Journal of
Virology Research And Advances In Vaccines

JScholar
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Research Article Open Access

SARS-Cov-2 Infection in Children and Adolescents During July-October 2020

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Received Date: April 24, 2023 **Accepted Date:** June 01, 2023 **Published Date:** June 03, 2023

Citation: Zahirović E, Salimovic Besic I, Muhić A, Dedeic Ljubovic A (2023) SARS-Cov-2 Infection in Children and Adolescents During July-October 2020. J Vir Res Adv Vac 2: 1-8

Abstract

Purpose: In this study we evaluated the extent and frequency of SARS-CoV-2 in school-attending children and adolescents during summer vacation and school attendance in the period from July-October 2020.

Methods: The study enrolled a group of children and adolescents aged 11- 24 years attending primary and secondary school, and colleges of Sarajevo Canton. Nasopharyngeal samples were collected for SARS-CoV-2 Real-time RT-PCR testing from July to October 2020. A bi-monthly monitoring period included number of SARS-CoV-2 positive adolescents, preventive behavior, and lifestyle information during the holidays and the beginning of the school year. The four-month follow-up period included the number of SARS-CoV-2 positive adolescents, information on preventive behavior and lifestyle during the holidays and the beginning of the school year.

Results: In the period July-October 2020, we processed a total of 2523 nasopharyngeal swabs of adolescents aged 11 to 24 years, of which 731 were confirmed SARS-CoV-2. Prevalence of SARS-CoV-2 infected for adolescents (15-18 age group 30.7%; 19-24 age group 29.7%) was greater then for children (11-14 age group 24,2%). The percentage of SARS-CoV-2 positive was significantly greater in July (49,4%) compared to other observed months.

Conclusions: In each month, the overall number of tested persons increased with age of group of adolescents although the percentage of positives varied. during vacations and school attendance

Keywords: COVID-19, SARS-CoV-2, Adolescents, Children

Implications and Contribution

Due to the prevention of COVID-19 infection, students attend a weekly combined model (half were in the classroom, the other half online). College students attended lectures online.

The months when the testing and positivity of all groups of adolescents reached the peaks followed the epidemiological situation of the general population.

In combating the spread of COVID-19, school closure was one of the most commonly used interventions. By mid-April 2020, 192 countries around the world had closed schools [1] and switched to online teaching.

From June to August 2020, school attenders and college students were on vacation in Sarajevo Canton. Return to school took place in early September 2020, and college lecture in October.

At the beginning of the school year (September 2020), the Cantonal Ministry of Education forwarded to schools the orders, measures, and recommendations of the Crisis Headquarters of the Federal Ministry of Health for the prevention of the spread of COVID-19 infection as follows: from 1st to 14th September 2020, the schools attended classes from I-IV according to the weekly combined model (half of the class, about 15 attenders, were in the classroom, and the other half followed the lessons online). The measures extended until the end of October, with classes V-IX included in the combined model.

From September 14 to October 9, 2020, classes for high school students of 1st and 4th grade realized a combined model of teaching in groups with a maximum of 15 students in a class, with a minimum distance between students of 1.5 meters. The students were arranged in two shifts. In the first week, one half ofstudents were in the school, and the other half of them received learning materials through an online platform by the subject teacher, and vice versa on the next week.

From October 23 to November 6, 2020, the combined model of the teaching process for primary schools continued. Attenders from V and/or VI grade and older followed e-learning model as well as high school students from I to IV grade.

College students had online lessons since the beginning of the school year. It was shown that school attenders and adolescents have lower COVID-19 morbidity and mortality (ref. kao ove u daljnjem tekstu), however, they can be potential carriers of the virus transmission. In fact, as per the WHO report in February 2020, there was no fatality due to coronavirus infection in the age group of 0–9 years, and the death rate was 0.2% in the age group of 10–19 years. The degree of mobility is higher in this age group of the population, so the probability of transmition is high (particularly to the high-risk population of the elderly) [2,3]. Many children infected with coronavirus often manifest fever, cough, and breathing difficulties, but also vomiting and diarrhea are among other symptoms [4]. It has been reported that children often have milder symptoms than adults and the elderly [5].

Upper respiratory infections caused by SARS-CoV-2 are also more prevalent in children than lower respiratory infections, which increases the ability to transmit the infection [6].

