/April and the period May/June, and it was found that there was an increase in seropositivity among both parents and children; however, it was higher and the difference statistically significant only in the parent group. This finding may indirectly indicate less efficient

transmission of SARS-CoV-2 in schools. Moreover, the design of the study did not allow to trace the sequence of infections within a family and therefore to evaluate the role of children in intrafamilial transmission. Finally, these seroprevalence results of our study concern the pre-Omicron period and, in particular, the Alpha period, just before the Delta wave. Further seroepidemiological studies are currently underway, in order to evaluate the seroprevalence during the Omicron period. It is known that Omicron variant is more transmissible and can cause milder disease compared to other variants [36]. So, it is expected that seroprevalence will be higher during the Omicron period compared to previous ones.

In conclusion, in this seroprevalence study, the spread of SARS-CoV-2 infection during a period of lockdown in Greece with the predominance of the Alpha variant was particularly low in children and comparable to adults, most likely due to intrafamilial transmission. These study findings will be useful for decisions regarding non-pharmaceutical interventions during the pandemic, and especially, to guide in designing and implementing appropriate containment measures for schools and social gatherings.

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Authors' contributions All authors contributed to the study conception and design. Maria Tsolia, Vassiliki Spoulou, Athanasios Michos, Despoina Gkentzi, Ekaterini Siomou, Vassiliki Papaevangelou, George Syrogiannopoulos, and Emmanouil Galanakis contributed to the conception and design of the work. Material preparation and data collection were performed by Dimitra Dimopoulou, Eleni Vergadi, Ekaterini Tsiligianni, Eleni Papadimitriou, Artemis Mavridi, Spyridon Giannakopoulos, Georgia Tsiourvopoulou, Maria Palyvou, Evangelia Angeli, Nikitas Brikos, and Irini Eleftheriou. Data analysis was performed by Maria Kyritsi, Katerina Dadouli, Christos Hadjichristodoulou, Maria Tsolia, and Ioanna Grivea. The first draft of the manuscript was written by Dimitra Dimopoulou, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Declarations

Ethics approval The study was approved by the “P. & A. Kyriakou” Children’s Hospital’s Ethical and Research Committee (approval number: 507/20.10.2020). All procedures were conducted in accordance with the 1964 Declaration of Helsinki and its later amendments.

Consent to participate For all participants, written informed consent was obtained from the parents at the time of the enrollment.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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