Infants younger than 6 months old infected by SARS-CoV-2 show the highest respiratory viral loads

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Summary of article's main point: Data obtained from over 45,000 SARS-CoV-2-positive nasopharyngeal swab samples processed in Buenos Aires, Argentina, show that infants younger than 6 months old have substantially higher viral loads than any other age group.

Footnote page

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Abstract page

There is a paucity of reports on the characteristics of SARS-CoV-2 infection in infants, since most studies have grouped infants with older children. We analyzed the viral loads of 45,318 SARS-CoV-2-positive nasopharyngeal swab samples obtained in Buenos Aires, Argentina. Infants younger than 6 months old presented higher viral loads than any other age group. Children older than 6 months showed significantly lower viral loads, similar to those founds in adults. This observation raises new questions regarding the role of infants in the spreading of SARS-CoV-2 infection.

Key words: SARS-CoV-2, COVID-19, viral load, children,

BACKGROUND

Children infected by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are usually asymptomatic or show a mild clinical course [1]. There is a paucity of reports specifically attending infants, since many studies have grouped them with older children, hiding potential differences between age subgroups [2,3,4,5]. Here, we compared the respiratory viral loads of younger infants with those of older children, making use of data from 175,808 nasopharyngeal swabs processed between October 2020 and June 2021.

METHODS

Nasopharyngeal swab samples were collected at public and private health institutions from the city of Buenos Aires and the Greater Buenos Aires area (Argentina), from either symptomatic individuals or close contacts to a confirmed COVID-19 case. An independent Institutional Review Board (Fundación Huésped Bioethics Committee) waived the requirement of informed consent for the use of de-identified data in this study.

Detection of SARS-CoV-2 was performed using SARS-CoV-2 Nucleic Acid Detection Kit (Transgen Biotech, China), which targets viral genes ORF1ab and N, and the human RPP30 gene as internal control. For the purpose of this study, samples were defined as positive when cycle threshold (Ct)

values were below 36 for both ORF1ab and N genes. The RT-PCR kit showed a dynamic range from Cts 15 to 36, with an efficiency of 89%, for ORF1ab amplification, as evaluated by plotting Ct versus $\log_{10}$ of serial dilutions of a low-Ct sample pool (r^2 = 0.9932).

The performance of the RT-PCR kit was controlled throughout the study by registering Ct values of positive controls in each run. When a positive control resulted in a Ct value outside of pre-established internal error limits, the run was repeated.

Variant determination was carried out using the SARS-CoV-2 Extended ELITe MGB kit (ELITech Group, Italy). Briefly, a real-time RT-PCR is performed on RNA extracted from nasopharyngeal swab samples and then the following mutations are detected by melting curve analysis: L452Q, L452R, E484K, E484Q and N501Y. Sanger sequencing of the S gene from selected samples confirmed the identity of the mutations.

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Statistical analysis was performed using nonparametric Kruskal-Wallis tests followed by Dunn's post hoc test for multiple comparisons for group analysis of Ct values, and chi-square test to compare demographic data.

RESULTS

A total of 175,808 samples were processed in the period between October 2020 and June 2021. We segmented our positive cohort (n = 45,318) by ten-year intervals to compare viral loads between different age groups. The median value of ORF1ab Ct in the 0-9 years old group (n = 528) was significantly higher when compared to any other age group (p value <0.001) (27.19 [21.5-34.09]) (median [interquartile range]) (**Figure 1A**)**.** The density plot showed a bimodal distribution of ORF1ab

Ct values across all age groups, being the distribution in the 0-9 age group skewed to higher Cts, suggesting lower viral loads in the upper respiratory tract (**Figure 1B**). Next, we analyzed the Ct values within the 0-9 age group by stratifying it in different subgroups: 0-6 months, 7-12 months, 1-4 years, and 5-9 years. Notably, the 0-6 months old group (n = 46) displayed the lowest median value of ORF1a Ct (20.77 [18.1-26.87]) (median [interquartile range]) compared to any other age groups, including adults (**Figure 1C**). In fact, the median Ct of the 0-6 months' group was between 6 to 10 cycles lower compared with either the 7-12 months (30.12 [22.01-34.56], p value = 0.0001), the 1-4 years old group (30.46 [22.34-34.69], p value < 0.0001) or the 5-9 years old group (26.58 [22.34-33.8], p value = 0.0001).