As there is no definite treatment to date for this illness, prevention has been the top priority [7]. The Centres for Disease Control and Prevention issued several instructions of the public interest intending to create awareness about coronavirus infection and its prevention among children. Parents, teachers, and other conscientious members of society should take responsibility for preventing the spread of coronavirus infection, as the child population can not care enough about the possible consequences of their behavior. They need to monitor the activities of children at home, school, and outside the home setting. During pandemics, one of the measures has been avoiding group activities, limiting playtime, and keep distance during playing and interaction. Furthermore, in the home and school setting, frequent cleaning of the surfaces and objects (walls, toilets, chairs, tables, boards, play items, reading materials) is recommended because of the possible transmission route of virus from infected individuals to healthy ones [8].

Choosing outdoor games in small groups may be more beneficial than indoor games for better ventilation, and the possibility of maintaining distance is higher during outdoor playing. Group travel, picnics, and study tours are to be strictly discouraged. Children have needed to be taught about hygiene regularly and monitored for the implementation of hygiene in practice [9].

Early isolation has been recommended for children with underlying disease manifestations [10]. Long-term monitoring of the consequences of SARS-CoV-2 infection on human growth and development, as well as the results of recommended preventive measures, treatment and vaccination options, is necessary to put the pandemic under control [9] .

This study aimed to evaluate the extent of SARS-CoV-2 infection in school-attending children and adolescents during vacations and school attendance in the period from July-October 2020 in Sarajevo Canton.

Materials and Methods

The work described has been carried out following The Code of Ethics of the World Medical Association (Declaration of Helsinki) for research involving humans. This research has been approved by the Ethics Committee of the Clinical Center of the University of Sarajevo, No. 06-04-9-44524/20. The research was conducted anonymously and all samples were coded.

The study was conducted from July to October, 2020. A total of 2523 clinical specimens from school-attending kids and adolescents (in the age of 11-24 years) who met the case definition [10]. Oropharyngeal and/or nasopharyngeal swabs were collected using universal viral transport collection kits (BD, Fisher Scientific, USA) containing 3 ml of the virus transport medium (VTM). The data were analyzed for the whole population and also divided into the three age groups of participants (11-14 years, 15-18 years and 19-24 years), attending primary and secondary school, and college, in the Sarajevo Canton.

Nucleic acids were extracted from nasopharyngeal/oropharyngeal swabs by automatic system Nextractor® (Genolution, Seoul, Republic of Korea) using the NX-48S Viral NA kit (Genolution, Seoul, Republic of Korea) to provide high-yield and quality DNA/RNA samples.

Following the manufacturer's instructions, 200 µl of each VTM specimen was used for nucleic acid extraction. Approximately 40–50 µl of the total nucleic acid eluate was recovered into a nuclease-free microplate and tested immediately by real-time RT-PCR.

The purified nucleic acids were amplified using the LabGun™ COVID-19 RT-PCR Kit (LabGenomics Co., Ltd, Republic of Korea). The LabGunTM COVID-19 RT-PCR Kit is a real-time reverse transcription-polymerase chain reaction (rtRT-PCR) test. Its primers and probes have been designed to target regions of RdRp and E gene of SARS-CoV-2 RNA genome from a variety of clinical specimens: nasopharyngeal, or oropharyngeal, anterior nasal, and mid-turbinate nasal swabs, as well as nasopharyngeal wash/aspirate or nasal aspirate specimens and sputum, of patients with signs and symptoms suspected of COVID-19.

Each 20 µl reaction mixture contained 10 µl of 2x One-step Buffer (One-step real-time RT-PCR buffer), 1 µl of the One-step Enzyme (DNA polymerase and reverse transcriptase), 1 µl Assay 1 or 2 (Assay 1 to detect RdRp gene- primers and probe mix for RdRp including Rox Dye, and Assay 2 targeting E gene-primers and probe mix for E including Rox Dye), and 5 µL of nucleic acid extract (Table 1). The rtRT-PCR program was set up as follows: reverse transcription (50°C, 30 minutes), initial denaturation (95°C, 15 minutes), amplification (45 cycles of the steps: 95°C, 15 seconds and 60°C, 60 seconds- data collection step) (Table 2).

The interpretation of the results was determined by reading the threshold cycle (Ct) value of each sample measurement for each target. The result was considered positive when Ct value was ≤ 40 for each target and negative if Ct value was above 40. The Ct value of the positive SARS-CoV-2 control and internal control (MS2) should be detectable at ≤ 40, and not detected for the negative control, for both virus genes. All test controls are included in the kit and examined before the interpretation of patient results.

