Infants Younger Than 6 Months Infected With SARS-CoV-2 Show the Highest Respiratory Viral Loads

Valeria Ochoa,1 Fernando Erra Díaz,2 Ezequiel Ramírez,2 María Clara Fentini,2 Mauricio Carobene,3 Jorge Gefner,2 Lourdes Arruotto,4 Federico Remes Lenicov1; on behalf of the INBIRS COVID-19 Study Group

1Universidad de Buenos Aires-Instituto de Investigaciones Biomédicas en Retrovirus y SIDA, Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina
2Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina

There is a paucity of reports on the characteristics of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in infants, because 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 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.

Keywords. SARS-CoV-2; COVID-19; viral load; children.

Children infected with 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, because many studies have grouped them with older children, hiding potential differences between age subgroups [2–5]. 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 coronavirus disease 2019 (COVID-19) case. An independent Institutional Review Board (Fundación Huésped Bioethics Committee) waived the requirement of informed consent for the use of deidentified data in this study.

Detection of SARS-CoV-2 was performed using SARS-CoV-2 Nucleic Acid Detection Kit (Transgen Biotech), 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 reverse transcription polymerase chain reaction (RT-PCR) kit showed a dynamic range from Ct 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 preestablished internal error limits, the run was repeated.

Variant determination was carried out using the SARS-CoV-2 Extended ELITe MGB kit (ELITech Group). Briefly, a real-time RT-PCR was performed on RNA extracted from nasopharyngeal swab samples and then the following mutations were 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.

Statistical analysis was performed using nonparametric Kruskal-Wallis tests followed by Dunn post hoc test for multiple comparisons for group analysis of Ct values, and χ^2 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 10-year intervals to compare viral loads between different age groups. The median value of ORF1ab Ct in the 0–9 years age group (n = 528) was significantly higher when compared to any other age group (median 27.19; interquartile range [IQR], 21.5–34.09; P value < .001; Figure 1A). The density plot showed a bimodal distribution of ORF1ab Ct values across all age groups, that is the distribution in the 0–9 years 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 years age group by stratifying it in subgroups: 0–6 months, 7–12 months, 1–4 years, and 5–9 years. Notably, the 0–6 months age group (n = 46) displayed the lowest median value of ORF1a Ct (median, 20.77; IQR, 18.1–26.87) compared to any other age group, including adults (Figure 1C). The median Ct of the 0–6 months age group was between 6 and 10 cycles lower compared with either the 7–12 months (median,
30.12; IQR, 22.01–34.56; *P* value = .0001), 1–4 years (median, 30.46; IQR, 22.34–34.69; *P* value < .0001), or the 5–9 years age group (median, 26.58; IQR, 22.34–33.8; *P* value = .0001).

These results could not be explained by known confounders of viral load determination. First, there were no differences among age groups in the median Ct values for the internal control gene (*RPP30*) or in the time between symptom onset and sample collection (Table 1). Second, there have been studies on the possible association between symptomatic infection and higher viral loads, with contrasting findings [6–8]. In this regard, it should be emphasized that in our cohort the frequency of symptomatic patients was similar across all age groups (Table 1).

Third, 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 1 reported case (an inbound traveler) of Delta variant in Argentina, according to genomic surveillance public data [9].

**Table 1.** Demographic and Sample Characteristics of 528 Children and Infants Younger Than 10 Years Who Tested Positive for SARS-CoV-2

| Characteristic                        | Group 1 0–6 mo (n = 46) | Group 2 7–12 mo (n = 40) | Group 3 1–4 y (n = 167) | Group 4 5–9 y (n = 275) |
|---------------------------------------|--------------------------|---------------------------|-------------------------|-------------------------|
| Sex                                   |                          |                           |                         |                         |
| Female, No. (%)                       | 21 (46)                  | 21 (53)                   | 83 (50)                 | 141 (51)                |
| Male, No. (%)                         | 25 (54)                  | 19 (48)                   | 84 (50)                 | 134 (49)                |
| Reported symptoms                     |                          |                           |                         |                         |
| Symptomatic, No. (%)                  | 28 (61)                  | 28 (70)                   | 101 (60)                | 165 (60)                |
| No reported symptoms, No. (%)         | 18 (39)                  | 12 (30)                   | 66 (40)                 | 110 (40)                |
| Time from symptom onset to sample collection, d, median (IQR) | 2 (1–3) | 3 (2–4) | 3 (2–4) | 3 (2–4) |
| PCR internal control RPP30 Ct, mean (SD) | 26.36 (2.03) | 25.79 (1.6) | 26.31 (2.21) | 26.60 (2.1) |

Abbreviations: Ct, cycle threshold value; PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

*χ*² analysis showed no significant differences among the analyzed groups (*P* value > .05).

*Kruskal-Wallis* analysis was performed. Dunn multiple comparisons test showed no significant differences. Group 1 vs group 2, adjusted *P* value = .81; group 1 vs group 3/group 4, adjusted *P* value > .99.
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, 0–87 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, because 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 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 showed higher viral loads than any other age group. Our observations are partially consistent with a previous study, which reported that infants younger than 12 months with symptomatic COVID-19 had 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, 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 at analyzing 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 a group aged 0 to 2 years [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 by Zachariah et al, which showed less-severe presentation but higher viral loads in infants [7].

