Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Letter to the Editor

Lower nasopharyngeal viral loads in pediatric population. The missing piece to understand SARS-CoV-2 infection in children? ☆

Dear Editor,

SARS-CoV-2 virus infects children but, contrary to other respiratory viruses, children tend to be asymptomatic or to have less symptoms than adults and are rarely the index case in household transmission chains[1], so they do not seem to be major drivers of transmission[2]. Understanding the role of children in the transmission of the virus would help designing appropriate control measures, planning the reopening of schools and restoring intergenerational contacts[3]. Several studies have addressed the viral loads of children, some have concluded that viral loads are not higher than adults[1,4] but some of them found the opposite[5,6]. Yet determination of viral loads is complicated by the lack of standardization and the nature of nasopharyngeal samples that are taken by surface swabbing[7]. Some studies use standard curves to translate Ct values into RNA copies/ml[1], one used the weight of collected secretions to give RNA copies/g of secretion[4] but these approaches do not address the variation in the amount of sample collected by the swabs. An accurate normalization assay should be based on a marker of collected cell mass like the human RNAseP[6,7].

The aim of this study was to compare the relative viral loads[7] in nasopharyngeal samples from children aged 0 to 17 years with those of an adult population. We recovered 126 positive nasopharyngeal samples from children (35 samples belonging to patients from 0 to 5 years old; 36 from 6 to 11 years old and 55 from 12 to 17 years old) and 127 positive samples from adults (>17 years old) collected from July to December 2020, during the second and third waves of the pandemic in Madrid. This period was largely dominated by clade 20E (EU1). The samples had been received at the Microbiology Department of Hospital Universitario La Paz from the hospital emergency rooms (pediatric and adult) and the associated primary care centers. Given the frequent changes in the nucleic acid extraction and RT-PCR systems used during the studied period in our department, we did not use the Ct data from the clinical registers. The samples had been stored frozen at −80 °C and all of them were recovered and re-tested simultaneously. Nucleic acids were extracted using a King Fisher Flex System with the MagMAX Pathogen RNA/DNA extraction kit (Thermofisher Scientific, Waltham, MA, USA). Relative viral loads were measured by RT-PCR and the comparative DCt method[7] using primers and probes targeting the viral gene E and the human RNAseP[8].

The Ct values obtained for the viral gene E were higher and more dispersed in children than in adults (mean values 26.6 and 24.5 respectively, *t*-test $p = 0.0049$, ranges 12.4 to 44.4 and 15.04 to 36.7) (Fig. 1A). The Ct values obtained for the human RNAseP were lower in children (mean values 28.5 and 29.5 respectively, *t*-test $p = 0.006$) and more dispersed in adult samples (range 24.4 to 36.9 in children vs 20.9 to 42.9 in adults) (Fig. 1B). Upon normalization the dispersion of the data was similar in the two groups (ranges −5.2 to 4.2 and −2.8 to 7.8 in children and adults respectively), but the distribution of the relative loads was displaced towards lower values in children (mean log(DCt)=0.59 vs 1.47, *t*-test $p = 0.0003$). As a consequence, the values of the first quartile of the children data were lower than the value of the 5th percentile of the adult data (Fig. 1C).

We found that the children samples had RNAseP Ct values more homogeneous than those of the adults. We do not know the reason for this, but it might be related to the fact that the swabs *adjust* better to the smaller size of children. In any case, the human RNAseP data show that there is a difference in the amount of material collected in the two populations and viral Ct values should not be used directly to compare them.

We also found that the relative viral loads were significantly lower in the children population than in the adult one. This could explain the lower sensitivity observed for antigen tests in pediatric population[9,10]. The sensitivity of antigen tests is higher when viral loads are high but the fraction of the population with very low loads is higher in the children than in the adults (Fig. 1C).