Table 1: Components of the PCR reaction mixture

Reagents	Volume (µL)
2x One-step Buffer	10
One-step Enzyme	1
Assay 1 or 2	4
Internal Control	1
Total volume master mix per reaction	16
Viral RNA	4
Total reaction volume	20

Table 2: PCR protocol

Step	Temperature	Time	Cycles
cDNA syntesis	50°C	30 minutes	1
Pre-denaturation	95°C	15 minutes	1
Denaturation	95°C	15 seconds	45
Annealing & Exstension*	60°C	1 minute	

*collection of the fluorescence data

Results

The study analyzed data on the prevalence of SARS-CoV-2 infection for the population of adolescents in Sarajevo Canton during July-October, 2020. Overall, **2523** adolescents aged 11-24 were tested, of whom 731 (28.9%) had a positive SARS-CoV-2 test result (Table 3).

Table 3: Total number of tested and SARS-CoV-2 positive persons during the July-October, 2020

	July		August		September		October		Total	
Age (group)	N	n (%)	N	n (%)	N	n (%)	N	n (%)	N	n (%)
11	21	6 (28.6)	21	3 (14.3)	23	1 (4.3)	42	13 (36.9)	107	23(21.5)
12	11	5 (45.4)	19	5 (26.3)	24	3 (12.5)	46	17 (36.9)	100	30(30.0)
13	8	7 (87.5)	26	5 (19.2)	15	1 (6.7)	38	11 (28.9)	87	24(27.6)
14	10	3 (30.0)	31	1 (3.2)	42	6 (14.3)	40	14 (35)	123	24(19.5)
(11-14)	50	21 (42.0)	97	14 (14.4)	104	11 (10.5)	166	55 (33.1)	417	101 (24.2)
15	17	6 (35.3)	22	6 (27.3)	29	5 (17.2)	72	25 (34.7)	140	42(30.0)
16	19	9 (47.3)	39	9 (23.1)	25	1 (4.0)	72	28 (38.8)	155	47(30.3)
17	21	13 (61.9)	37	7 (18.9)	25	5 (20.0)	73	30 (41.1)	156	55(35.2)
18	18	6 (33.3)	46	13 (28.3)	31	4 (12.9)	56	18 (32.1)	151	41(27.1)
(15-18)	75	34 (45.3)	144	35 (24.3)	110	15 (13.6)	273	101 (40.3)	602	185 (30.7)
19	27	15 (55,5)	40	8 (20.0)	31	2 (6.4)	51	24 (47)	149	49(32.8)
20	31	19 (61.3)	41	11 (26.8)	44	4 (9.1)	65	22 (33.8)	181	56(30.9)
21	38	19 (50.0)	62	14 (22.6)	48	5 (10.4)	83	35 (42.2)	231	73(36.3)
22	61	29 (47.5)	65	12 (18.5)	48	7 (14.6)	78	31 (39.7)	252	79(31.3)
23	47	23 (48.9)	106	16 (15.1)	57	9 (15.8)	111	46 (41.4)	321	94(29.3)
24	41	18 (43.9)	98	16 (16.3)	82	18 (21.9)	181	53 (29.3)	402	105(26.1)
(19-24)	245	123 (50.2)	412	77 (18.7)	310	45 (14.5)	569	211 (37.1)	1536	456 (29.7)
Total:	338	167 (49.4)	653	126 (19.3)	524	71 (13.5)	1 008	367 (36.4)	2523	731 (28.9)

The lowest number of tested persons was observed in July 2020. However, the percentage of the positives in this month was the highest (167/338; 49.4%), especially for respondents of 13 years of age (87.5%). The youngest population (11 years of age) showed the lowest percentage of SARS-CoV-2 positives in this month (28.6%) (Table 3).

The month with the second highest number of tested was August (653) with 19.3% of positives. We noticed that in comparison with other periods of study, respondents in the age of 14 years was the least affected by infection (3.23%) (Table 3).

The lowest proportion of positive adolescents (71/524; 13.5%) was recorded in September. During this month, the lowest number of infected respondents (1/25; 4.0%) was among 16-year-olds, while the highest percentage was in 24-year-olds (18/82; 23.8%) (Figure 1).

The largest number of test requests was in October of which 36.4% (367/1008) were positive for SARS-CoV-2 infection. The lowest percentage of SARS-CoV-2 positives in October was 28.9% in 11-year-old adolescents, while the highest percentage was in 19-year-olds (47%).

