

COVID-19 Seroprevalence in Children during Pandemic Waves in Sarajevo, a Single Center Experience

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Abstract

Objective. The aim of this study was to present data on the prevalence of seropositive children tested in the laboratory of the Eurofarm polyclinic in Sarajevo, from September 2020 to May 2021. **Material and Methods.** Peripheral blood samples were collected and anti-SARS-CoV-2 antibodies were detected using an electrochemiluminescence immunoassay. **Results.** In the total of 762 tested children, 187 were positive (24.5%), based on cut-off value. Of all the positive cases 42.8% were female while 57.2% were male. There were 10.1% of positive children in the first age group (0-5 years), 44.4% in the second group (6-13 years), and 45.5% in the third group (14-18 years). There was no statistically significant difference in seroprevalence between age groups and gender. The lowest seroprevalence (3.6%) was observed in October 2020 after the first pandemic wave, and the highest seroprevalence (60.3%) was observed in April 2021, corresponding to the third pandemic wave. **Conclusion.** The results of our study showed that the seroprevalence in children was low, especially during the first year of the pandemic. In the second year of the pandemic, there was an evident statistically significant increase in the number of seropositive children. Similar data have been shown in studies for adults.

Key Words: Seroprevalence ▪ COVID-19 ▪ SARS-CoV-2 ▪ Antibodies ▪ Children.

Introduction

During the coronavirus disease 2019 (COVID-19) pandemic, children were less represented and had a milder form of the disease compared to adults. In the United States, children <18 years accounted for approximately 13% (1), in China less than 1% of cases in children <10 years (2). Most of the children with acute COVID-19 had a mild form of the disease, and a smaller number required hospitalization (3). A strong innate immune response, immune training with previously given vaccines (4, 5), as well as previous exposure to respiratory viruses, less comorbidity and different angiotensin converting enzyme-2 (ACE2) receptors expression in children may have led to the lower incidence

and mostly mild forms of COVID-19 in children (6). Post-infectious manifestations, such as multisystem inflammatory syndrome associated with SARS-CoV-2 (MIS-C) and long COVID, were a cause for concern. Serological tests are important in the diagnosis of these complex manifestations (7). During the pandemic, a large number of children had asymptomatic infection (8). A systematic review article and meta-analyses show that the percentage of asymptomatic children was 15-42% (9). Recent research in Bosnia and Herzegovina (B&H) showed that the percentage of asymptomatic children was 35.7% (10). The gold standard for the diagnosis of acute infection is the polymerase chain reaction (PCR) test. Serological tests are helpful for assessing the total number of

infected, including asymptomatic cases, monitoring the course of the pandemic, and planning public health measures (11-13). One of the measures to monitor the number of infected people in a certain location and in a certain period of time is the COVID-19 serology surveillance strategy. Positive serology test results are a sign of a past or recent infection. Antibodies can be detected 1-3 weeks after infection. Available serological antibody tests usually reveal antibodies to the nucleocapsid or spike proteins of the virus (11, 14). Seroprevalence studies provide valuable information for vaccination strategies because they help quantify what proportion of the population has been exposed to the virus, help achieve herd immunity, and help identify populations that may be at greater risk of infection (15).

The aim of this study was to present the results of seroprevalence in children tested in the laboratory of the Eurofarm polyclinic in Sarajevo, from September 2020 to May 2021, which can be useful for planning public health measures, and help in assessing herd immunity for COVID-19.

Materials and Methods

Participants

A total of 762 children were tested in the period between September 2020 and May 2021. The participants were divided into three groups: group one (children from 0-5 years of age) consisting of 73 individuals (9.6%), group two (children from 6-13 years of age) consisting of 373 individuals (48.9%) and group three (children from 14-18 years of age) consisting of 316 individuals (41.5%). A total of 368 participants were female (48.3%), while 394 (51.7%) were male.

Materials

Peripheral blood samples were collected by venipuncture in Vacusera vacutainers with CAT serum (BD Vacutainer1, Germany). Samples were transferred for centrifuge at 500×g for 10 minutes and analyzed the same day.