One limitation of our study is the use of Ct values as a proxy for 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 Ct values obtained under different assay conditions.

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

Notes

Acknowledgments. We are grateful to Dr Horacio Salomón and the members of the Instituto de Investigaciones Biomédicas en Retrovirus y SIDA COVID-19 working group for processing the samples and generating the data: Alan Adamczyk, Sabrina Azzolina, Lucía Baquero, Paula Benencio, Carolina Berini, Mirna Biglione, Lucía Bleichmar, Gonzalo Cabrerizo, Silvia Cassime, Ricardo Cassime, Ana Ceballos, Cintia Cevallos, Alejandro Czernikier, Victoria Delpino, Facundo Di Diego García, Nicolás Ducasa, Mercedes Elizalde, Diego Flichman, Ramiro Gatti, Yanina Ghiglione, Denise Giannone, Claudio Gómez, Virginia Gonzalez Polo, Natalia Laufier, Luz Leitaj, Yesica Longueira, Alvaro Lopez Malizzia, Ignacio Mazzitelli, Claudia Melucci Ganzarain, Fernando Montesano, Nicolás Morando, Matías Ostrowski, Sandra Pampuro, Ana Paletta, María Pando, Federico Penna, Paula Pérez, Claudio Piccardo, Azul Peralisi, Mónica Pippo, Laura Polo, Florencia Quiroga, Juan Sabatté, Melina Salvatori, Inés Sananes, Vanesa Seery, Micaela Speroni, Gabriela Turk, Augusto Varese, Belén Vecchione, and Douglas Vera Aguilar.

Financial support. This work was supported by the University of Buenos Aires; and by the National Ministry of Science Technology and Innovation of Argentina.

Potential conflicts of interests. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Shekerdemian LS, Mahmood NR, Wolfe KK, et al. International COVID-19 PICU collaborative. characteristics and outcomes of children with coronavirus disease 2019 (COVID-19) infection admitted to US and Canadian pediatric intensive care units. JAMA Pediatr 2020; 174:868–73.
2. Ade C, Pum J, Abele I, Raggub L, Bockmühl D, Zöllner B. Analysis of cycle threshold values in SARS-CoV-2-PCR in a long-term study. J Clin Virol 2021; 138:104791.

3. Madera S, Crawford E, Langelier C, et al. Nasopharyngeal SARS-CoV-2 viral loads in young children do not differ significantly from those in older children and adults. Sci Rep 2021; 11:3044.

4. Baggio S, L’Huillier AG, Yerly S, et al. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) viral load in the upper respiratory tract of children and adults with early acute coronavirus disease 2019 (COVID-19). Clin Infect Dis 2021; 73:148–50.

5. Heald-Sargent T, Muller WJ, Zheng X, Rippe J, Patel AB, Kociolek LK. Age-related differences in nasopharyngeal severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) levels in patients with mild to moderate coronavirus disease 2019 (COVID-19). JAMA Pediatr 2020; 174:902–3.

6. Chung E, Chow EJ, Wilcox NC, et al. Comparison of symptoms and RNA levels in children and adults with SARS-CoV-2 infection in the upper respiratory tract of children and adults with early acute coronavirus disease 2019 (COVID-19). JAMA Pediatr 2021; 175:e21025.

7. Zachariah P, Halabi KC, Johnson CL, Whitter S, Sepulveda J, Green DA. Symptomatic infants have higher nasopharyngeal SARS-CoV-2 viral loads but less severe disease than older children. Clin Infect Dis 2020; 71:2305–6.

8. Kociolek LK, Muller WJ, Yee R, et al. Comparison of upper respiratory viral load distributions in asymptomatic and symptomatic children diagnosed with SARS-CoV-2 infection in pediatric hospital testing programs. J Clin Microbiol 2020; 59:e02593-20.

9. Ministerio de Salud de la Nación Argentina. Nuevo coronavirus COVID-19. Información epidemiológica. Informes vigilancia genómica. Junio 2021. https://www.argentina.gob.ar/salud/coronavirus-COVID-19/informacion-epidemiologica/junio-2021. Accessed 4 August 2021.

10. Torres C, Mojsiejczuk I, Acuña D, et al. Cost-effective method to perform SARS-CoV-2 variant surveillance: detection of alpha, gamma, lambda, delta, epsilon and zeta in Argentina [accepted for publication 2 November 2021]. Front Med. doi:10.3389/fmed.2021.755463

11. Ministerio de Salud de la Nación Argentina. Datos abiertos del Ministerio de Salud. http://datos.salud.gob.ar/dataset/vacunas-contra-covid19-dosis-aplicadas-en-la-republica-argentina. Accessed 4 August 2021.

12. Paul LA, Daneman N, Schwartz KL, et al. Association of age and pediatric household transmission of SARS-CoV-2 infection. JAMA Pediatr 2021; 175:1151–8.

13. Hyde Z. Difference in SARS-CoV-2 attack rate between children and adults may reflect bias [published online ahead of print 26 February 2021]. Clin Infect Dis doi: 10.1093/cid/ciab183.

14. Soriano-Arandes A, Gatell A, Serrano P, et al. Household severe acute respiratory syndrome coronavirus 2 transmission and children: a network prospective study. Clin Infect Dis 2021; 73:e1261–9.