The main limitation of this study is that it is a retrospective analysis with samples stored in our institution. Most had been sent from associated primary care centers, we did not have access to their clinical records or the data related to the clinical event is limited and could not register the time after or before symptoms onset and other clinical features of the patients. Most of them were followed in the primary care centers. This implies that they were asymptomatic or mild to moderate COVID19 patients.

In conclusion, in our study the average viral load was lower in the children population than in the adult one. More important, in one fourth of the children the relative loads were lower than the 5th percentil of the adult population. Despite the limitations of the study, this is a significant fraction of the population, and could have an impact on the transmission rates, the sensitivity of the antigen tests or the proportion of COVID19 cases reported.

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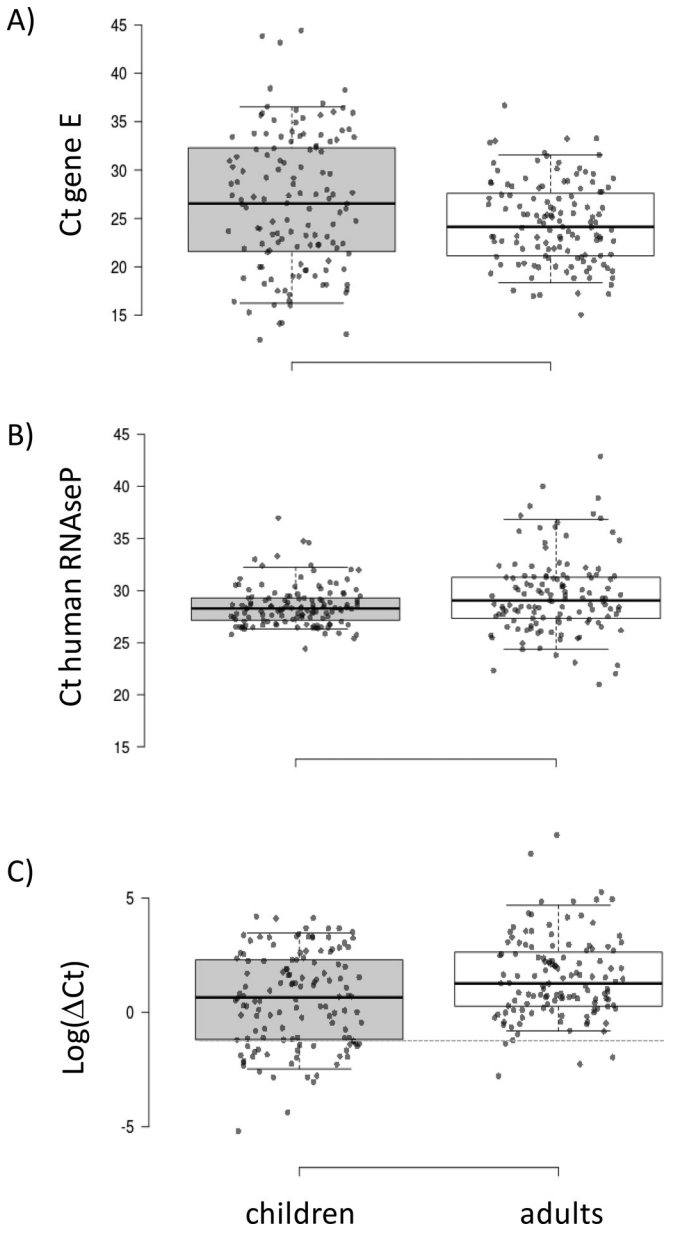

Fig. 1. Boxplots showing the RT-PCR Ct values of **A)** the viral gene E, **B)** the human RNAseP, and **C)** the normalization in logarithmic scale, log(DCt). gray boxes in the left are children data, white boxes in the right are adult data. Box center lines show the medians; box limits indicate the 25th and 75th percentiles; whiskers extend to 5th and 95th percentiles and outliers are represented by dots. The gray dashed line in panel C marks the 25th percentile of the children population. Graphics drawn with BoxPlotR (http://shiny.chemgrid.org/boxplotr/).

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