Methods

Anti-SARS-CoV-2 antibodies were quantified using an electrochemiluminescence immunoassay (ECLIA). The ELISA (enzyme-linked immunosorbent assay) method was used for testing the control group. The results were interpreted according to the protocols of the American Society for Microbiology (16). Prior to testing and analysis, 60 control samples, selected randomly from the tested cohort, were used for verification and correlation between the ECLIA and ELISA methods. Verification shown that 58/60 tests gave the same results. During the ECLIA tests, 18 were positive, while the ELISA tests gave 16 positive results, and the difference was not significant ($\chi^2=0.164$ $P=0.685$). For the ECLIA test the serum samples were analyzed using CE.IVD Roche CobasElecys1 Anti SARS-CoV-2, an electrochemiluminescence immunoassay, for the qualitative detection of total pan-Ig, IgG, IgM, and IgA (Roche Diagnostics, Rotkreuz, Switzerland). The test was performed according to the manufacturer's instructions (Roche Diagnostics, Rotkreuz, Switzerland). The assay uses a recombinant protein representing the nucleocapsid (N) antigen in a double-antigen sandwich assay format, which favors detection of high affinity antibodies against SARS-CoV-2. The results are generated by an interpolating ECLIA signal, with a threshold produced during calibration. The result is given either as reactive or non-reactive in the form of a cutoff index (COI; signal sample/cut-off). If the COI is <1.0 then the result is considered non-reactive (negative for anti-SARS-CoV-2 antibodies); if the COI is ≥ 1.0 then the result is considered reactive (positive for anti-SARS-CoV-2 antibodies). Elecys1Anti-SARS-CoV-2 exhibits a high overall clinical specificity of 99.8%, and sensitivity from 60.2-99.5%, depending on the number of days that have passed from the primary infection, where for up to 6 days the sensitivity is 60.2%, after 14 days the sensitivity is 99.5% (17). The ELISA was performed using a commercial ELISA kit based on the recombinant spike glycoprotein (S) and nucleocapsid protein (N) antigens of SARS-CoV-2 (ELISA COVID-19

IgG; Vircell Microbiologists, Granada, Spain). The results were expressed through the antibody index: $AI = (\text{sample optical density (OD)} / \text{cut-off serum mean OD}) \times 10$, and interpreted as follows: IgG <4, negative; 4–6, borderline; >6, positive. The results were interpreted according to the protocols of the American Society for Microbiology (16).

Ethics Statement

The research was conducted at the Eurofarm Centre Laboratory, from September 2020 to May 2021. All the parents of the tested children signed written informed consent (prepared in accordance with the principles of the Declaration of Helsinki), and this was reviewed and approved by the local ethics committee, No: 7-EC-12/22.

Statistical Analysis

Standard descriptive statistical methods were used in the data analysis. The Chi-square (χ^2) test was used to determine a statistically significant difference by age and gender. The exact test of goodness-of-fit was used to compare the seroprevalence by month (September 2020 – May 2021). Data analysis was performed in MS Excel, as described by McDonald in 2014 (18) and IBM SPSS Statistics for Windows v27 (IBM, Armonk, NY). The level of statistical significance was $P < 0.05$. The Bonferroni correction for 36 comparison (seroprevalence by month) was used to reduce type I error, where statistical significance was $P < 0.0014$.

Results

Individuals were considered to have tested positive for anti-SARS-CoV-2 antibodies if COI levels were ≥ 1.0 . On the basis of this cut-off level, a total of 187 individuals tested positive ($N=762$), which accounts for 24.5%. On the basis of the sensitivity and specificity of the ECLIA test, we calculated the true prevalence, where the result was 0.2948 (29.5%). We used the following formula (19): True

prevalence = $(\text{Apparent prevalence} + \text{Specificity} - 1) / (\text{Sensitivity} + \text{Specificity} - 1)$. The apparent prevalence was 0.2454 (24.5%), the specificity of ECLIA was 99.8% and median sensitivity 79% (60.2%–99.5%). True prevalence = $(0.24 + 0.99 - 1) / (0.79 + 0.99 - 1) = 0.23 / 0.78 = 0.2948$ (29.5 %). In the first age group (0–5 years) there were 19 (10.1%) positive cases, while 83 (44.4%) in the second age group (6–13 years) and 85 (45.5%) in the third age group (14–18 years) (Table 1).