According to the age group of adolescents, the highest prevalence of positives was noticed in the age group of 19 to 24 years (50.2%; 123/245) and 15-18 years (45.3%; 34/75) during July. The second month with the peak of positivity was September putting in the focus the same two age groups of adolescents (15-18 years, 40.3%, 101/273; and 19-24 years, 37.1%, 211/569, respectively), (Table 3, Figure2).

In each month, the overall number of tested persons increased with age of group of adolescents although the percentage of positives varied (Table 3).

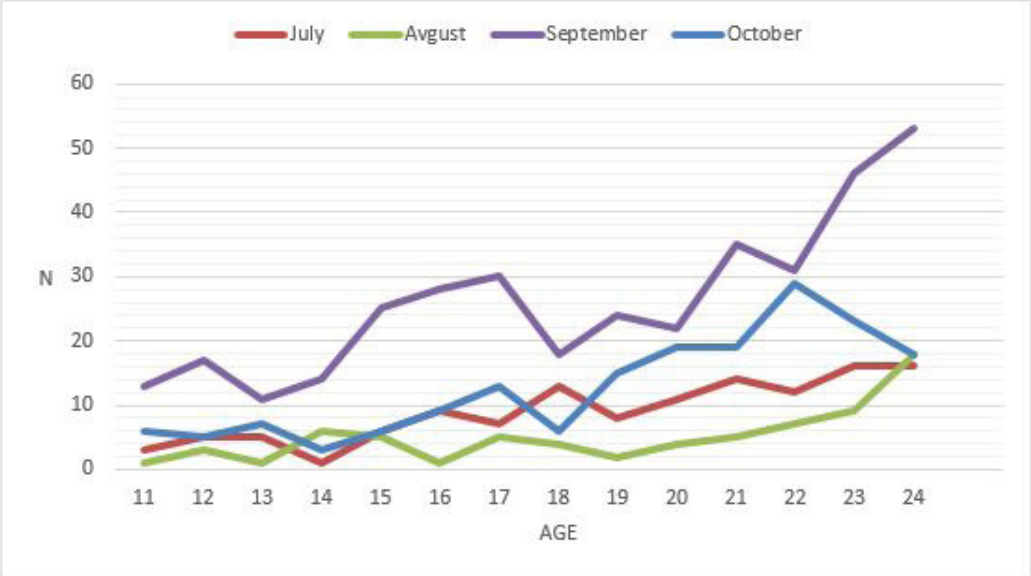

Figure 1: SARS- CoV- 2 infected adolescent per observed months and age

n – number

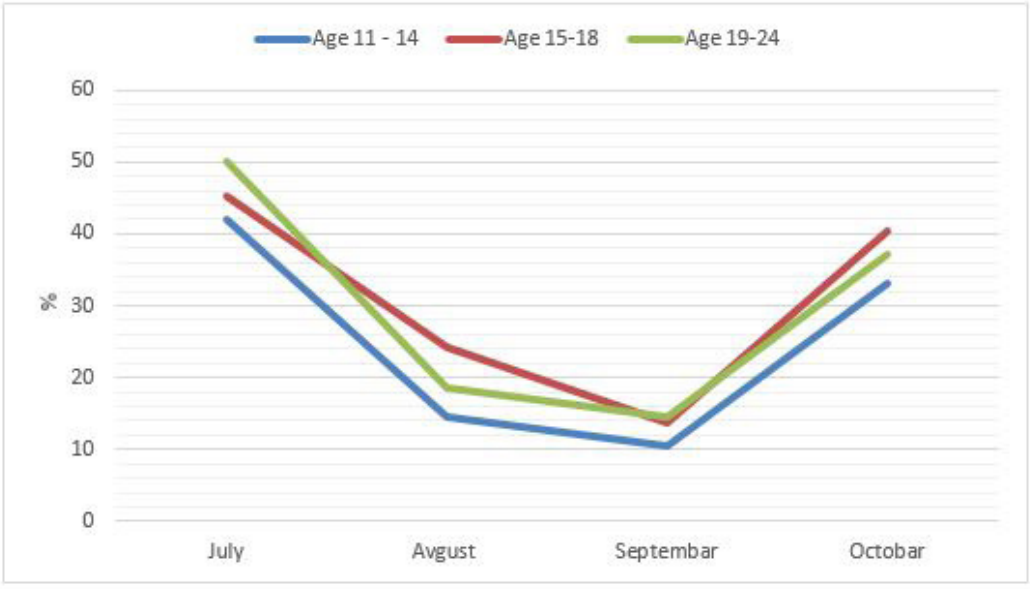

Figure 2: Prevalence of SARS-CoV-2 infected adolescents per month and age group

%- percentage of SARS-CoV-2 infected adolescents

Discussion

A worsening epidemic situation related to COVID-19 has been observed in Sarajevo Canton since mid-June. In the period from June 1 to July 24, 2020, 1,726 cases of COVID-19 were confirmed in the Sarajevo Canton. The increase in the number of newly infected has been registered since June 9, 2020. In the following period, the trend of increasing the number of cases continued, and the largest daily number of infected was on July 2, 2020, when 107 newly infected persons were confirmed. In July, the average number of newly infected was 57 people a day[10].

In this study, we showed that during the four month research period between July 1, and October 31, 2020, the highest percentage of SARS-CoV-2 positive adolescents in Sarajevo Canton followed the same pattern (during July- holidays and October- beginning school). Although the number of tested in October was the highest (1008/2523), the percentage of positives was not higher than in July (36,4% vs. 49,4%, respectively), when the maximum was observed.

According to the Sarajevo Canton Public Health Institute, a worsening of the COVID-19 epidemiological situation in general population has been observed from mid-June, which was also reflected on high positivity of our study population in July [11].

At the beginning of the school year, further epidemiological measures were implemented, by reducing the number of students (combined model) in primary schools. From 1th to 14th September, the high school was attended by students from I-IV classes according to the weekly combined model. Half of the class (about 15 students) were in the classroom, and the other half followed the lessons online. The measures have been extended until the end of October. The college students have had online classes since the beginning of the school year.

The epidemiological measures applied during that period included obligatory wearing of masks indoors, as well as in open areas where it was not possible to keep a distance of two meters. Also, the number of people indoors was reduced up to 50, by adhering to the recommended distance and wearing masks, as well as 100 people in the open area. During the stay indoors, including public spaces, as well as the use of public transport, the use of protective masks was mandatory, without exception.