These results could not be explained by known confounders of viral load determination. To begin with, there were no differences among age groups in the median Ct values for the internal control gene (RPP30) nor in the time between symptom onset and sample collection (**Table 1**). Secondly, there have been studies about the possible association between symptomatic infection and higher viral loads, with contrasting findings [6,7,8]. In this regard, it should be emphasized that in our cohort the frequency of symptomatic patients was similar across all age groups (**Table 1**). Another potential confounder when analyzing viral loads across time is the displacement of circulating variants by the Delta variant, which is characterized by high viral shedding. By the end of our study, in June 2021, there was only one reported case (an inbound traveler) of Delta variant in Argentina, according to genomic surveillance public data [9]. Community circulation of the Delta variant in the Buenos Aires area was first demonstrated in samples obtained during August 2021 [10]. Furthermore, we retrospectively assessed 66 samples obtained between April and June (the final months of our study) to determine the presence of variant-associated mutations in the S gene. All age groups were represented in the sampling (range 0 to 87 years old). We found 34 samples (52%) carrying mutations E448K and N501Y (compatible with Gamma variant of concern) and 30 (45%)

samples carrying mutation L452Q (compatible with the Lambda variant of interest). Two other samples presented either no mutation or only the N501Y mutation. We did not find the Delta-associated L452R mutation in any of the samples, further indicating that our data distribution was not skewed by Delta variant introduction.

Finally, vaccination status could not be considered as a confounder factor, since by the end of our study only 6.72% of the general population in the Buenos Aires area had been vaccinated, and at this time vaccination was concentrated in health care workers and the elderly and did not include children [11].

DISCUSSION

While it is clear that SARS-CoV-2 infection in children is mostly mild and often asymptomatic, its contribution to the spreading of the infection has not been well defined [12,13]. In this regard, it should be noted that most previous studies have analyzed pediatric COVID-19 considering children as a homogeneous population. We found that infants younger than 6 months old show higher viral loads than any other age group. Our observations are partially consistent with a previous study, which reported that symptomatic COVID-19 in infants younger than 12 months have higher nasopharyngeal viral loads compared with older children and adolescents [7]. Here, by studying a larger cohort of patients, we found that SARS-CoV-2-infected children younger than 6 months old, either symptomatic or asymptomatic, show the highest viral loads. In contrast, our data indicate that children older than 7 months display lower viral loads compared to those found in adults.

These findings are consistent with recent studies directed to analyze the role of children in SARS-CoV-2 transmission. A large epidemiological study by Paul et al. found that children aged 0 to 3 years showed the highest probability of transmitting SARS-CoV-2 to household contacts when compared to older children [12]. Previously, a study from Spain had found a similar result for the group aged 0

to 2 years old [14]. Our results suggest that higher viral loads in the infant population could be a contributing factor explaining increased transmission by this age group compared to older children.

Our results reinforce the notion that there is not a direct correlation between viral load and disease severity. In our cohort, younger infants (0-6 months old) showed Ct values between 6 and 10 cycles lower than other children, while they were reported to have equal or even a lower proportion of symptomatic infections. Our observations are in agreement with those presented earlier by Zachariah et al. which showed less severe presentations but higher viral loads in infants [7].

One limitation of our study is the use of Ct values as a proxy to viral load. While Ct values are inversely correlated to the logarithm of viral load, the actual conversion depends on the PCR design and efficiency. Thus, raw Ct values reported in this study cannot be directly compared to Cts values obtained under different assay conditions.

In conclusion, we found that children younger than 6 months old display higher SARS-CoV-2 viral loads compared to all other age groups. Whether this reflect a lower ability to control SARS-CoV-2 replication at the upper respiratory tract, remains to be established.

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Figure legends

Figure 1. ORF1a Ct values across age groups. A, 45,318 positive samples segmented by ten years old intervals; *** 0-9 vs other groups p <0.001**. B,** Density plot showing Ct value distribution (colors correspond to groups in 1A). **C,** Ct values from infants and children subgroups; *** 0-6 months vs any other age group p<0.001.

Table

Table 1. Demographic characteristics of 528 children and infants less than 10 years old who tested positive for SARS-CoV-2.

	Group 1 0-6 months n = 46	Group 2 7-12 months n = 40	Group 3 1-4 years n = 167	Group 4 5-9 years n = 275
Sex [a]				
Female, No. (%)	21 (46)	21 (53)	83 (50)	141 (51)
Male, No. (%)	25 (54)	19 (48)	84 (50)	134 (49)
Reported Symptoms [a]				
Symptomatic, No. (%)	28 (61)	28 (70)	101 (60)	165 (60)
Non reported symptoms, No. (%)	18 (39)	12 (30)	66 (40)	110 (40)
Sample collection: days after symptom onset				
Median [IQR]	2 [1-3]	3 [2-4]	3 [2-4]	3 [2-4]
PCR - internal control values				
RPP30 Ct, mean (SD) [b]	26.36 (2.03)	25.79 (1.6)	26.31 (2.21)	26.60 (2.1)

[a] Chi-square analysis showed no significant differences among the analyzed groups (p value > 0.05).

[b] Krustall-Wallis analysis was performed. Dunn's multiple comparisons test showed no significant differences. Group 1 vs Group 2 (Adjusted P value = 0.81); Group 1 vs Group 3/Group 4 (Adjusted P value > 0.99)

Figure 1