The chi-square test revealed the following association between the age groups: there was no significant difference in the number of positive cases between the first and second age groups ($\chi^2=0.493$; $P=0.482$), or between the first and third age groups ($\chi^2=0.023$; $P=0.879$). Also, there was no significant difference in the number of positive cases between the second and third age groups ($\chi^2=2.003$; $P=0.157$). A total of 80 anti-SARS-CoV-2 antibody positive individuals were female (42.8%) while 107 (57.2%) were male, and this difference in frequency was not found to be significant ($\chi^2=3.016$; $P=0.082$). The seroprevalence throughout the nine-month time frame, with error bars indicating the 95% confidence interval, is shown in Figure 1.

The exact test of goodness-of-fit showed a significant increase in positive cases in November–December 2020 and February–April 2021 (Figure 1; Table 2). The lowest seroprevalence (3.6%) was observed in October 2020, and the exact test of goodness-of-fit showed that the number of cases was significantly lower when compared to March and April 2021 (Figure 1; Table 2). The highest seroprevalence (60.3%) was observed in April 2021, and the exact test of goodness-of-fit showed that the number of cases was significantly higher when compared to September and October 2020 (Figure 1; Table 2).

Table 1. The Frequency and Number of Total Tested and Positive Cases by Age Groups

Participants	Total tested N (%)	Positive cases N (%)
0-5 years	73 (9.6)	19 (10.1)
6-13 years	373 (48.9)	83 (44.4)
14-18 years	316 (41.5)	85 (45.5)

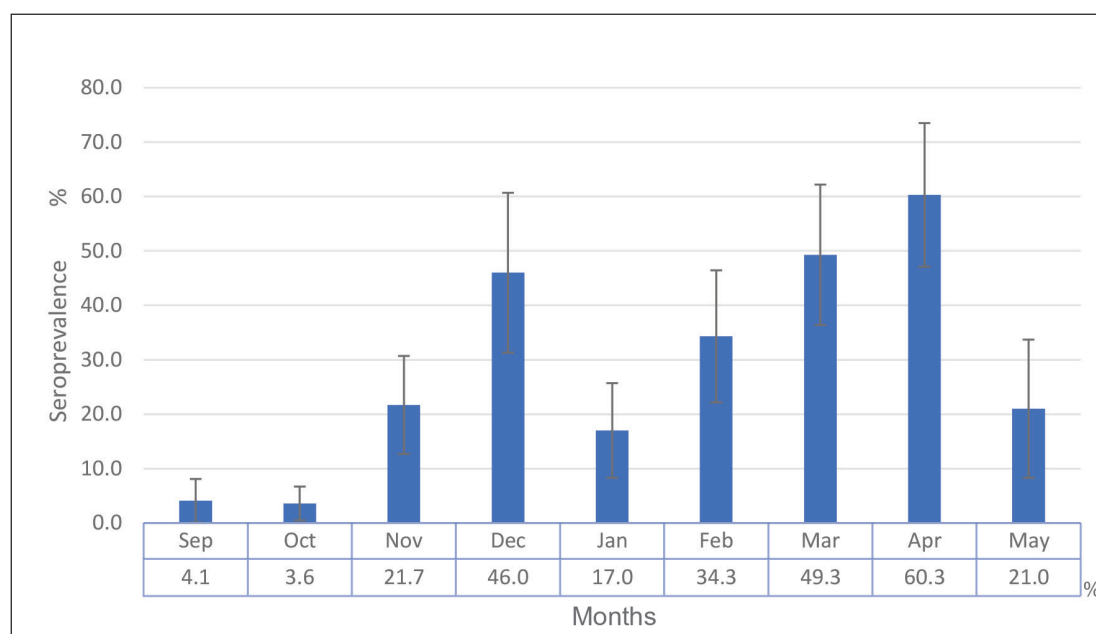


Figure 1. Seroprevalence throughout the nine-month time frame, with error bars indicating the 95% confidence interval.