Scientific research shows different results when it comes to COVID-19 infection in children. Especially when it comes to the transmission of COVID-19 among children, and from children to adults, in the study conducted in Australia was found that the secondary "attack rate" (AR) among students in most cases ranged from 0 to 1.2%, while it was recorded to be high (35.1%) in only one school [12]. Another study from Ireland showed no cases of secondary transmission of COVID-19 in schools among 1,025 contacts [13]. The only case of transmission was recorded among adults in a working environment, but outside of school. However, both of these studies were done on a smaller sample and in the early stages of a pandemic [12,13].

Two related studies were conducted in France, where the authors found high AR among students and staff in secondary schools (38% and 59%, respectively), and significantly lower among parents, siblings (11% and 10%, respectively) [14], and even lower AR in primary schools among students, staff or household (10% for all) [15]. They also did not find AR in primary schools convincing evidence for secondary transmission of infection. Research from South Korea showed a high transmission within the household if the index case was between 10 and 19 years old (19%)[16], compared to younger age (5%).

During the presented research period, it was noticed that the youngest group of adolescent was tested in the lowest number (Figure 1).

Ultimately, the incidence of COVID-19 in children and the youngest adolescent has likely been underestimated because of their propensity for asymptomatic or mild disease [17]. This could be the main reason for fewer tests and positivity of adolescent aged 11-14, which does not give an objective incidence when it comes to this population.

Conclusions

The hint of a second wave of pandemics resulted in an increase in the number of tests for all groups of adolescents reaching the maximum in October, although the proportion of positives did not reach the percentage recorded in July.

Deterioration of the epidemiological situation of COVID-19 in the general population during mid-June in Sarajevo Canton was reflected in high positivity of the study population in July, especially for adolescents of 19-24 years of age.

The higher number of SARS-CoV-2 positives among older adolescents was probably due to potentially higher transmission rate of this population. However, a smaller number of tests done on youngest adolescents aged 11-14 years, was a consequence of more prevalent asymptomatic or mild disease, which does not give an objective incidence when it comes to this population.

Regardless of the epidemiological measures taken for school activities, the number of tests and the positivity of adolescents followed the epidemiological situation of the general population in the Sarajevo Canton.

Further studies are needed to elucidate the role of adolescent population in the ongoing pandemic and particularly in schools reopening in a full.

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