Table 2. Comparison of Seroprevalence by Months (September 2020-May 2021) Based on Exact Test of Goodness-of-fit

SM* (%)	Sep (4.1)	Oct (3.6)	Nov (21.7)	Dec (46.0)	Jan (17.0)	Feb (34.3)	Mar (49.3)	Apr (60.3)	May (21.0)
	P values†								
Sep (4.1)	-	0.501	<0.001	<0.001	0.004	<0.001	<0.001	<0.001	<0.001
Oct (3.6)	0.501	-	<0.001	<0.001	0.001	<0.001	<0.001	<0.001	<0.001
Nov (21.7)	<0.001	<0.001	-	0.002	0.314	0.052	<0.001	<0.001	0.439
Dec (46.0)	<0.001	<0.001	0.002	-	<0.001	0.109	0.419	0.103	0.001
Jan (17.0)	0.004	0.001	0.314	<0.001	-	0.012	<0.001	<0.001	0.314
Feb (34.3)	<0.001	<0.001	0.052	0.109	0.012	-	0.062	0.005	0.029
Mar (49.3)	<0.001	<0.001	<0.001	0.419	<0.001	0.062	-	0.169	<0.001
Apr (60.3)	<0.001 [†]	<0.001	<0.001	0.103	<0.001	0.005	0.169	-	<0.001
May (21.0)	<0.001 [†]	<0.001	0.439	<0.001	0.314	0.029	<0.001	<0.001	-

*Seroprevalence by months; †Significant level ($P < 0.0014$). The Bonferroni correction was used to reduce type I error in 36 comparisons of seroprevalence by months.

Discussion

In this study, among the pediatric population tested in the Eurofarm laboratory in Sarajevo the number of positive cases throughout the nine-month period showed a significant increase in the number of positive cases in November-December 2020 and February-April 2021. These data coincide with the two major pandemic waves, one after the relaxation of public health measures in the autumn of 2020, and the next in February 2021

(20, 21). Similar trends were observed in studies of adults (22). The seroprevalence in Bosnia and Herzegovina among adults for the period from April to July 2020 was 3.77%, and one year later for the same population it was 29.9% (23). In Bosnia and Herzegovina, the first confirmed cases of COVID-19 were found at the beginning of March 2020, in Sarajevo Canton on 20th March. On that day, there were three positive cases (24). By mid-May 2020, in both entities, more than 2,200 cases

of COVID-19 were recorded, where children <18 years were represented by 9.5% cases (25). This was the period of the so-called “zero” wave of the pandemic. The first pandemic wave was from the middle of July to the beginning of September 2020. In our study, the lowest seroprevalence (3.6%) was registered in October 2020 after first pandemic wave. A systematic review and meta-analysis by Rostami et al. showed that SARS-CoV-2 seroprevalence in the general population in 23 countries after first six months of the pandemic varied from 0.37% to 22.1%, with a pooled estimate of 3.38%. This prevalence suggests, however, that about 96% of the world’s population are still susceptible to infection (12). Most published studies provided data on the seroprevalence of COVID-19 for the adult population. Only a few studies have reported seroprevalence data in children. A study from Seattle Children’s Hospital of 1076 children (ages 0-15) who sought medical care during March and April 2020, showed that about 1% of the children had antibodies to SARS-COV-2 (26). Data from Spain for the period April-May 2020 among children aged 0-19 years, showed that their seroprevalence was 3.8% (27). In Croatia, in the first pandemic wave in May 2020, the seroprevalence among the pediatric population was 2.9%, while for the period October-November 2020 it was higher, at 8.4% (28). In Sarajevo Canton the second major pandemic wave lasted from the middle of October to the end of December 2020. During this wave, the seroprevalence in our study increased from 21.7% in November to 46.0% in December. The increase in the number of cases corresponds to the time it takes for a person to create antibodies after infection. In our study, the children were divided into three age groups. In the 0-5 years group seroprevalence was 10.1%, in the 6-13 years group 44.4% and the 14-18 years group 45.5%, without any statistically significant difference. Generally, the incidence of COVID-19 in children increased with increasing age (1, 29). In a study from India, the highest number of seropositive children were aged 10-17 years (30), and in Italy, seropositivity was highest at the age of 12-17 years (31). In our study, in the total of anti-SARS-CoV-2 Ig-positive

individuals 42.8% were female while 57.2% were male, without a statistically significant difference. Other similar studies also did not point out differences in seroprevalence in relation to gender (28, 30, 31). On the basis of current data, the seroprevalence in children was generally low during the first year of the pandemic (26, 27). Strict public health prevention measures were in force. Most employers enabled work from home, population movement was limited, closure of schools and kindergartens resulted in children being less exposed (32). Initial studies indicated that children were asymptomatic carriers (33). Recent data suggest that most of the children were infected by transmission from a sick parent or household member (10, 34). Studies dated from 2021 showed an increase in seroprevalence in children. In Italy, in July 2020 the seroprevalence in children was 1%, while in January 2021 it increased to 9.5% (31). In our study, the highest seroprevalence (60.3%) was observed in April 2021, corresponding to the third pandemic wave which lasted from February to April 2021. Our results show that in the second year of the pandemic, the seroprevalence among children was significantly higher. According to a recent study from India SARS-CoV-2 seropositivity rate among children was high and comparable to that of the adult population. The seroprevalence was 55.7% in the <18 years age group, and 63.5% in the >18 years age group (30). Data from United States, as of February 2022, reported that approximately 75% of children and adolescents had serological evidence of previous infection with SARS-CoV-2 (35). From previous serostudies, children and adults had a similar risk of SARS-CoV-2 infection, but SARS-CoV-2 infections among children were mostly asymptomatic compared to adults.

Limitations of the Study

During the pandemic, serological testing in Sarajevo Canton was available in hospitals and private laboratories. In this research, we present data on seroprevalence among children in Sarajevo, tested in only one laboratory (the Eurofarm polyclinic) from September 2020 to May 2021. The

data were analyzed almost a year later. The main limitation of this study was that we collected blood samples from all the patients who came for serological testing, without collecting data regarding the reasons for testing. We do not have data on whether these were healthy children or children with comorbidities, whether they had previously been infected with COVID-19, or if they had any symptoms. During the study period, vaccines for COVID-19 were not available for children so they could not affect the antibody level. This report did not include the seroprevalence for two more recent pandemic waves in Sarajevo, the fourth wave from August to October 2021 and the fifth from late December 2021 to early February 2022. This research did not include children tested in hospitals and other laboratories. Despite all the limitations, this study indicates the importance of serological testing in children.

Conclusion

The results of our study show that the seroprevalence in children was low, especially during the first year of the pandemic. In the second year of the pandemic, there was an evident statistically significant increase in the number of seropositive children. Similar data have been shown in studies for adults.

What Is Already Known on This Topic:

During the pandemic, a large number of children had asymptomatic infection. Serological tests are helpful for assessing the total number of infected, including asymptomatic cases, monitoring the course of the pandemic, and planning public health measures. Seroprevalence studies provide valuable information for vaccination strategies because they help quantify what proportion of the population has been exposed to the virus, help achieve herd immunity, and help identify populations that may be at greater risk of infection.

What This Study Adds:

This is the first study about seroprevalence in children in B&H. The data we have presented can be helpful in planning public health measures in our country, and can help in assessing herd immunity for COVID-19. Also, this report can be useful for other databases on the seroprevalence of COVID-19 in children that are monitored worldwide.

Authors' Contributions: Conception and design: SMD and MLK; Acquisition, analysis and interpretation of data: SMD, MLK and JPM; Drafting the article: SMD; Revising it critically for important intellectual content: SMD, MLK, JPM and

AJ; Approved final version of the manuscript: AJ, JPM, MLK and SMD.

Conflicts of Interest: The authors declare that they have no conflict of interest.

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